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(54) Arzneimittel mit einem Gehalt an Ciclosporin

(57) Es wird ein Arzneimittel mit einem Gehalt an Ciclosporin vorgeschlagen, das als Nanoemulsion vorliegt. Außerdem wird die Verwendung eines derartigen Arzneimittels zur Behandlung von Hautkrankheiten und zur Behandlung des menschlichen Auges vorgeschlagen. Ein Verfahren zur Herstellung des Arzneimittels wird ebenfalls bereitgestellt.

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Beschreibung

Die Erfindung betrifft ein Arzneimittel mit einem Gehalt an Ciclosporin.

Derartige Arzneimittel sind allgemein zur Behandlung von Transplantationspatienten bekannt.

Der Wirkstoff Ciclosporin ist ein zyklisches, aus elf Aminosäuren bestehendes Peptid, mit der Summenformel $C_{62}H_{111}N_{11}O_{12}$. Es wird auch als Cyclosporin A (WHO) bezeichnet. Ursprünglich wurde es aus Pilzen isoliert. Inzwischen sind auch Verfahren zu seiner synthetischen Herstellung bekannt.

Ciclosporin ist ein sogenannter Immunmodulator mit immunsuppressiver Wirkung. Es blockiert die Aktivierung von Helfer- und Killerzellen des Immunsystems durch Inhibition der Lymphokinproduktion. Ciclosporin unterdrückt dabei sowohl die humorale als auch die zelluläre Immunreaktion, indem es die Freisetzung von Interleukinen, insbesondere von IL-1 aus Monozyten und IL-2 aus T-Helfer-Zellen in den frühen Phasen der Immunantwort unterbindet.

Aufgrund dieser immunsuppressiven Wirkung wird Ciclosporin zur Vorbeugung der Transplantatabstoßung nach allogenen Transplantationen von Niere, Leber, Herz, Herz-Lunge, Lunge und Pankreas sowie nach Knochenmark-Transplantationen eingesetzt.

Außerdem wird Ciclosporin zur Behandlung der Graft-Versus-Host-Krankheit eingesetzt, einer Krankheit, die bei Transplantationspatienten nach Übertragung fremder immunkompetenter Zellen durch zelluläre Immunreaktionen auftritt.

Weitere Anwendungsgebiete für Ciclosporin sind die Behandlung von schwerer endogener Uveitis, einer schweren Entzündung der Aderhaut des Auges, sowie von schwersten therapieresistenten Formen der Psoriasis (Schuppenflechte). Auch die therapeutische Wirksamkeit von Ciclosporin zur Behandlung des steroidabhängigen und steroidresistenten nephrotischen Syndroms, also von Nierenerkrankungen, die mit einem ausgeprägten Eiweißverlust einhergehen, sind bekannt.

Bisher wurde Ciclosporin als Infusionslösung sowie als Trinklösung von der Sandoz bzw. Novartis AG, Basel dargereicht. Da Ciclosporin ein hydrophobes Peptid ist, das in wäßriger Lösung nicht lösbar ist, enthielten die bisherigen Darreichungsformen als Emulgatoren bzw. Löslichkeitsvermittler in großen Mengen Ethanol (ca. 12 Vol.-%) sowie Lipide in Form von Maiskeimöl und Triacylglycerid-Derivaten.

Die bisher bekannten Verabreichungsformen von Ciclosporin sind lediglich zur systemischen Anwendung geeignet. Wenn Ciclosporin in Form einer Trinklösung verabreicht wird, so erfolgt die Aufnahme in den Körper über den Darm. Bei einer Infusion der Ciclosporin-Lösung gelangt der Wirkstoff direkt ins Blut und verteilt sich über das Blut im gesamten Körper.

Eine topische, d. h. örtlich begrenzte Anwendung von Ciclosporin ist aufgrund seiner lipophilen Eigenschaften, die die Verwendung von Ethanol und Lipiden zum Löslichmachen des Ciclosporins erfordern, problematisch.

Eine andere pharmazeutische Zubereitung, die unter anderem auch für Ciclosporin vorgeschlagen wird, ist in der US 5,154,930 beschrieben. Diese Verabreichungsform umfaßt ein salzfreies geladenes Lipid, wie bspw. Phosphatidylethanolamin oder Phosphatdidylserin sowie ein Lösungsmittel wie Polyethylenglykol oder Ethanol. Dabei bilden sich in der pharmazeutischen Zusammensetzung Liposomen-Komplexe zwischen dem Wirkstoff und den Lösungsmitteln. Die beschriebene pharmazeutische Zubereitung erlaubt es, besonders hohe Konzentrationen an Wirkstoff, bspw. Ciclosporin, zu verabreichen.

Als Verabreichungsformen werden Tabletten, Kapseln, Dragees und ähnliches vorgeschlagen. Zur örtlich begrenzten Verwendung an besonders empfindlichen Körperbereichen ist diese Darreichungsform aufgrund der Anwesenheit der zum Löslichmachen der hydrophoben Wirkstoffe notwendigen Lösungsmittel Ethanol bzw. Polyethylenglykol jedoch nicht geeignet.

Die topische Verwendung von hydrophoben Wirkstoffen ist immer dort besonders problematisch, wo stark wasserhaltige bzw. hydrophile Körperteile behandelt werden sollen, da eine Voraussetzung zur Aufnahme solcher Wirkstoffe in den Körper darin besteht, zunächst einen Kontakt zwischen Wirkstoff und Körperoberfläche herzustellen.

Vor diesem Hintergrund ist es die Aufgabe der Erfindung, ein Arzneimittel mit einem Gehalt an Ciclosporin bereitzustellen, das zur topischen Verabreichung auch an stark wasserhaltigen und/oder sehr empfindlichen Körperbereichen geeignet ist, und mit dem eine gute Wirkstoffaufnahme ermöglicht wird.

Es ist eine weitere Aufgabe der Erfindung, neue Anwendungsgebiete für ein derartiges Arzneimittel vorzuschlagen.

Erfindungsgemäß wird diese Aufgabe dadurch gelöst, daß das Ciclosporin in einer Öl-in-Wasser-Nanoemulsion vorliegt. Unter einer Nanoemulsion im Sinne der Erfindung wird jede Öl-in-Wasser-Emulsion verstanden, die Tröpfchengrößen im Nanometerbereich, also mit Durchmessern von kleiner als 1 µm enthält. Derartige Nanoemulsionen haben eine ölige bzw. Lipid-Phase und eine wäßrige Phase, wobei die wäßrige Phase Wasser oder physiologisch verträgliche wäßrige Lösungen wie bspw. physiologische Kochsalzlösung (0,9 Gew.-% Natriumchlorid in Wasser) aufweist.

In einer derartigen Nanoemulsion wird der hydrophobe Wirkstoff Ciclosporin in den winzigen öligen Tröpfchen gelöst, die wiederum in der wässrigen Phase dispergiert sind. Somit ist der Wirkstoff Ciclosporin optimal verteilt. Bei einer Applikation in stark wasserhaltigen Körperbereichen kann so eine besonders gute Verteilung von Ciclosporin und damit eine optimale Wirkstoffaufnahme erreicht werden.

So zeigte sich in einer über sechs Monate andauernden Studie in der Universitätsklinik Tübingen, daß die Verwendung einer erfindungsgemäßen Nanoemulsion sowohl im Bereich des hochempfindlichen Auges als auch im Hautbereich eine gegenüber herkömmlichen Therapieformen wesentlich verbesserte Wirksamkeit und Verträglichkeit aufweist. Dies ist vor allem darauf zurückzuführen, daß aufgrund der neuen Darreichungsform auf die Verwendung physiologisch bedenklicher bzw. unverträglicher Lösungsmittel vollständig verzichtet werden kann, ohne daß dadurch die Wirkung oder Aufnahme von Ciclosporin an den betreffenden Körperteilen beeinträchtigt wird.

Die der Erfindung zugrundeliegende Aufgabe wird somit vollkommen gelöst.

In einer vorteilhaften Ausgestaltung weist die Nanoemulsion Tröpfchengrößen von kleiner als etwa 500 nm auf.

Diese Maßnahme hat den Vorteil, daß der Wirkstoff Ciclosporin besonders gut dispergiert wird und sich damit optimal auf Gewebeoberflächen verteilt und folglich auch besonders gut in das Gewebe aufgenommen wird.

In einer besonders vorteilhaften Ausgestaltung weist das Arzneimittel einen Gehalt an zumindest einem Phospholipid auf.

Unter Phospholipiden versteht man eine Gruppe von Lipiden, die Derivate entweder von Glycerin oder von dem komplexen Alkohol Sphingosin sind. Phospholipide enthalten im Allgemeinen zwei Fettsäuren, die den hydrophoben Bestandteil des Phospholipids bilden, und eine sogenannte

polare Kopfgruppe, die aus einem über eine Phosphodiestergruppe gebundenen Alkohol besteht. Durch diese Struktur sind die Phospholipide amphiphil, d. h. sie enthalten sowohl hydrophobe als auch hydrophile Gruppen. Dadurch sind sie besonders gut als Emulgatoren von hydrophoben Stoffen in wäßrigen Phasen einsetzbar.

Die Verwendung von Phospholipiden in dem erfindungsgemäßen Arzneimittel hat den Vorteil, daß Phospholipide Bestandteile aller Zellmembranen sind und somit eine besonders hohe physiologische Verträglichkeit aufweisen. Dadurch ist das Arzneimittel auch an besonders sensiblen Organen, wie bspw. dem menschlichen Auge, einsetzbar.

In einer weiteren vorteilhaften Ausgestaltung ist das Phospholipid Lecithin.

Lecithin oder Phosphatidylcholin ist eines der am weitesten verbreiteten Membranlipide des Menschen. Von der WHO ist dem Lecithin Unbedenklichkeit als Lebensmittel zuerkannt worden, es wurden keine ADI-Werte (Acceptable Daily Intake) zuerkannt. Lecithin entspricht ferner den Normen der US-amerikanischen Behörde FDA und besitzt den GRAS-Status (Generally Recognized As Safe, CFR Nr. 182.1400/184.1400).

In Fettemulsionen für die parenterale Ernährung wird Lecithin in Kliniken in großem Umfang eingesetzt.

Lecithin ist somit ein besonders gut verträglicher Emulgator, der aufgrund seines Vorkommens in menschlichen Zellen ohnehin Bestandteil des menschlichen Körpers und damit gesundheitlich unbedenklich ist. Als Emulgator von Ciclosporin ist es aufgrund seiner stark amphiphilen Eingenschaften besonders gut geeignet.

In einer weiteren Ausgestaltung der Erfindung liegt der Gehalt an Phospholipid im Bereich von 0,1 bis 20 Gew.-%, vorzugsweise im Bereich von 1 bis 10 Gew.-%.

Hierbei ist vorteilhaft, daß diese Konzentrationen eine besonders feine Emulgierung des Ciclosporins in einer wäßrigen Lösung erlauben.

In einer weiteren Ausgestaltung weist die Nanoemulsion einen Gehalt an Triacylglyceriden, bevorzugt mittelkettigen Triacylglyceriden auf.

Triacylglyceride sind neutrale Lipide, bei denen Fettsäuren über Esterbindungen an einen Glycerinrest gebunden sind. Die Fettsäuren können kurz-, mittel- oder langkettig sein, sie können gesättigt oder ungesättigt vorliegen. Triacylglyceride sind stark hydrophobe Stoffe und dienen z. B. als Energiespeicher im Körper, wo sie in den Fettzellen abgelagert werden.

Die Verwendung von Triacylglyceriden, insbesondere mittelkettigen Triacylglyceriden hat den Vorteil, daß diese problemlos in Arzneimittelqualität erhältlich und zum Inlösungbringen des Ciclosporin in einer Nanoemulsion besonders gut geeignet sind.

Dies gilt vor allem dann, wenn der Gehalt an Triacylglyceriden im Bereich von 10 bis 40 Gew.-%, bevorzugt im Bereich von 20 bis 30 Gew.-% vorliegt.

In einer besonders vorteilhaften Ausgestaltung liegt der Gesamtgehalt an Lipiden im Bereich von 1 bis 50 Gew.-%, bevorzugt im Bereich von 20 bis 30 Gew.-%.

Dabei umfaßt der Gesamtgehalt an Lipiden sowohl rein hydrophoben Lipide wie Triacylglyceride als auch amphiphile Phospholipide wie bspw. Lecithin.

Diese Maßnahme hat den Vorteil, daß bei einem möglichst effizienten Emulgieren des hydrophoben Wirkstoffs Ciclosporin gleichzeitig eine Anwendung an hydrophilen Oberflächen und eine gute Wirkstoffaufnahme möglich ist.

In einer weiteren Ausgestaltung der Erfindung liegt der Gehalt an Ciclosporin im Bereich von 0,1 bis 10 Gew.-%, bevorzugt im Bereich von 1 bis 3 Gew.-%.

Hierbei ist vorteilhaft, daß eine gute therapeutische Wirksamkeit von Ciclosporin erzielt wird.

In einer besonders vorteilhaften Ausgestaltung weist das Arzneimittel 2 Gew.-% Ciclosporin, 5 Gew.-% Lecithin, 23 Gew.-% mittelkettige Triacylglyceride und 70 Gew.-% physiologische Kochsalzlösung sowie ggf. ein physiologisch verträgliches Konservierungsmittel auf.

Es hat sich nämlich in einer klinischem Studie mit dem erfindungsgemäßen Arzneimittel herausgestellt, daß mit dieser Zusammensetzung eine besonders gute Verträglichkeit bei optimaler Wirkstoffaufnahme in den Körper erreicht wird.

Der Zusatz eines Konservierungsmittels ist notwendig, wenn das Arzneimittel für längere Zeitspannen aufbewahrt werden soll. Es versteht sich, daß Konservierungsmittel bei allen Verabreichungsformen des erfindungsgemäßen Arzneimittels enthalten sein können.

In einer weiteren Ausgestaltung liegt das erfindungsgemäße Arzneimittel mit physiologisch verträglichen Trägerstoffen zum Auftragen auf die Haut vermischt vor.

Derartige Trägerstoffe können bspw. Cremes, Gele oder Salben mit den üblichen Bestandteilen sein.

Hierbei ist von Vorteil, daß bei der Verwendung der erfindungsgemäßen Nanoemulsion auf der Haut oder einer Schleimhaut die Verteilung sowie das Zurückhalten auf der Haut verbessert wird.

Darüber hinaus wird die Anwendung des Arzneimittels für den Patienten erleichtert.

In einer weiteren Ausgestaltung weist das Arzneimittel viskositätserhöhende Zusätze auf.

Derartige Zusätze können bspw. Cellulosederivate, Polyacrylate oder andere physiologisch verträgliche Polymere sein.

Hierbei ist von Vorteil, daß der Verbleib des Wirkstoffs Ciclosporin an dem Ort, wo er wirken soll, verlängert wird.

Die Erfindung betrifft auch die Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik zur Behandlung von Hautkrankheiten.

Bei in der Hautklinik der Universitätsklinik Tübingen durchgeführten Versuchen wurde nämlich erstmals die topische Anwendung von Ciclosporin zur Behandlung von Hautkrankeiten untersucht. Dabei stellte sich heraus, daß durch die topische Verabreichung von Ciclosporin gegenüber den üblicherweise verwendeten Therapien, insbesondere der Verabreichung von Cortisonpräparaten, eine überlegene therapeutische Wirkung bei gleichzeitiger guter Verträglichkeit erzielt wird.

Da Ciclosporin bisher nur systemisch angewendet wurde, kam die Behandlung von gewöhnlichen lokal begrenzten Hautkrankheiten aufgrund der schweren Nebenwirkungen einer systemischen Anwendung nicht in Betracht. Bei der systemischen Verabreichung kommt es aufgrund der immunsupprimierenden Wirkung des Ciclosporins nämlich zu einer erhöhten Anfälligkeit gegen Infektionen jeglicher Art. Diese Nebenwirkungen wurden bisher nur bei schweren, sonst nicht behandelbaren Krankheiten in Kauf genommen.

Galeniken, die bei einer erfindungsgemäßen Verwendung in Betracht kommen, umfassen z. B. die Formulierung als Cremes, Gele, Salben oder auch in Form von Liposomen oder Mikroemulsionen.

Besonders bevorzugt ist jedoch die Verwendung von Ciclosporin in Form einer Nanoemulsion, wie sie weiter oben beschrieben wurde.

Hierbei ist vorteilhaft, daß sich der Wirkstoff Ciclosporin bei einer Verabreichung als Nanoemulsion in der oberen Hautschicht, der Hornschicht, anreichert. Dadurch wird ein besonders langer Wirkstoffverbleib in diesen Hautbezirken erreicht, was erwünscht ist, da bei den meisten Hautkrank-

heiten die obersten Hautzellschichten befallen sind.

In einer besonders vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel im Bereich der Mundschleimhaut und/oder der Schleimhäute des Genitalbereichs verwendet.

Im Bereich dieser stark wasserhaltigen Oberflächen ist eine rasche Aufnahme des hydrophoben Wirkstoffs zwingend erforderlich, da er an diesen Körperoberflächen nicht anhaftet und vor allem im Mundbereich durch Speichel schnell weggespült wird. Die erfindungsgemäße Nanoemulsion sorgt dabei für die Anlagerung von Ciclosporin an die Schleimhäute und fördert somit eine schnelle Aufnahme.

In einer weiteren vorteilhaften Ausgestaltung wird das Arzneimittel zur Behandlung von Lichen ruber eingesetzt.

Diese Krankheit ist eine sehr verbreitete entzündliche Erkrankung der Haut und Schleimhaut, die auch als kleinpapulöses Exanthem oder Flechte bezeichnet wird.

Zur Behandlung dieser Hautkrankheit wurden bisher lediglich Schälkuren mit Vitamin A-Säure und anschließende Hydrocortisonbehandlung oder Behandlung mit anderen Cortisonpräparaten eingesetzt. Im Genitalbereich waren zur Behandlung von Lichen ruber bisher sogar operative Eingriffe erforderlich, die durch die topische Anwendung von Ciclosporin nun unterbleiben können.

In einer weiteren Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Behandlung von Neurodermitis eingesetzt.

Bei einem Vergleich der Ciclosporin-Anwendung und der bisher üblichen Hydrocortisonanwendung konnten verbesserte Therapieerfolge mit Ciclosporin bei der Behandlung von Neurodermitis beobachtet werden.

In einer weiteren vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Behandlung von Neurodermitis im Bereich des Auges verwendet.

Hierbei ist vorteilhaft, daß Ciclosporin, insbesondere wenn es in Form einer erfindungsgemäßen Nanoemulsion vorliegt, keine Reizungen im Auge oder in den Bereichen um das Auge herum hervorruft, wobei es gleichzeitig gut aufgenommen wird, und daß es hoch effizient gegen Neurodermitiden im Augenbereich wirkt, wie in an der Augenklinik der Universitätsklinik Tübingen durchgeführten Versuchen mit Patienten nachgewiesen werden konnte.

Die Erfindung betrifft auch die Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik zur Behandlung von Allergien.

In einer an der Augenklinik der Universitätsklinik Tübingen durchgeführten Studie zeigte sich nämlich, daß Ciclosporin bei topischer Anwendung therapeutisch hochwirksam gegen Allergien eingesetzt werden kann. Ciclosporin kann dabei in allen zur topischen Verabreichung geeigneten Galeniken eingesetzt werden. Besonders bevorzugt ist dabei eine Darreichung als Nanoemulsion, wie sie oben näher beschrieben wurde.

Insbesondere bei einer Verabreichung von Ciclosporin als Nanoemulsion zur Bekämpfung von Allergien im Augenbereich konnten hervorragende Therapieerfolge erzielt werden.

In einer weiteren vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur prophylaktischen und/oder therapeutischen Behandlung des Auges verwendet.

Eine therapeutische Behandlung des menschlichen Auges mit Ciclosporin ist z. B. bei Hornhaut-Transplantationen zur Verhinderung von Abstoßungsreaktionen erforderlich.

Bei der Verwendung von Ciclosporin als Nanoemulsion verteilt sich der hydrophobe Wirkstoff besonders gut über den gesamten Augapfel, so daß aufgrund der großen Resorptionsfläche eine optimale Wirkstoffaufnahme gegeben ist. Die Nanoemulsion verteilt sich außerdem im Kammerwasser selbst, das auch die Linse und die Hornhaut umspült. Da das Kammerwasser nur ca. alle vier Stunden ausgetauscht wird, kann Ciclosporin besonders dauerhaft auf die von dem Kammerwasser benetzten Augenbereiche einwirken. So kann das Risiko von Gewebeabstoßungen im Bereich des Auges sicher vermieden werden.

In klinischen Versuchen, bei denen die Verwendung des erfindungsgemäßen Arzneimittels am Auge getestet wurde, kam es in keinem einzigen Fall zu Sehbeeinträchtigungen oder einer Verstopfung der Schlemm-Kanäle, die dem Abfließen der Tränenflüssigkeit in die Nase dienen. Darüber hinaus wurden keine Schmerzfälle beobachtet.

In einer besonders vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Verhinderung von Abstoßungsreaktionen nach Transplantationen, vorzugsweise im Bereich des Auges, verwendet.

Hierbei ist bspw. an die bereits erwähnten Hornhaut-Transplantationen oder Transplantationen anderer Bestandteile des Auges, jedoch auch an Haut-Transplantationen zu denken.

Da der Wirkstoff nun direkt am Zielort aufgetragen werden kann und dort auch gut aufgenommen wird, kann das Ciclosporin mit im Vergleich zur systemischen Anwendung geringen Nebenwirkungen effizient therapeutisch wirken.

In einem Verfahren zur Zubereitung des erfindungsgemäßen Arzneimittels werden die folgenden grundsätzlichen Schritte durchgeführt:

a) Lösen von Ciclosporin in einer öligen Phase;
b) Hinzufügen eines Anteils einer wäßrigen Phase;
c) Rühren;
d) Hinzufügen des verbleibenden Anteils der wäßrigen Phase;
e) Behandeln des Gemischs mit Ultraschall; und
f) Sterilfiltrieren.

Hierbei ist von Vorteil, daß eine erfindungsgemäße Nanoemulsion mit Ciclosporin in einem zügigen Verfahren ohne technischen Aufwand hergestellt werden kann.

Die ölige Phase kann dabei z. B. Triacylglyceride und Lecithin, die wäßrige Phase physiologische Kochsalzlösung oder Wasser enthalten. Das Lösen des Ciclosporins in Schritt a) sowie das Rühren in Schritt c) kann z. B. durch auf einem Magnetrührer oder mit einem Flügelrührer durchgeführt werden. Es versteht sich, daß das erfindungsgemäße Verfahren unter sterilen Bedingungen durchgeführt werden muß, wobei zwischen den einzelnen aufgeführten Schritten jeweils Sterilfiltrationsschritte zwischengeschaltet werden können.

Die Ultraschallbehandlung dient der Dispersion der öligen Phase in der wäßrigen Phase, wobei die Tröpfchengrößen in der entstehenden Suspension durch die Dauer der Ultraschallbehandlung und die Leistung bestimmt wird.

Es versteht sich, daß die vorstehend genannten und die nachstehend noch zu erläuternden Merkmale nicht nur in den angegebenen Kombinationen, sondern auch in anderen Kombinationen oder in Alleinstellung einsetzbar sind, ohne den Rahmen der vorliegenden Erfindung zu verlassen.

Weitere Merkmale und Vorteile der Erfindung ergeben sich aus den nachfolgenden Ausführungsbeispielen.

Beispiel 1

Herstellung einer Ciclosporin-Nanoemulsion

Es wird eine Ciclosporin-Nanoemulsion hergestellt, die aus 2 Gew.-% Ciclosporin, 23 Gew.-% Oleum neutrale

DAB, 5 Gew.-% Lecithin und 70 Gew.-% 0,9%iger Natriumchloridlösung besteht. Die fertige Emulsion ist u. a. zur Verwendung als Augentropfen geeignet.

Inhaltsstoffe und ihre Bezugsquellen:

Ciclosporin: Firma Synochem, Hamburg;

Oleum neutrale DAB (MIGLYKOL): Firma Henkel, Düsseldorf;

Lecithin (80 Gew.-% Phosphatidylcholin): Firma Lipoid, Ludwigshafen;

Natriumchlorid, 0,9 Gew.-%: Firma Braun, Melsungen.

Alle verwendeten Hilfsmittel, bspw. Bechergläser, Rührer, Filter, usw. werden bei 121°C für 15 Minuten lang durch Autoklavieren sterilisiert.

Zunächst wird der lipophile Wirkstoff Ciclosporin zusammen mit dem Lecithin in Oleum neutrale gelöst.

Dazu werden in ein steriles Becherglas 5 g Lecithin, 2 g Ciclosporin und 23 ml Oleum neutrale eingefüllt und mit einem Magnetrührer in Lösung gebracht.

Die Lösung wird in ein zweites Becherglas sterilfiltriert. Dann werden 40 ml 0,9%ige Natriumchloridlösung zugefügt und für 1 Stunde bei 400 upm (Umdrehungen pro Minute) mit einem Flügelrührer gerührt.

Dadurch wird eine Voremulsion erzeugt, die in eine sterile Durchflußzelle gegeben und mit der restlichen Menge an 0,9%iger Natriumchloridlösung aufgefüllt wird.

Diese Lösung wird für 15 Minuten mit 70 Watt Leistung in einem Ultraschallgenerator (Firma Branson, Schwäbisch Gmünd) beschallt.

Dabei wird eine Nanoemulsion mit Tröpfchengrößen von kleiner als 500 nm erzeugt, die über einen 0,45 µm-Sterilfilter direkt in Augentropfflaschen abgefüllt wird.

Alle Arbeitsschritte werden unter einer sterilen Werkbank (Laminar Airflow Bank, Firma Ehret, Emmendingen) durchgeführt. Die Augentropfen sind bei einer Lagertemperatur von 4°C für drei Monate lang steril.

Beispiel 2

Studie mit Patienten an der Universitätsklinik Tübingen

1. Augenklinik

In der Augenklinik der Universitätsklinik Tübingen wurden die in Beispiel 1 hergestellten Augentropfen für einen Zeitraum von sechs Monaten mit insgesamt über 200 Präparationen bei Patienten mit Hornhaut-Transplantationen eingesetzt. Die Studie dauert noch an.

Über den gesamten Zeitraum der Behandlung von Patienten mit dem erfindungsgemäßen Arzneimittel in Form von Augentropfen wurde kein einziger Fall von Schmerzentwicklung bei der Verabreichung der Tropfen beobachtet.

Obwohl die Nanoemulsion ein milchiges Aussehen aufweist, kam es bei der Applikation am Auge in keinem Fall zu Sehbeeinträchtigungen.

Die erfindungsgemäße Ciclosporin-Nanoemulsion wurde außerdem zur Behandlung von Neurodermitiden im Bereich des menschlichen Auges sowie zur Behandlung von Allergien im Augenbereich eingesetzt. Bei beiden Krankheitsbildern konnten überragende Therapieerfolge erreicht werden, ohne daß es zu einer Entwicklung von Schmerzen oder Sehbeeinträchtigungen bei den Patienten gekommen wäre.

2. Hautklinik

Die Ciclosporin-Nanoemulsion wurde darüber hinaus vier Monate lang in der Hautklinik der Universitätsklinik Tübingen zur Behandlung von Lichen ruber im Gesichtsbereich und im Genitalbereich eingesetzt.

Durch den Einsatz von Ciclosporin bei der Behandlung im Gesichtsbereich konnten die bisher üblichen Schälkuren mit Vitamin A-Säure und anschließender Hydrocortisonbehandlung vermieden werden.

Bei der Verwendung im Genitalbereich war es darüber hinaus möglich, auf die bisher üblichen operativen Eingriffe zu verzichten.

Die erfindungsgemäße Ciclosporin-Nanoemulsion wurde außerdem zur Behandlung von Neurodermitis eingesetzt. Hier konnte ein verbesserter Therapieerfolg im Vergleich zu einer Hydrocortisonbehandlung erreicht werden.

In der Hautklinik wurde das erfindungsgemäße Arzneimittel sowohl stationär als auch ambulant eingesetzt. Auch die Studien in der Hautklinik dauern noch an. Darüber hinaus werden derzeit Versuche zur Verabreichung von Ciclosporin zur Behandlung von Hautkrankheiten in Form von Liposomen durchgeführt.

Patentansprüche

1. Arzneimittel mit einem Gehalt an Ciclosporin, **dadurch gekennzeichnet**, daß das Ciclosprorin in einer Öl-in-Wasser-Nanoemulsion vorliegt.
2. Arzneimittel nach Anspruch 1, dadurch gekennzeichnet, daß die Nanoemulsion Tröpfchengrößen von kleiner als etwa 500 nm aufweist.
3. Arzneimittel nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß es einen Gehalt an zumindest einem Phospholipid aufweist.
4. Arzneimittel nach Anspruch 3, dadurch gekennzeichnet, daß das Phospholipid Lecithin ist.
5. Arzneimittel nach Anspruch 3 oder 4, dadurch gekennzeichnet, daß der Gehalt an Phospholipid im Bereich von 0,1 bis 20 Gew.-%, vorzugsweise im Bereich von 1 bis 10 Gew.-% liegt.
6. Arzneimittel nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß die Nanoemulsion einen Gehalt an Triacylglyceriden, vorzugsweise mittelkettigen Triacylglyceriden aufweist.
7. Arzneimittel nach Anspruch 6, dadurch gekennzeichnet, daß der Gehalt an Triacylglyceriden im Bereich von 10 bis 40 Gew.-%, insbesondere im Bereich von 20 bis 30 Gew.-% liegt.
8. Arzneimittel nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß der Gesamtgehalt an Lipiden im Bereich von 1 bis 50 Gew.-%, vorzugsweise im Bereich von 20 bis 30 Gew.-% liegt.
9. Arzneimittel nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß der Gehalt an Ciclosporin im Bereich von 0,1 bis 10 Gew.-%, vorzugsweise im Bereich von 1 bis 3 Gew.-% liegt.
10. Arzneimittel nach einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, daß es 2 Gew.-% Ciclosporin, 5 Gew.-% Lecithin, 23 Gew.-% mittelkettige Triacylglyceride und 70 Gew.-% physiologische Kochsalzlösung sowie ggf. ein physiologisch verträgliches Konservierungsmittel aufweist.
11. Arzneimittel nach einem der Ansprüche 1 bis 10, dadurch gekennzeichnet, daß es mit physiologisch verträglichen Trägerstoffen zum Auftragen auf die Haut vermischt vorliegt.
12. Arzneimittel nach einem der Ansprüche 1 bis 11, dadurch gekennzeichnet, daß es einen Gehalt an zumindest einem viskositätserhöhenden Zusatz aufweist.
13. Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik, vorzugsweise nach einem der Ansprüche 1 bis 12, zur Behandlung von Hautkrankhei-

ten.

14. Verwendung nach Anspruch 13 zur Behandlung der Mundschleimhaut und/oder der Schleimhäute des Genitalbereichs.

15. Verwendung nach einem der Ansprüche 13 oder 14 zur Behandlung von Lichen ruber.

16. Verwendung nach einem der Ansprüche 13 oder 14 zur Behandlung von Neurodermitits.

17. Verwendung nach Anspruch 16 zur Behandlung von Neurodermitis im Bereich des Auges.

18. Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik, vorzugsweise nach einem der Ansprüche 1 bis 12, zur Behandlung von Allergien.

19. Verwendung nach Anspruch 18 zur Behandlung von Allergien im Bereich des Auges.

20. Verwendung eines Arzneimittels nach einem der Ansprüche 1 bis 12 zur prophylaktischen und/oder therapeutischen Behandlung des Auges.

21. Verwendung eines Arzneimittels nach einem der Ansprüche 1 bis 12 zur Verhinderung von Abstoßungsreaktionen nach Transplantationen, vorzugsweise im Bereich des Auges.

22. Verfahren zur Zubereitung eines Arzneimittels nach einem der Ansprüche 1 bis 12, gekennzeichnet durch die grundsätzlichen Schritte:

a) Lösen von Ciclosporin in einer öligen Phase;
b) Hinzufügen eines Anteils einer wäßrigen Phase;
c) Rühren;
d) Hinzufügen des verbleibenden Anteils der wäßrigen Phase;
e) Behandeln des Gemischs mit Ultraschall; und
f) Sterilfiltrieren.

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(54) Solubilization reagent for biological test samples.

(57) A solubilization reagent for use in analytical systems for the determination of hydrophobic analytes in a biological test sample, particularly analytical systems employing specific binding proteins for such analytes, such as in fluorescent polarization immunoassays, is disclosed. The solubilization reagent dissociates analytes from various components of a biological test sample, such as cellular material, phospholipids, proteins and the like, at substantially low concentrations of such solubilization reagent while, at the same, minimizing the denaturation of specific binding proteins, such as, for example, antibodies, which may be present in an analytical system. Preferably, such surfactant is alkyl-oxy-(polyethylene-oxy-propylene-oxy-sopropanol) or N-tetradecyl-n,n-demethyl-3-ammonio-1-propane sulfonate, and may further comprise saponin.

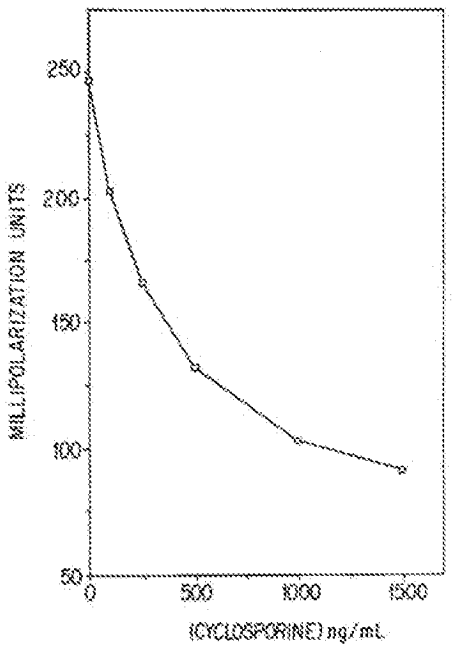

FIG. 1

EP 0 471 293 A2

Field of the Invention

The present invention relates to reagents which are useful for extracting analytes from a liquid test sample. In particular, the present invention relates to reagents which facilitate the dissociation of analytes, particularly hydrophobic analytes, from the components of a biological test sample to permit the measurement of such analytes present therein.

Background of the Invention

The monitoring of therapeutic drug levels and other analytes in biological fluids such as serum, plasma, whole blood, urine and the like has become very useful to provide physicians with information to aid in proper patient management. For example, adjustment of patient dosage, achievement of optimal therapeutic effects, and avoiding useless subtherapeutic or harmful toxic dosage levels can be provided. Conventional techniques which are employed to monitor drug levels or detect other analytes are known and include radioimmunoassays and nonisotopic assays such as fluorescence polarization immunoassays. However, such techniques produce inconsistencies in results when determining the amount or presence of hydrophobic analytes because of their intracellular relationship with various cellular components of a biological test sample. Accordingly, when such analytes remain associated with such cellular components, the detection of such analytes in an analytical system is difficult, and in some instances impossible, particularly when such analytes are present at particularly low levels.

Although various reagents have been described to extract various analytes for analysis, such as Triton X-100®, Tweens®, sodium dodecyl sulfate and saponin, the use of such reagents suffer from a number of disadvantages, particularly where such analysis involves reagents such as specific binding proteins, antibodies, and the like. For example, such reagents, in many cases, do not achieve complete cell lysis wherein in the case of hydrophobic analytes, a significant amount of such analytes could remain associated with cellular components and thereby not made available for analysis. Similarly, the presence of such reagents in, for example, an immunoassay system, will result in significant denaturation of specific binding proteins or antibodies employed in such immunoassays to thereby reduce the binding activity of such proteins and antibodies. Moreover, the use of such reagents to dissociate analytes from various cellular components and other materials which may be present in a liquid test sample can have a dramatic effect on the integrity of reagents employed in various analytical systems, particularly where such reagent are employed at high concentrations in order to achieve such dissociation.

Summary of the Invention

The present invention relates to the discovery that analytical systems for the determination of hydrophobic analytes in a biological test sample, particularly analytical systems employing specific binding proteins for such analytes, can be substantially improved by employing the solubilization reagent of the present invention which serves to dissociate analytes from various components of a biological test sample, such as cellular material, phospholipids, proteins and the like. In particular, such solubilization reagent has unexpectedly and surprisingly been found to dissociate hydrophobic analytes from such components, particularly cellular components, at substantially low concentrations of such solubilization reagent while, at the same, minimize the denaturation of specific binding proteins, such as, for example, antibodies, which may be present in an analytical system. The solubilization reagent of the present invention is particularly useful in a fluorescent polarization immunoassay for the determination of hydrophobic analytes such as cyclosporine and the like.

The solubilization reagent of the present invention comprises from between about 1.5% (w/v) and about 10% (w/v), preferably about 2% (w/v), of a surfactant having either nonionic characteristics or zwitterionic characteristics wherein such surfactant is capable of dissociating substantially all of a hydrophobic analyte from the components of a biological test sample. Preferably, such surfactant is either a nonionic polyglycol detergent, such as alkyl-oxy(polyethylene-oxy-propylene-oxy-isopropanol), or a zwitterionic detergent, such as N-tetradecyl-N,N-demethyl-3-ammonio-1-propanesulfonate. The solubilization reagent may further comprise from between about 0% (w/v) and about 25% (w/v) of saponin.

Brief Description of the Drawings

Fig. 1 illustrates a calibration curve employed to determine the amount cyclosporine from a whole blood sample in a fluorescent polarization immunoassay employing the solubilization reagent of the present invention.

Detailed Description of the Invention

The solubilization reagent of the present invention dissociates analytes, particularly hydrophobic analytes, from a biological test sample such as whole blood, serum, plasma, urine, spinal fluid, and the like. As contemplated by the present invention,

hydrophobic analytes include, but are not intended to be limited to, steroids, drugs such as cyclosporine, and the like.

In particular, the solubilization reagent dissociates such analytes from cellular material, such as erythrocytes, populations of leucocytes, such as lymphocytes, phospholipids, proteins, and the like, which may be present in a biological test sample, to thereby render such analytes readily available for measurement by a desired analytical system. Although the solubilization reagent is particularly useful in analytical systems for determining hydrophobic analytes employing specific binding proteins, especially immunoassay systems, the solubilization reagent can be be employed in other assay systems as well, such as radioactive assays and the like.

Where it is desireable to employ a non-ionic surfactant in the solubilization reagent according to the present invention, such non-ionic surfactant is preferably a nonionic polyglycol detergent such as alkyloxy(polyethyleneoxypropyleneoxy)-isopropanol and the like, also commonly known as Tergitol®. Where it desireable to employ a zwitterionic surfactant in the solubilization reagent according to the present invention, such zwitterionic surfactant is selected from the group consisting of N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-hexadecyl-N,N-dimethyl 3-ammonio-1-propanesulfonate, and the like.

In addition to either a non-ionic surfactant or a zwitterionic surfactant as described above, the solubilization reagent according to the present invention may further comprise a glycoside, such as saponin.

According to the present invention, the solubilization reagent is capable of lysing substantially all of the various cellular components which may be present in a biological test sample, and dissociate substantially all of the desired analyte from other biological test sample components in order to render such analyte available for analysis. In particular, the solubilization reagent according to the present invention is capable of providing substantially complete cell lysis of, for example, cellular populations such as erythrocytes, leukocytes and the like, for recovery of substantially all of the desired analyte contained therein. In addition, the solubilization reagent is also capable of dissociating the desired analyte from other components which may be present in a biological test sample, such as cellular material, phospholipids, proteins, and the like, to which such analyte could nevertheless remain associated with and thereby not made available for analysis.

The solubilization reagent according to the present invention has unexpectedly and surprisingly been found to achieve such cell lysis and dissociation of analyte at substantially low concentration. In particular, the use of the solubilization reagent at a concentration as low as 1.5% (w/v) of the non-ionic or zwitterionic surfactant, with saponin, has been found to be effective for such cell lysis and dissociation of the analyte. Preferably, the concentration of the surfactant is from between about 1.5% (w/v) and about 10% (w/v), more preferably about 2% (w/v), and, the solubilization reagent may further comprise from between about 0% and about 25% saponin, preferably 2%.

It is to be understood that the use of reagents to treat a biological test sample prior to the use thereof in an analytical system, such as described herein, will be present in the biological test sample during subsequent analysis thereof. Accordingly, the solubilization reagent of the present invention is particularly useful where the biological test sample is to be employed in an analytical system employing specific binding proteins or antibodies which are sensitive to the presence of, for example, detergents or other pretreatment reagents which are typically employed for the purposes described herein. For example, the use of the solubilization reagent according to the present invention prior to the analysis thereof in an immunoassay system minimizes denaturation of antibody reagents employed therein, thereby having substantially no effect on the binding activity of such antibody reagents.

When employing the solubilization reagent of the present invention for performing an immunoassay, the test sample is first treated with the solubilization reagent wherein cellular populations present in the test sample are lysed and the hydrophobic analyte dissociated from other components as described above. The resulting solution is then treated with a precipitation reagent, such as described in the copending U.S. Patent Application Serial No. 567,853 , entitled "Protein Precipitation Reagent", filed on August 15, 1990 and incorporated by reference herein. Such precipitation reagent precipitates any interfering proteins, including the cellular material resulting from treatment of the test sample with the solubilization reagent of the present invention. Although the precipitated material resulting from such pretreatment step with the precipitation reagent may settle by gravity, extraction of the resulting dissociated analyte is preferably accomplished by centrifuging the treated test sample wherein the resulting supernatant contains the desired analyte, substantially free of such cellular material and components. The supernatant is then combined with a detectable tracer com-

pound as would be known by one skilled in the art, and an appropriate antibody to, or binding agent for, the analyte prepared according to methods known in the art. According to such general immunoassay procedure, the analyte present in the test sample and the tracer compound compete for a limited number of binding sites, resulting in the formation of analyte and tracer compound complexes. By maintaining a constant concentration of the tracer compound and the antibody, the ratio of the formation of analyte complex to tracer complex is directly proportional to the amount of analyte present in the test sample.

The solubilization reagent of the present invention is particularly useful in fluorescence polarization immunoassay systems wherein the amount of analyte in a test sample is determined by exciting an assay mixture with polarized light and measuring the polarization of the fluorescence emitted by any of the free or unbound tracer compound and tracer-antibody complex. Any of the tracer compound which is not complexed to an antibody is free to rotate in less than the time required for adsorption and re-emission of fluorescent light. As a result, the re-emitted light is relatively randomly oriented so that the fluorescence polarization of any of the tracer compound not complexed to the antibody is low, approaching zero. Upon complexing with a specific antibody, the tracer-antibody complex thus formed assumes the rotation of the antibody molecule, which is slower than that of the relatively small tracer compound molecule, thereby increasing the polarization observed. When making such determination, the analyte competes with the tracer compound for antibody sites wherein the observed polarization of fluorescence of the tracer-antibody complex becomes a value between the value of the free tracer compound and the value tracer-antibody complex. Accordingly, if the test sample contains a high concentration of analyte, the observed polarization value is closer to that of the free tracer compound, i.e., low. Conversely, if the test sample contains a low concentration of analyte, the polarization value is closer to that of the tracer-antibody complex, i.e., high. By sequentially exciting the reaction mixture of an immunoassay with vertically and then horizontally polarized light, and analyzing only the vertical component of the emitted light, the polarization of the fluorescence in the reaction mixture can be accurately determined. The precise relationship between polarization and concentration of the analyte is established by measuring the polarization values of calibrators having known concentrations, and the concentration of the analyte can be interpolated from a standard curve prepared therefrom.

When employing fluorescence polarization techniques, the results can be quantified in terms of "millipolarization units", "span" (in millipolarization units) and "relative intensity". The measurement of millipolarization units indicates the maximum polarization when a maximum amount of the tracer compound is bound to the antibody in the absence of any phenylclorobenzene (PCB) in the test sample. The higher the net millipolarization units, the better the binding of the tracer compound to the antibody. For the purposes of the present invention, a net millipolarization value of at least about 130 is preferred.

The "span" is an indication of the difference between the net millipolarization and the minimum amount of the tracer compound bound to the antibody. A larger span provides for a better numerical analysis of the data. For the purposes of the present invention, a span of at least about 15 millipolarization units is preferred.

The "relative intensity " is a measure of the strength of the fluorescence signal above the backgound fluorescence. Thus, a higher intensity will give a more accurate measurement. The intensity is determined as the sum of the vertically polarized intensity plus twice the horizontally polarized intensity. The intensity can range from a signal of about three times to about thirty times the backgound noise, depending upon the concentration of the tracer compound and other assay variables. For the purpose of the present invention, an intensity of about three to about twenty times that of background noise is preferred.

The solubilization reagent according to the present invention is particularly useful for performing a fluorescent polarization immunoassay for cyclosporine and metabolites thereof employing a fluorescent tracer compound comprising 4-aminomethylfluorescein coupled to the hydroxyl group of MeBmt at the first position of cyclosporine, as described by the copending U.S. Patent Application Serial No. 567,842 , entitled "Immunoassay Reagents And Method For Determining Cyclosporine", filed on even date herewith and incorporated by reference herein, and a monoclonal antibody to cyclosporine, such as described by International Patent Application Publication No. WO 86/02080. According to such method, a precipitation reagent comprising zinc sulfate, ethylene glycol and methanol, such as described in the copending U.S. Patent Application Serial No. 567,853 , entitled "Protein Precipitation Reagent", filed on August 15, 1990 and incorporated by reference herein, a dilution buffer, and calibrators and controls are also employed. Such precipitation reagent is employed to precipitate interfering proteins, hemoglobin, and other interfering substances while, at the same time, maintaining hydrophobic analytes in solution in order to render such analytes available for binding to, for example, a spe-

cific binding protein such as an antibody.

Once the the test sample has been treated with the solubilization reagent of the present invention and the precipitation reagent as described above, the supernatant containing cyclosporine, or cyclosporine and metabolites of cyclosporine, is then combined with the antibody. Prior to addition of the tracer compound and dilution buffer, a background fluorescence reading is taken, wherein after an incubation period of from between about ten minutes and about thirty minutes, a fluorescence polarization reading is taken as described above.

The present invention will now be illustrated, but is not intended to be limited, by the following example:

Fluorescent Polarization Immunoassay For Cyclosporine

Reagents

The reagents for performing a fluorescence polarization immunoassay employing the solubilization reagent according to the present invention were prepared as follows:

a) Cyclosporine Tracer Reagent:

(i) Preparation of [O-(Chloroformyl)MeBmt]^1 cyclosporine (Cyclosporine chloroformate):

Cyclosporine (24.2 mg, 0.020 mmoles) was dissolved in a 25%w/w solution of phosgene in benzene (2.0 mL) in a 10mL round bottom flask fitted with stopper and stirbar. The reaction was stirred for 5 minutes to dissolve the cyclosporine, then was allowed to stand undisturbed at room temperature for 24 hours. The reaction was concentrated in vacuo, and the product could be stored as a solid at 0° C for up to six months. For subsequent reactions, a 0.02M solution in DMF was used.

(iii) Preparation of [O-(Fluorescein-4'-yl-methylaminoformyl)MeBmt]^1 cyclosporine:

Cyclosporine chloroformate (0.2mL, 4 moles), as a 0.02M solution in DMF as described in step (i) above was combined with 4'-aminomethylfluorescein hydrochloride (2.0 mg, 5 moles) in a stoppered vial fitted with a stirbar. Pyridine was added until the apparent pH (by moist pH paper) was approximately 7. The reaction was stirred at room temperature for 24 hours. The solvent was removed in vacuo, and the residue was taken up in methanol and loaded onto a 1mm silica gel plate. The plate was developed with 15% methanol/methylene chloride. The product band, Rf 0.55, was eluted from the silica gel with methanol.

(iii) Preparation of Tracer Reagent:

A 60 nanomolar cyclosporine tracer reagent was prepared comprising the cyclosporine tracer compound prepared according to step (ii) above in 0.1 M sodium phosphate buffer, pH 7.5, containing 0.01 % (w/v) bovine gamma globulin, 0.1 % (w/v) sodium azide, 5.0% (w/v) ethylene glycol and 0.05% (w/v) Tween™ 20.

(b) Monoclonal Antibody Formulation:

A monoclonal antibody reagent was prepared comprising mouse (ascites) monoclonal antibody to cyclosporine (Sandoz AG, Basle, Switzerland) diluted with a citrate buffer including sodium azide.

(c) Pretreatment Reagent:

A pretreatment reagent was prepared comprising 0.1 M Tris™ buffer, pH 7.5, 0.1% (w/v) sodium azide, 0.5% (w/v) copper sulfate and 10.0% (w/v) 5-sulfosalicylate.

(d) Dilution Buffer:

A dilution buffer was prepared comprising 0.1 M sodium phosphate, pH 7.5, and 0.1 % (w/v) bovine gamma globulin.

(e) Whole Blood Precipitation Reagent:

A whole blood precipitation reagent was prepared comprising 60 mM zinc sulfate, 50% (w/v) methanol and 33% (w/v) ethylene glycol.

(f) Solubilization Reagent:

A solubilization reagent was prepared comprising 2.0% (w/v) Tergitol min foam 1X ™, 2.0% (w/v) saponin and 0. 1% (w/v) sodium azide.

(g) Calibrators:

Cyclosporine monoclonal whole blood calibrators were prepared comprising cyclosporine and an artificial human whole blood matrix. The calibrators were prepared at concentrations of 0.0, 100, 250, 500, 1000, and 1500 nanograms per milliliter, with sodium azide as a preservative.

(h) Controls:

Cyclosporine monoclonal whole blood controls were prepared comprising cyclosporine and an artificial whole blood matrix. The controls were prepared at concentrations of 150, 400 and 800 nanograms per milliliter with sodium azide as a preservative.

Cyclosporine Whole Blood FPIA Assay Protocol

A fluorescent polarization immunoassay for determining cyclosporine in a whole blood test sample employing an Abbott TDx® Therapeutic Drug Monitoring Analyzer was performed as follows:

One hundred-fifty microliters each of patient whole blood samples containing cyclosporine, controls and calibrators were pipetted into labeled centrifuge tubes, and 50 microliters of the solubilization

reagent were added to each of the tubes. A pipette was filled with the whole blood precipitation reagent, purged of air bubbles, and 300 microliters were dispensed into each centrifuge tube by touching the end of the pipette tip to the wall of each centrifuge tube while dispensing the reagent. The centrifuge tubes were then capped and mixed on a vortex mixer for ten seconds and placed into a centrifuge head so that the tubes were evenly distributed so that the centrifuge head was balanced. The tubes were centrifuged for approximately five minutes at 9,500 x g until a clear supernatant and a hard, compact pellet of denatured protein was obtained. After centrifugation was complete, each tube was uncapped and the supernatant was decanted into the corresponding sample well of a TDx Sample Cartridge.

The fluorescence polarization value of each calibrator, control and sample was determined and printed on the output tape of the Abbott TDx Analyzer. A standard curve was generated in the instrument by plotting the polarization, P, of each calibrator versus its concentration using a nonlinear regression analysis wherein, the concentration of each control and sample was read off the stored calibration curve (Figure 1) and printed on the output tape.

The sensitivity of the preferred fluorescence polarization assay according to the present invention is 15.0 nanograms/milliliter of cyclosporine and metabolites. When compared to an available radioimmunoassay using 60 clinical samples, a linear least squared regression analysis gave a slope of 0.947, an intercept of 7.15, and a correlation coefficient of 0.969.

Where a test kit according to the present invention is being used in conjunction with the TDx Analyzer, the reagents for performing the fluorescent polarization immunoassay according to the present invention can be contained in separate vials of a TDx Reagent Pack wherein vial caps from each of the vials in the Reagent Pack are removed and placed into designated wells inside the Reagent Pack. Accordingly, once the Reagent Pack is placed inside the TDx Analyzer, the assay procedure heretofore is fully automated.

If a manual assay is being performed, the test sample is first treated with the precipitation reagent as described above, and then mixed with the dilution buffer. The antibody reagent and the pretreatment solution are then placed into the test tube containing the sample, and a backgound fluorescence reading is taken. The tracer compound and dilution buffer are added to the sample, and after incubation, a fluorescence polarization reading is taken.

It will be apparent that many modifications and variations of the present invention as herein set forth are possible without departing from the spirit and scope hereof, and that, accordingly, such limitations are imposed only as indicated by the appended claims.

Claims

1. A solubilization reagent useful for dissociating hydrophobic analytes from components of a biological test sample, said reagent comprising a non-ionic or a zwitterionic surfactant.

2. The reagent of claim 1 wherein said non-ionic surfactant is a non-ionic polyglycol surfactant.

3. The reagent of claim 1 wherein said zwitterionic surfactant is selected from the group consisting of N-tetradecyl-N,N-demethyl-3-ammonio-1-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate and N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

4. The reagent of claims 1-3 further comprising a glycoside.

5. The reagent of claims 1-4 comprising from between about 1.5% (w/v) and about 10% (w/v) of said surfactant in aqueous solution.

6. The reagent of claim 4 comprising less than about 25% (w/v) of said glycoside.

7. An immunoassay method for determining hydrophobic analytes in a biological test sample characterized in that said assay comprises a solubilization reagent according to claims 1-3.

8. The immunoassay method of claim 7 wherein said reagent further comprises a glycoside.

9. The immunoassay method of claim 7 wherein said reagent comprises from between about 1.5% (w/v) and about 10% (w/v) of said surfactant in aqueous solution.

10. The immunoassay method of claims 7-9 wherein said reagent comprises less than about 25% (w/v) of said glycoside.

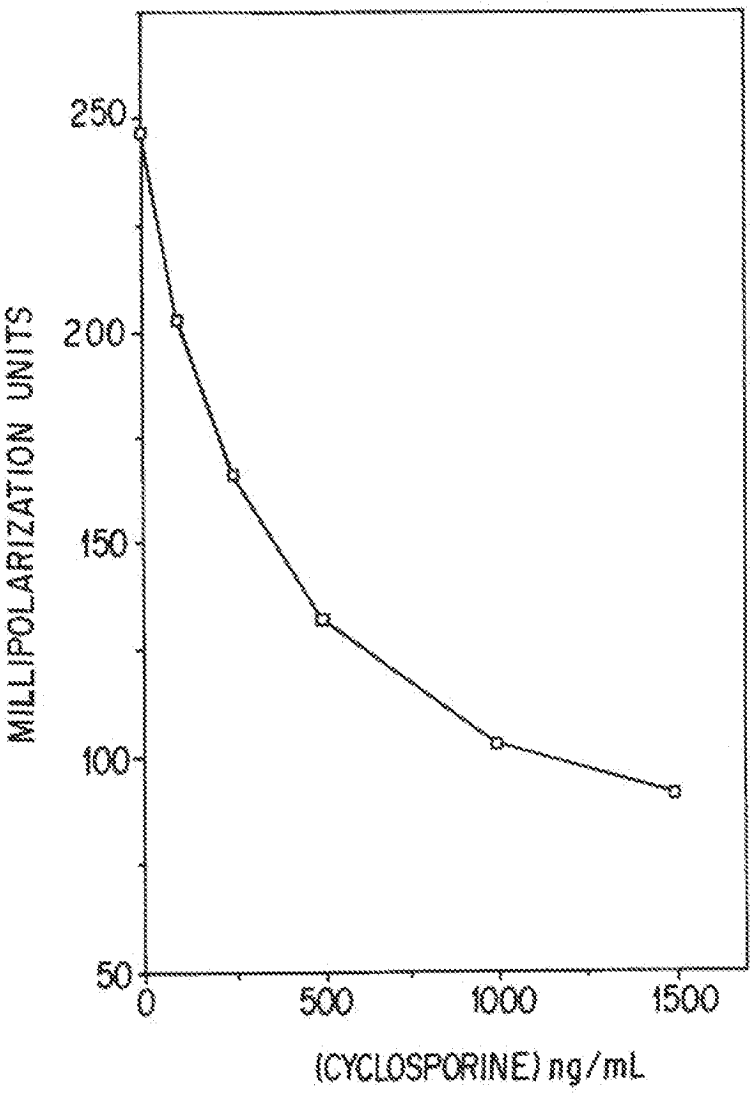

FIG. 1

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(54) EXTERNAL PREPARATION CONTAINING CYCLOSPORIN.

(57) An external preparation containing cyclosporin as the active ingredient, characterized by comprising (a) cyclosporin, (b) an organic solvent for dissolving the same, (c) a fatty acid ester of a monohydric alcohol, which is liquid at 25 °C and bears at least 8 carbon atoms in total, and/or an alkanolamine which is liquid at 25 °C, (d) an oleaginous substance which is solid at 25 °C, and (e) a surfactant, wherein the cyclosporin content ranges from 0.1 to 10 wt.% and the content of the ester and/or alkanolamine ranges from 1 to 15 wt.%. The preparation has an excellent efficacy of curing atopic dermatitis, psoriasis, allergic contact dermatitis, and so forth.

EP 0 547 229 A1

TECHNICAL FIELD

The present invention relates to topical preparations containing cyclosporin as a major active component. The topical preparations containing cyclosporin include topical preparations in the form of an emulsion or a non-emulsion.

The term "cyclosporin" referred to in this application is intended to mean a single substance or a mixture of a group of cyclosporin antibiotics which are described in detail in Japanese Patent Laid-open Publication (kokai) No. 2-17,127.

BACKGROUND ART

Cyclosporin is known as an immune inhibitor and it has extensively been employed in the field of the transplant of organs including the kidney. Recently, cyclosporin becomes apparent as being effective to various diseases that are caused mainly from autoimmune reaction, in addition to the efficacy for the transplant of the organs. A number of literature has already been published which reports the efficacy of cyclosporin forarthritis. Autoimmune diseases to which cyclosporin has been applied or proposed to be applied may include, for example, autoimmune blood diseases, chronic bronchial asthma, systemic erythematosus, polymyositis, systemic scleroderma, Wegner syndrome, myasthenia gravis, psoriasis vulgaris, autoimmune intestinal diseases (idiopathic ulcerative colitis, Crohn disease), sarcoidosis, multiple sclerosis, juvenile diabetes mellitus, uveitis, psoriatic rheumatoid, glomerulonephritis, and the like.

As described hereinabove, cyclosporin contributes largely to the inhibition of rejection at the time of transplanting organs and autoimmune therapy; however, it is also known that it may often cause severely adverse affect upon the kidney when administered orally over a long period of time so that this toxicity to the kidney has been the cause of suppressing cyclosporin from being extensively employed. It can be noted that there are many cases where morbid states are caused to occur at the skin, eye or joint to which topical preparations can be applied. In the case of diseases that can be administered with topical preparations, it is advantageous to avoid systemic administration that might cause disturbances to occur in the kidney. If the focus of a disease is restricted to a layer of the dermis, topical administration through the epidermis is more advantageous than other ways of administration because it can save the amount of a medicine to be administered and further the efficacy of the medicine can be enhanced in association with a local rise in the concentration of the medicine, while systemic side effects can be reduced. The way of administration in the form of topical preparations can be said to be one of the most effective drug delivery systems (DDS) for cyclosporin.

On the other hand, it is extremely difficult to formulate cyclosporin into topical preparations so as to maintain its highly therapeutical effect, unlike water-soluble or low-molecular weight, pharmaceutically effective substances. One of the reasons for this difficulty is because the cyclosporin is a large cyclopolypeptide having a molecular weight of larger than 1,200 so that it suffers from the difficulty in allowing cyclosporin to infuse or penetrate through the horny skin layer into the focal site present in the dermis layer. Another reason for the difficulty is because the cyclosporin is insoluble in water and there is the restriction upon the kind of organic solvents in which the cyclosporin can be dissolved. As such specific organic solvents, a lower alkanol such as ethanol or isopropanol may be generally employed. However, such a lower alkanol is too highly irritative to the skin when it is employed for topical preparations in a relatively high concentration, so that safe topical preparations cannot be provided. On the other hand, when the lower alcohol is employed in a relatively low concentration for topical preparations, the ability of the cyclosporin to be dispersed uniformly in the topical preparations may be impaired, thereby providing no topical preparations with a highly therapeutical effect.

Reports on clinical research of cyclosporin ointments have been published to the effect that a 10% cyclosporin formulation may be pharmaceutically effective or ineffective, so that its pharmaceutical effects may or may not be reproduced. Some reports describe specific compositions of cyclosporin formulations yet no clear pharmaceutical effects therefor are described.

For example, Japanese Patent Laid-open Publication No. 2-17,127 discloses compositions which contain, as essential components, cyclosporin and a mono- or polyunsaturated fatty acid or an unsaturated alcohol, each having from 12 to 24 carbon atoms. The mono- and polyunsaturated fatty acids may include, for example, vaccenic acid, linoleic acid, linolenic acid, elaidic acid, erucic acid, and the like. The unsaturated alcohol may include, for example, vaccenyl alcohol, linoleyl alcohol, linolenyl alcohol, elaidyl alcohol, erucyl alcohol, and the like. Further, it describes the compositions are effective to various skin diseases; however, that publication does not specify its pharmaceutical effects and refers merely to the ability of the cyclosporin to infuse or penetrate through the skin and to the concentration of the cyclosporin.

The publication is thoroughly silent about the extent, for example, to which the cyclosporin is effective against psoriatic diseases.

Several cases of skin diseases are reported; many of the literature states that cyclosporin is effective against the skin diseases.

For example, atopic dermatitis is reported in Acta. Derm. Venerol.: Suppl. 144, 136 - 138 (1989) where an alcoholic oily gel of containing cyclosporin at the rate of 10% by weight is effective against atopic dermatitis. Further, Arch. Derm.: 125, p. 570 (1989) reports that an alcoholic oily gel of a 10% (by weight) cyclosporin is effective.

There are reports of contact-type dermatitis, for example, in Arch. Dermato-1: 125, 568 (1989) which reports to the effect that cyclosporin is employed for a human DNCB test with no effect. Further, Contact Dermatitis: 19, 129-132 (1988) makes a review on three formulations: a 10% cyclosporin formulation in Labrafil (polyoxyl-5-oleate, olive oil and ethanol), a 5% cyclosporin formulation in castor oil, and a 5% cyclosporin formulation in castor oil containing 20% propylene glycol; however, it states the results of this review are not so satisfactory that a more effective solvent is required. In addition, Contact Dermatitis, 20, 155-156 states that none of three formulations, or 0.1%, 1% and 10% cyclosporin formulations, are effective at all against contact dermatitis.

Pharmaceutical effect of cyclosporin upon psoriasis is described, for example, in Clin. Res., 34, 1007A (1986), in which it is described that topical administration of cyclosporin is not effective for the therapy to psoriasis, although neither the concentration of cyclosporin nor the composition thereof are specified. It is also described in Lancet 1, 806 (1987) that a 2% by weight cyclosporin (on ointment base) is as effective upon psoriasis as placebo. Further, J. Amer. Acad. Dermatol., 18, 378-379 (1988) describes that a 5% cyclosporin solution in olive oil is equal to the sole use of olive oil that is employed as the base in the previous case. In addition, J. Amer. Acad. Dermatol., 22, 126-127 (1990) states that a gel comprising 10% cyclosporin, 43% olive oil, 10% ethanol, 30% polyoxyl-5-oleate and 7% colloidal silica did not produce any effect upon psoriasis. Furthermore, it is reported in Brit. J. Derm., 122, 113-114 (1990) that a 5% (by weight) cyclosporin ointment was not effective.

Reports on alopecia areata are made, for example, in Lancet, 2, 803-804 (1986) where it is described that a 2% cyclosporin oily solution was effective. In addition, Lancet 2, 971-972 (1986) reports that a 5% (wc) cyclosporin formulation in oil was effective against alopecia areata. On the other hand, Acta. Derm. Venereol., 69, 252-253 (1989) describes that a 10% cyclosporin oily preparation was not effective. Furthermore, J. Amer. Acad. Dermatol 22, 251-253 (1989) reports that a 5% cyclosporin formulation was effective against male alopecia, although no specific compositions are described therein.

As long as literature as described hereinabove has been reviewed, it is considerably difficult to draw a conclusion that cyclosporin is topically effective against the skin diseases as specified hereinabove. Even if it could be said that cyclosporin would be effective against the skin diseases, it can be said that cyclosporin should be employed in a considerably large amount. If cyclosporin preparations are not topically effective against the skin diseases or the effect is not satisfactory, it can be said in many occasions that the kinds of formulation components and the dosage are inappropriate. In summary, no conventional topical cyclosporin preparations can achieve the object to utilize cyclosporin effectively as topical preparations.

DISCLOSURE OF INVENTION

The primary object of the present invention is to provide a topical preparation containing cyclosporin, which acts effectively upon skin diseases, is useful therefor, and is highly safe.

Another object of the present invention is to provide a topical preparation containing cyclosporin, which is lower in the concentration of a lower alcohol and high in safety.

A further object of the present invention is to provide a highly safe topical preparation containing cyclosporin, which does not yet contain any quantity of a lower alcohol.

As a result of extensive research and reviews on cyclosporin-containing topical preparations which are superior in the ability of infusion or penetration through the skin or the horny skin layer yet which are less in irritation to the skin and high in safety, the present invention has been completed on the basis of the new finding as will be described hereinafter.

One aspect of the present invention provides the topical preparation containing cyclosporin, which is characterized by (a) cyclosporin; (b) an organic solvent in which the cyclosporin is to be dissolved; (c) an ester of an fatty acid with a monovalent alcohol, which is in liquid state at 25° C and which has a total number of carbon atoms of 8 or more, and/or an alkanol amine in liquid form at 25° C; (d) an oily substance in a solid form at 25° C; and (e) a surfactant, wherein an amount of the cyclosporin ranges from 0.1% by weight to 10% by weight and a total amount of the ester of the fatty acid with the monovalent

alcohol and/or the alkanol amine ranges from 1% by weight to 15% by weight.

Another aspect of the present invention provides a topical preparation containing cyclosporin, which is characterized by (a) cyclosporin; (b) a lower alcohol; (c) an fatty acid ester in liquid state at 25° C and/or an alkanol amine in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant, wherein an amount of the cyclosporin ranges from 0.1% by weight to 10% by weight, an amount of the lower alcohol ranges from 2% by weight to 15% by weight; and a total amount of the fatty acid ester and/or the alkanol amine ranges from 1% by weight to 15% by weight.

The cyclosporin-containing topical preparations according to the present invention are characterized by the features that the compositions are different from those of the conventional cyclosporin topical preparations as reported in the aforesaid literature and it can achieve the objects of the present invention in an effective way by using a reduced amount of cyclosporin.

The topical preparations containing cyclosporin according to the present invention is provided with the features as follows:

1. They are superior in therapeutic effect;
2. They are highly stable (i.e., cyclosporin does not become free from the topical preparations, no crystallization of cyclosporin is caused to occur, and no chemical reaction of cyclosporin is caused to occur with any other components of the compositions);
3. They are easily administered topically;
4. They contain cyclosporin in a highly uniformly dispersed state; and
5. They are highly safe.

In order to determine the formulations of the topical preparations according to the present invention, the selection of each component of the formulation and the rates of the components are of significant factors. For example, when the topical preparations are employed in the form of ointment, the pharmaceutical effect of the ointment, the biological activity of the ointment, and the physicochemical stability of the ointment should be taken into account. Heretofore, in usual cases, a higher saturated fatty acid or an fatty acid such as oleic acid or 12-hydroxystearic acid has been employed as an ointment base. Among those fatty acids, lauric acid, myristic acid, palmitic acid and stearic acid have been employed to form soap, together with an alkali, particularly potassium hydroxide, which in turn helps emulsify the formulated medicine.

It should be noted herein, however, that the fatty acid, whether it is employed as it is or in the form of potassium soap as an ointment base, for the cyclosporin-containing topical preparations according to the present invention, is little effective for emulsifying cyclosporin in the topical preparations, whereby no topical preparations with an highly pharmaceutical effect can be provided, and the stability of ointment may be impaired.

BEST MODES FOR CARRYING OUT THE INVENTION

The topical preparations according to the present invention contains cyclosporin, as a major active component, at a rate ranging from 0.1% to 10% by weight, preferably from 1% by weight to 7% by weight. It is to be noted herein that the topical preparations of the present invention can demonstrate highly therapeutic effects in such a low concentration.

The topical preparations according to the present invention contains the organic solvent for cyclosporin, which is in liquid state at ambient temperature (25° C) and which can dissolve the cyclosporin. Such organic solvents may include an aliphatic alcohol and a fatty acid ester with a polyvalent alcohol.

As the aliphatic alcohols, there may be employed any lower alcohol and higher alcohol as long as they are liquid at ambient temperature. The alcohol may be a straight or branched one or may be saturated or unsaturated one. Specific examples of such aliphatic alcohols may include a lower alcohol such as ethanol, propanol, isopropanol, butanol, and the like, and a higher alcohol such as octyl alcohol, nonyl alcohol, decyl alcohol, 2-octyl dodecanol, 2,6-dimethyl-4-heptanol, oleyl alcohol, and the like. The branched higher alcohol is preferably appropriate as the organic solvent for the cyclosporin.

The polyvalent alcohol-fatty acid ester may be represented by the following formula:

R^1COOR^2OH

where

R^1 is an alkyl group having from 4 to 12 carbon atoms, preferably from 6 to 10 carbon atoms; and

R^2 is an alkyl group having from 2 to 4 carbon atoms.

Specific examples of the polyvalent alcohol-fatty acid ester may include, for example, propylene glycol caprylate, propylene glycol caprate, butylene glycol caprylate, butylene glycol caprate, glycol butyrate, and

propylene glycol butyrate.

The organic solvents as described hereinabove may be employed solely or in admixture with the other organic solvents. The mixture advantageously contains the lower alcohol in the range from approximately 5% to 60% by weight, preferably from approximately 10% to 50% by weight.

The organic solvents may be admixed with the cyclosporin at the rate ranging from approximately 0.5 part to 10 parts by weight, preferably from approximately 1 part to 5 parts by weight, per part by weight of cyclosporin. As the organic solvents, the lower alcohol, particularly ethanol, is preferred. The lower alcohol can serve as a solvent for the cyclosporin as well as acts for accelerating the ability of the cyclosporin to infuse or penetrate through the skin.

The rate of the lower alcohol to be admixed with the cyclosporin may preferably be determined so as to amount to 2% by weight or more with respect to the total weight of the topical preparation, in order to accelerate the ability of the cyclosporin for infusion or penetration through the skin. If the concentration of the lower alcohol increases, the extent of irritation becomes so severer that the concentration of the lower alcohol may be reduced to 15% by weight or lower with respect to the total weight of the topical preparation. It is to be noted, however, that the concentration of the lower alcohol may preferably range from 3% to 6% by weight with respect to the total weight of the topical preparation, in order to focus on improvements in the ability of the cyclosporin for infusion or penetration through the skin and a low degree of irritation.

It is noted that for the topical preparations according to the present invention, it is preferred to use such an organic solvent as having a boiling point of 160 ° C or higher, preferably 180 ° C or higher and being sparingly volatile or volatilizable. Such organic solvents may include, for example, a higher aliphatic alcohol having 8 carbon atoms or more and a divalent alcohol-fatty acid ester.

The topical preparations according to the present invention contains the ester of the fatty acid in liquid state at ambient temperature with the monovalent alcohol and/or the alkanol amine. The fatty acid ester with the monovalent alcohol may have 8 carbon atoms or more, preferably 12 carbon atoms or more.

The monovalent alcohol component of the monovalent alcohol-fatty acid esters may be a residue of a straight- or branch-chained aliphatic alcohol having from 1 to 22 carbon atoms, preferably from 2 to 18 carbon atoms. The fatty acid component may be a straight-chained or branch-chained, monovalent or divalent fatty acid having from 4 to 22 carbon atoms, preferably from 6 to 18 carbon atoms. The monovalent alcohol and fatty acid components may in each case contain an unsaturated bond. The monovalent alcohol component thereof may include, for example, ethanol, propanol, isopropanol, butanol, hexanol, octanol, isooctanol, dodecanol, isododecanol, myristyl alcohol, cetyl alcohol, hexadecyl alcohol, 2-ethylhexyl alcohol, 2-octyl dodecanol and the like. The fatty acid component may include, for example, a monovalent fatty acid such as butyric acid, octanoic acid, nonanoic acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolic acid, and erucic acid, and a divalent fatty acid such as succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, and dodecane diacid. Preferred examples of the fatty acid esters with the monovalent alcohols may include, for example, a monovalent fatty acid ester such as ethyl myristate, isopropyl myristate, isotridecyl myristate, isopropyl laurate, isopropyl caprylate, isopropyl palmitate, isopropyl butyrate, amyl butyrate, and octyl butyrate, and a divalent fatty acid ester such as diethyl succinate, diisopropyl succinate, diethyl adipate, diisopropyl adipate, diisooctyl adipate, dioctyl adipate, didecyl adipate, decyl isooctyl adipate, diethyl azelate, diisopropyl azelate, diisooctyl azelate, diethyl sebacate, diisopropyl sebacate, dibutyl sebacate, and dioctyl sebacate.

Specific examples of the alkanol amines may include, for example, diethanol amine, triethanol amine, isopropanol amine, triisopropanol amine, dibutanol amine, tributanol amine, and the like.

The monovalent alcohol-fatty acid ester and the alkanol amine can serve as improving the ability of the cyclosporin solution in the organic solvents to infuse or penetrate through the skin as well as demonstrate the action of homogeneously dispersing the cyclosporin, dissolved in the organic solvents, in the oily substance in solid form. The rate of these compounds may range usually from approximately 1% to 15% by weight, preferably from approximately 3% to 10% by weight, with respect to the total weight of the topical preparation. Further, these compounds may be employed at the rate ranging usually from approximately 2 parts to 5 parts by weight, preferably from approximately 2.5 parts to 4 parts by weight, with respect to part by weight of the organic solvent or solvents.

The topical preparations according to the present invention contains the oily substance in solid form at ambient temperature. It is noted herein that the term "solid" referred to herein is intended to mean semi-solid as well as solid. The oily substances may include, for example, an alcohol, an fatty acid, an ester, a triglyceride, wax, vaseline, and the like. The alcohol may include, for example, palmityl alcohol, stearyl alcohol, eicosyl alcohol, glycerine, polyglycerin, and the like. The fatty acid may include, for example, palmitic acid, stearic acid, oleic acid, arachic acid, behenic acid, montanic acid, melissic acid, sebacic acid,

and the like. The ester may include, for example, butyl stearate, hexyl laurate, myristyl myristate, dodecyl oleate, 2-octyldodecyl myristate, hexyl decyl octanoate, cetyl lactate, glyceryl caprate, glyceryl caprilate, and the like. As the triglyceride, there may be employed a variety of materials originating from sources such as animals or naturally occurring plants or vegetables, which are generally called fats and oils and which can be commercially available. It may include, for example, a large variety of vegetable oils, cow fats, liver fats, lanolin, lard, and the like. Preferable ones are vegetable oils, particularly olive oil, camellia oil, soybean oil, rapeseed oil, corn oil, castor oil, safflower oil, and the like. There may also be employed fish oil rich in eicosapentadecanoic acid that recently draws increasing attention due to its action for allergy or malignant tumor.

The rate of the oily substance is not restricted to a particular one and may be formulated at any arbitrary rate in accordance with the desired properties of the topical preparations. Generally, the rate of the oily substance may range from approximately 1 part to 10 parts by weight, preferably from approximately 2 parts to 8 parts by weight, with respect to part by weight of the total weight of the organic solvent and the monovalent alcohol-fatty acid ester and/or the alkanol amine, which is in liquid state at room temperature.

The surfactant is contained in the topical preparations according to the present invention. As the surfactants, there may be employed a variety of surfactants, including anionic, cationic, non-ionic or amphoteric ones. The non-ionic surfactants may preferably be employed in terms of a low degree of irritation to the skin. As the non-ionic surfactants, there may be mentioned, for example, an ethylene oxide type surfactant, a polyhydroxy type surfactant, a polymer type surfactant, and the like. The ethylene oxide type surfactants may include, for example, an ethylene oxide adduct of a higher alcohol, an ethylene oxide adduct of a higher fatty acid, an ethylene oxide adduct of an alkyl phenol, an ethylene oxide adduct of an fatty acid amine, an ethylene oxide adduct of an fatty acid amide, an ethylene oxide adduct of a polyvalent alcohol, an ethylene oxide/propylene oxide block copolymer, and the like. The polyhydroxy type surfactants may include, for example, a glycerin monofatty acid ester, a pentaerythritol fatty acid ester, a sorbitan fatty acid ester, a sucrose fatty acid ester, an fatty acid amide of ethanol amine and an alkylene oxide adduct thereof, and the like. Among these polyhydroxy type surfactants, there may be advantageously employed a polyoxy ethylene sorbitan fatty acid ester, a polyoxy ethylene glyceryl monofatty acid ester, a polyoxy propylene monofatty acid ester, the sorbitan fatty acid ester, a polyoxy ethylene alcohol ether, and the like. These surfactants may be employed solely or in admixture with the other surfactant or surfactants.

The amount and the rate of the surfactant is not restricted to a particular one and may vary depending upon the desired properties of the topical preparation, although the surfactant may be generally contained in the range of from approximately 5% to 50% by weight, preferably from approximately 20% to 45% by weight, with respect to the total weight of the topical preparation in the case of the topical preparation being of a non-emulsion type and from approximately 1% to 20% by weight, preferably from approximately 5% to 15% by weight, with respect to the total weight thereof in the case of the topical preparation being of an emulsion type.

The topical preparation in accordance with the present invention may, as desired, contain an additive such as a filler, an aid for dissolving cyclosporin, a thickening agent, a colorant, a flavor, water, liquid paraffin, squalane, an emulsification stabilizer, a bactericide, a fungicide, and the like. The filler may be finely divided powder of an organic type or of an inorganic type. The particle size of the filler may range usually from approximately 0.1 μm to 20 μm, preferably from approximately 0.5 μm to 10 μm. Appropriate examples of the fillers may include silica, alumina, titania, resin powder, silicate powder, clay powder, sepiolite powder, montmorilonite powder, fluorinated mica powder, hydroxypropyl cellulose powder, and the like. The aid of dissolving cyclosporin may include, for example, an alkylene glycol and a polyalkylene glycol such as ethylene glycol, propylene glycol, isopropylene glycol, polyethylene glycol, polypropylene glycol, and the like. The rate and the amount of the dissolving aid may range from approximately 0.2 part to 5 parts by weight with respect to part of the total weight of the organic solvent. The alkylene glycol serves as accelerating the infusion or penetration of the cyclosporin through the skin.

The topical preparations according to the present invention may be applied in the form of an emulsion or a non-emulsion. When the topical preparations are formulated in a non-emulsion form, they may preferably comprise the following composition:

a. Cyclosporin: from approximately 0.1% to 10% by weight, preferably from approximately 1% to 7% by weight;

b. Organic solvent: from approximately 1% to 40% by weight, preferably from approximately 2% to 20% by weight;

c. Monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine: from 1% to 15% by weight, preferably from approximately 3% to 10% by weight;

d. Oily substance in solid state at ambient temperature: from approximately 20% to 80% by weight, preferably from approximately 35% to 60% by weight;

e. Surfactant: from approximately 5% to 50% by weight, preferably from approximately 20% to 45% by weight; and

f. Filler: from 0% to approximately 15% by weight, preferably from approximately 5% to 10% by weight.

When the lower alcohol is employed solely as the organic solvent for the topical preparation of the non-emulsion type, the lower alcohol may conveniently be contained at a rate ranging from approximately 2 to 15% by weight, preferably from approximately 3% to 10% by weight. In this case, the surfactant may conveniently be contained at a rate ranging from approximately 20% to 45% by weight, preferably from approximately 20% to 40% by weight and the oily substance may conventionally be contained at a rate in the range of from approximately 35% to 60% by weight, preferably from approximately 40% to 55% by weight. Further, the surfactant to be employed may have an HLB of 8 to 25, preferably from 9 to 12.

The topical preparation of the non-emulsion type may be formulated by mixing a cyclosporin solution in the organic solvent and the monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine, mixing the resulting mixture with the oily substance and the surfactant, and adding the filler to the resulting mixture as needed, and then homogenizing the mixture.

The topical preparations in accordance with the present invention in an emulsion form may preferably comprise the composition as follows:

a. Cyclosporin: from approximately 0.1% to 10% by weight, preferably from approximately 1% to 7% by weight;

b. Organic solvent: from approximately 1% to 20% by weight, preferably from approximately 2% to 12% by weight;

c. Monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine: from 1% to 15% by weight, preferably from approximately 3% to 10% by weight;

d. Oily substance in solid state at ambient temperature: from approximately 10% to 35% by weight, preferably from approximately 15% to 30% by weight;

e. Surfactant: from approximately 1% to 20% by weight, preferably from approximately 5% to 15% by weight;

f. Filler: from 0% to approximately 10% by weight, preferably from approximately 0.1% to 5% by weight; and

g. Sterilized water: from approximately 30% to 75% by weight, preferably from approximately 40% to 50% by weight.

The topical preparations in the form of an emulsion may be prepared by mixing the components (a) to (f), inclusive, at elevated temperature to give an oily mixture in a liquid state, referred to hereinafter as "mixture A", and adding sterilized pure water, referred to hereinafter as "water B" to the mixture A with stirring at elevated temperature. The water B may be added at a rate of from approximately 30% to 75% by weight with respect to the total weight of the mixture A and the water B. To the water B may in advance be added an aid of infusion or penetration of cyclosporin through the skin, a viscosity adjusting agent, the bactericide, a water-soluble substance such as an alkanol amine. The infusion or penetration aid may include, for example, an alkylene glycol such as ethylene glycol, propylene glycol, butylene glycol, and the like. The viscosity adjusting agent may include, for example, a polyalkylene glycol such as polyethylene glycol, polypropylene glycol, and the like; a polyvalent alcohol such as glycerin and the like; and a water-soluble polymer such as carboxyvinyl polymer and the like. The topical preparations in the emulsion form may be of an oil/water type and of a water/oil type. For the topical preparations of the oil/water type, the surfactant having an HLB of 9 to 18 may preferably be employed; for the topical preparations of the water/oil type, the surfactant having an HLB of 2 to 8 may preferably be employed. To the topical preparations of the emulsion type may be added, as needed, a viscous oily substance such as liquid paraffin, glycerin, vaseline, and the like.

The topical preparations according to the present invention may be administered by applying them directly to the affected part of the skin or by applying them in the form of a patch, plaster, poultice, or the like to the affected part thereof, several times, e.g. once to thrice, per day. The number of applications may appropriately be increased or reduced depending upon the extent of the disease to be applied.

In accordance with the topical preparations of the present invention, a mixture of the cyclosporin solution in the organic solvent with the liquid monovalent alcohol-fatty acid ester and/or alkanol amine is contained in the oily substance in homogeneously dispersed manner. Hence, the topical preparations is so highly likely to infuse or penetrate through the skin that they can demonstrate highly therapeutic effects upon autoimmune or allergic skin diseases merely by applying them to the affected part of the skin. Further, the topical preparations are little irritative or extremely low in irritation to the skin so that they are highly

safe.

The topical preparations according to the present invention are highly effective for the therapy of various dermal diseases such as atopic dermatitis, psoriasis, contact dermatitis, allergic contact dermatitis, alopecia, and the like. Further, they are effective for treating other dermal diseases, such as scald. The topical preparations can assist adapt a skin piece grafted to the site of skin grafting.

The present invention will be described more in detail by way of examples.

Example 1:

For a topical preparation, there were employed the components as follows:

Cyclosporin:	1% by weight
95% Ethanol:	3% by weight
Isopropyl myristate:	5% by weight
Olive oil:	48% by weight
Polyoxyethylene (5) glyceryl monostearate:	35% by weight
Finely divided silica (Aerosil 200)	8% by weight

The topical preparation was formulated by mixing isopropyl myristate, polyoxyethylene (5) glyceryl monostearate and olive oil with stirring at 50° C to give a homogenous solution to which a solution of cyclosporin in ethanol was added, and the resulting mixture heated to 30° - 35° C was mixed with aerosil to give an ointment.

Example 2:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	1% by weight
95% Ethanol:	5% by weight
Isopropyl myristate:	5% by weight
Olive oil:	47% by weight
Polyoxyethylene (5) glyceryl monostearate:	35% by weight
Finely divided silica (Aerosil 200)	7% by weight

Example 3:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	2% by weight
95% Ethanol:	10% by weight
Isopropyl myristate:	5% by weight
Camellia oil:	44% by weight
Polyoxyethylene (5) glyceryl monostearate:	32% by weight
Finely divided silica (Aerosil 200)	7% by weight

Example 4:

After the skins of guinea pigs were sensitized with dinitrofluorobenzene (DNFB), DNFB were applied again, thereby causing the strong allergic reaction to emerge on the skins of the guinea pigs.

The efficacy of the topical preparations according to the present invention was observed with this experimental model.

Cyclophosphamide was intraperitoneally administered at the rate of 200 mg per kg three days before the sensitization of male Hartley guinea pigs, weighing from 40 grams to 500 grams, and 50 µl of a 10% DNFB solution in a 1:1 mixture of acetone and olive oil) was applied to one earlobe of each of the guinea pigs. At day 8, a dose of 20 µl of 0.5% or 0.1% DNFB solution in a 4:1 mixture of acetone and olive oil was applied to the both sides of the depilated abdominal portions of the guinea pigs, whereby contact dermal allergic reaction was induced.

After DNFB was then applied as an antigen to the corresponding sites of the both abdominal portions, the topical preparations prepared in Example 1 (containing cyclosporin at the rate of 0.1%, 1% and 10%) were applied in the amount of 50 µl thereto. This application was repeated twice a day at an interval of 8 hours. The first application of each topical preparation was conducted immediately after DNFB had been air dried.

The allergic reaction was evaluated at 24 hours, 48 hours and 72 hours after the application of the antigen in accordance with the following criteria: Rating 4 = swell in red; rating 3 = colored in red; rating 2 = colored in pink; rating 1 = a spot colored in pink; and rating 0 = no change. The values as shown in Table 1 below represent the mean value plus or minus the standard error (SE).

The statistical treatment was conducted with Student's t-test, and a significant difference was justified if the error rate was $p<0.05$.

The application of the 0.5% DNFB solution caused the strongest allergic reaction over the time range from 24 hours to 48 hours after the application. The 0.1% cyclosporin ointment suppressed the allergic reaction to a considerable extent with no significant difference. On the other hand, the ointment containing 1% cyclosporin reduced the allergic reaction to a remarkable extent at 24 hours with the significant difference of $p<0.01$. Even at 48 hours and 72 hours, the allergic reaction was suppressed with the significant difference. Further, the ointment containing 10% cyclosporin demonstrated the significant suppression of the allergic, like the 1% cyclosporin ointment. As a control, the ointment base only did not suppress the allergic reaction at all. The results are shown in Table 1 below.

TABLE 1

Test Samples		24 hours	48 hours	72 hours
Cyclosporin (%)	No. of guinea pigs			
0	9	3.4 ± 0.2	3.4 ± 0.2	2.7 ± 0.2
0.1	9	2.4 ± 0.3	2.7 ± 0.3	1.8 ± 0.3
1.0	9	0.7 ± 0.3**	1.0 ± 0.3**	1.0 ± 0.3**
0	4	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.3
10	4	0.8 ± 0.5*	1.0 ± 0.6*	1.0 ± 0.5

* $p < 0.05$
** $p < 0.01$

When the 0.1% DNFB solution was applied, the strongest allergic reaction was caused to appear at 48 hours after the application. The 0.1% cyclosporin topical preparation suppressed the allergic reaction to a remarkable extent with the significant difference of $p < 0.01$. The allergic reaction was likewise suppressed even at 48 hours and 72 hours. On the other hand, the topical preparations containing 1% and 10% cyclosporin showed the reduction in the allergic reaction with the significant difference, like the topical preparation containing 0.1% cyclosporin. As a control, the ointment base only did not suppress the allergic reaction at all. The results are shown in Table 2 below.

TABLE 2

Test Samples		24 hours	48 hours	72 hours
Cyclosporin (%)	No. of guinea pigs			
0	8	2.1 ± 0.3	3.1 ± 0.2	2.5 ± 0.2
0.1	8	0.3 ± 0.2**	1.0 ± 0.2**	0.8 ± 0.2**
1.0	8	0.1 ± 0.1**	0.4 ± 0.3**	0.1 ± 0.1**
0	4	2.0 ± 0.4	3.0 ± 0	2.3 ± 0.3
10	4	0 ± 0*	0.5 ± 0.3*	0.3 ± 0.3*

* $p < 0.05$
** $p < 0.01$

Example 5:

Case 1:

A male patient, 27 years old, has been affected with atopic dermatitis since his age of 22 although a temporary remission had been gained at his age of 8 years from the atopic dermatitis since his age of 3. Various steroidal ointments were applied so far; they were found hardly effective. With the 10% cyclosporin ointment according to the present invention, an itch on his skin disappeared four to five hours after the application of the ointment and the lichenized erythra peculiar in the atopic dermatitis disappeared completely at day 3 after its application when the ointment was applied twice per day.

Case 2:

A male child, 6 years old, has been affected with atopic dermatitis since his age of 3 and was administered with Azeptin, Zaditen, and Rizaben as well as ointments such as Rinderon V, Locorten and Methaderm; however, no effect was recognized. The application of a 5% cyclosporin ointment according to the present invention eliminated an itch to his skin within 5 hours after the topical administration and the itch, erythema and wet erosion of the affected part had disappeared within 24 hours after the application thereof.

Case 3:

A male patient, 52 years old, was affected with psoriatic arthritis, and the 1% cyclosporin ointment according to the present invention was applied to the wet erythema with a clear borderline and the scales on the surface thereof. The 1% cyclosporin ointment improved the Auspitz phenomenon within 24 hours after the application with the erythema disappearing at day 3 from the application of the ointment.

Example 6:

In order to demonstrate the efficacy of the topical preparations according to the present invention, the ointments were prepared from the components as shown in Table 3 below and the efficacy thereof was evaluated in substantially the same manner as in Example 4. The evaluation results are shown in Table 3 below.

TABLE 3

Components	Contents (% by weight)						
	Experiment Nos.						
	1*	2*	3	4	5	6	7
Cyclosporin	5	5	5	5	10	5	10
95% Ethanol	0	0	2	5	10	5	10
Isopropyl myristate	5	5	5	5	5	0	3
Olive oil	48	48	48	45	35	36	36
Polyoxyethylene glycol monostearate	35	35	35	35	35	36	36
Aerosil	5	7	5	5	5	6	6
Triethanol amine	2	0	0	0	0	3	2
Efficacy	None	None	Yes	Yes	Yes	Yes	Yes

* Comparative Examples

Comparative Examples:

The following topical preparations containing cyclosporin were prepared for comparative purposes in conventional manner:

i. A caster oil suspension containing 5% by weight of cyclosporin;

ii. A suspension of 5% by weight of cyclosporin in castor oil containing 20% by weight of propylene glycol; and

iii. An ointment containing 10% by weight of cyclosporin, 43% by weight of olive oil, 10% by weight of ethanol, 7% by weight of polyoxyethylene (5) oleate, and 30% by weight of silicon dioxide in colloidal state.

The topical preparations prepared in the manner as described hereinabove were evaluated for their pharmaceutical efficacy in substantially the same manner as in Example 4; however, none of them were found significantly effective.

Example 7:

For a topical preparation, there were employed the components as follows:

Cyclosporin:	5% by weight
95% Ethanol:	2% by weight
Isopropyl myristate:	7% by weight
Camellia oil:	40% by weight
Polyoxyethylene (5) glyceryl monostearate:	41% by weight
Finely divided silica (Aerosil 200)	5% by weight

The topical preparation was formulated in substantially the same manner as in Example 1.

Example 8:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	5% by weight
95% Ethanol:	5% by weight
Isopropyl myristate:	5% by weight
Camellia oil:	39% by weight
Polyoxyethylene (5) glyceryl monostearate:	39% by weight
Finely divided silica (Aerosil 200)	5% by weight

Example 9:

After each of the topical preparations prepared in Examples 7 and 8 were stored in a closed state for 6 months at relative temperature of 75% and temperature of 40° C, the content of cyclosporin within the topical preparation was measured. As a result, it was found that no substantial changes were observed between before and after storage. Thus, it is confirmed that cyclosporin is sustained in a stable state for a long period of time.

Example 10:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 70 grams of 2-octyl dodecanol, 30 grams of isopropyl myristate, 20 grams of isotridecyl myristate, 10 grams of polyoxyethylene sorbitan monooleate (20), 50 grams of polyoxyethylene glyceryl monostearate (5), 10 grams of sorbitan monostearate, 30 grams of cetanol, 40 grams of sebacate and 30 grams of olive oil at 80 C. On the other hand, a mixture (B) was prepared by adding 30 grams of propylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 1 gram of methyl p-hydroxybenoate, and 1 gram of propyl p-hydroxybenzoate to 596 ml of sterilized water and heating the mixture to approximately 82° C. As the two mixtures reached the predetermined temperatures, the mixture A was gradually added with vigorous stirring to the mixture B, thereby producing an emulsion. After the addition was completed, the heating was ceased and the temperature of the emulsion was stirred and cooled down to 60° - 55°. Then, sterilized water was added to make the total volume of the mixture 1 kg. The whole mixture was allowed to stand and defoamed, followed by filling in a vessel.

In the above composition, polyoxyethylenel glyceryl monostearate (5) can be replaced by 2.0% by weight of polyoxyethylene (2) cetyl ether; sorbitan monostearate can be replaced by squalane SK; and cetanol can be replaced by behenyl alcohol. Further, the total volume of the sterilized water used for the mixture (B) can be replaced by liquid paraffin.

Example 11:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 50 grams of ethanol, 50 grams of isopropyl myristate, 50 grams of polyethylene glycol (400), 30 grams of dithyl sebacate, 80 grams of olive oil, 30 grams of polyoxyethylene monostearate (5), 30 grams of polyethylene glycol monostearate (40), and 20 grams of sorbitan monostearate at elevated temperature. On the other hand, a mixture (B) was prepared by dissolving 50 grams of polythylene glycol, 20 grams of diisopropanol amine, 10 grams of carboxyvinyl polymer, 1 gram of methyl p-hydroxybenoate, and 1 gram of propyl p-hydroxybenzoate in 528 ml of sterilized water at elevated temperature. The mixture A was gradually added with vigorous stirring to the mixture B, thereby producing an emulsion. After the addition was completed, the total volume of the mixture was increased to make 1 kg by adding sterilized water to the mixture.

In the above composition, ethanol can be replaced by behenyl alcohol, and diisopropanol amine can be replaced by triethanol amine.

Example 12:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 10 grams of octyl alcohol, 50 grams of olive oil, 30 grams of isopropyl myristate, 25 grams of isotridecyl myristate, 20 grams of polyoxyethylene sorbitan monooleate (20), 60 grams of polyoxyethylenel glyceryl monostearate (5), 20 grams of sorbitan stearate, 30 grams of cetanol, 25 grams of stearic acid, and 35 grams of diethyl sebacate at 80° C. On the other hand, a mixture (B) was prepared by adding and dissolving 20 grams of polythylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5

gram of propyl p-hydroxybenzoate to and in approximately 400 ml of sterilized water by heating the mixture to 82° C or higher. The mixture B was gradually added with vigorous stirring to the mixture A, thereby producing an emulsion. After the addition was completed, the heating was ceased and sterilized water was added at 80° C to the resulting mixture with stirring at room temperature, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the ointment in cream form was filled in a container.

In the above composition, isopropyl myristate can be replaced by isopropyl palmitate.

Example 13:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 30 grams of bees wax, 80 grams of 2,6-dimethyl-4-heptanol, 30 grams of olive oil, 40 grams of isotridecyl myristate, 20 grams of polyoxyethylene solbitol hexastearate (20), 60 grams of polyoxyethylenel glyceryl monostearate (5), 20 grams of polyoxyethylene (60) hardened castor oil, 40 grams of cetostearyl alcohol, and 40 grams of diethyl sebacate at 80° C. On the other hand, a mixture (B) was prepared by adding and dissolving 30 grams of polyethylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5 gram of propyl p-hydroxybenzoate to and in 510 ml of sterilized water by heating the mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C. After the addition was completed, the heating was ceased and the mixture was cooled down to 60° - 55° C with stirring. Then, sterilized water heated to 80° C was added to the resulting mixture with stirring at room temperature, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the resulting mixture was filled in a container.

In the above composition, the bees wax can be replaced by polyoxyethylene lanolyl alcohol or a bees wax derivative; isotridecyl myristate can be replaced by 0.2% by weight of silicone oil; polyoxyethylenel sorbitan oleate (20) can be replaced by polyoxyethylenel sorbitan-fatty acid ester; sorbitan monostearate can be replaced by squalane SK; and sterilized water can be replaced by liquid paraffin.

Example 14:

A mixture (A) was prepared by mixing 50 grams of cyclosporin, 80 grams of propylene glycol monocaprylate, 30 grams of isopropyl myristate, 30 grams of PEG monostearate (25EO), 30 grams of polyethylene glycol, 20 grams of isotridecyl myristate, 20 grams of cetanol, 50 grams of olive oil, 80 grams of whale wax, 30 grams of sorbitan monostearate, 30 grams of polyoxyethylene glyceryl monostearate (5), 30 grams of stearic acid, 20 grams of diisopropanol amine, and 40 grams of diethyl sebacate and heating the resulting mixture at 80° C. On the other hand, a mixture (B) was prepared by adding 30 grams of propylene glycol, 15 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5 gram of propyl p-hydroxybenzoate to approximately 400 ml of sterilized water and heating the resulting mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C or higher. After the addition was completed, the heating was ceased and the mixture was stirred to cool the temperature of the mixture to 60° - 55° C, followed by adding sterilized water heated at 80° C to the resulting mixture to increase the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the resulting mixture was filled in a container.

Example 15:

A mixture (A) was prepared by mixing 50 grams of cyclosporin, 70 grams of 2-octyl dodecanol, 30 grams of isoprene glycol, 40 grams of diethyl sebacate, 30 grams of isopropyl myristate, 30 grams of isotridecyl myristate, 60 grams of whale wax, 30 grams of cetanol, 40 grams of stearic acid, 20 grams of POE (5) glyceryl monostearate, 20 grams of PEG monostearate (40EO), 10 grams of sorbitan monostearate, 50 grams of olive oil, and 1 gram of propylparaben and heating the mixture to 80° C. On the other hand, a mixture (B) was prepared by adding 30 grams of butylene glycol, 20 grams of diisopropanol amine, and 1 gram of methylparaben to 460 ml of sterilized water and heating the resulting mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C or higher. After the addition was completed, the heating was ceased and the temperature of the mixture was cooled down to 60° - 55° C with stirring. Then, sterilized water headed at 80° C was added to the resulting mixture, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to

stand and defoamed; then the resulting mixture was filled in a container.

Example 16:

A solution of 50 grams of cyclosporin in 80 grams of 2-octyl dodecanol was added to a warmed mixture of 40 grams of isopropyl myristate, 370 grams of olive oil, 378 grams of polyoxyethylene (5) glyceryl monostearate, 2 grams of polyoxyethylene (9) lauryl ether, and 10 grams of sorbitan monostearate, and 70 grams of aerosil was added to the resulting mixture, thereby yielding a topical preparation of a non-emulsion type.

Example 17:

The efficacy of the creamy ointment containing cyclosporin, prepared in substantially the same manner as in Example 15, was confirmed by applying it to the transplant of the skin sections of mice.

The skin sections of 10 male CBA mice of 5 weeks were transplanted to male C3H/HeN mice of the same week. To the transplanted sites and the portions surrounding them was applied approximately 0.1 gram of the ointment prepared in Example 12 two times per day until the transplanted sections eventually fell off. The results are shown in Table 4 below.

TABLE 4

Cyclosporin (%)	Period of Transplantation*	Effect of Extension	Significant Difference**
5.0	>60	>397	$p < 0.001$
1.0	31.3 ± 1.43	207	$p < 0.001$
0.0 (as control)	15.1 ± 0.78	100	-

* mean value ± SE

** Student's t-test

For a control group in which a cream without cyclosporin was applied, the transplanted skin specimens fell off for an average period of transplantation of 12.7 days, while a group in which a cream containing 5% cyclosporin was applied had all the transplanted skin sections grow for 60 days or longer. For a group in which a cream containing 1% cyclosporin was applied, the period of transplantation for which the transplanted skin sections grew was extended with significant difference to mean 31.3 days.

Example 18:

Eight Hartley male guinea pigs weighing approximately 300 grams were intraperitoneally administered with 150 mg/kg of cyclophosphamide, and 50 µl of a 10% dinitrofluorobenzene (DNFB) solution was applied to one earlobe of each guinea pig in three days after the intraperitoneal administration. The DNFB solution was prepared by dissolving the predetermined amount of the DNFB in a 1:1 mixture of acetone with olive oil. After 8 days, the hairs on the both abdominal parts were cut off and 20 µl of a 0.1% DNFB solution was applied to the depilated abdominal parts of the guinea pigs to induce contact dermal allergy. Immediately thereafter, the cyclosporin ointment prepared in substantially the same manner as in Example 15 was applied to the parts to which the DNFB solution was applied, followed by applying the cyclosporin ointment thereto in 8 hours. To a control group, the base used in Example 15 without cyclosporin was applied in accordance with the same schedule as described hereinabove.

The allergic reaction was determined in 24 hours, 48 hours and 72 hours after the application of the DNFB solution as the antigen, and the rating was: 4 = swell in red; 3 = colored in red; 2 = inflammation causing the skin to turn pink; 1 = inflammation causing the skin to turn pale pink; and 0 = no change. The results are shown in Table 5 below.

TABLE 5

Cyclosporin (%)	Severity of Dermal Reaction (mean value ± SE)		
	24 hours	48 hours	72 hours
1.0	0.0 ± 0.0**	0.3 ± 0.2**	0.1 ± 0.1**
0.1	0.3 ± 0.3**	0.9 ± 0.2**	0.7 ± 0.3**
0.0 (control)	2.2 ± 0.3**	3.1 ± 0.2**	2.4 ± 0.2**

** $p < 0.001$ in Student's t-test

In these experiments, the strongest allergic reaction was induced over the range extending from 24 hours to 48 hours after the application of the DNFB solution. The ointment containing 1.0% cyclosporin strongly suppressed the allergic reaction and the ointment containing 0.1% cyclosporin suppressed the allergic reaction with significant difference.

Claims

1. A topical preparation comprising (a) cyclosporin; (b) an organic solvent in which said cyclosporin is to be dissolved; (c) an ester of a fatty acid with a monovalent alcohol having a total number of carbon atoms of 8 or more and/or an alkanol amine, each being in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant; wherein said cyclosporin is contained at a rate ranging from approximately 0.1% to 10% by weight, and said ester and/or said alkanol amine are/is contained at a rate ranging from approximately 1% to 15% by weight.

2. A topical preparation as claimed in claim 1, wherein said organic solvent is an aliphatic alcohol in liquid state at 25° C.

3. A topical preparation as claimed in claim 2, wherein said aliphatic alcohol is a lower alcohol.

4. A topical preparation as claimed in claim 3, wherein said lower alcohol is ethanol.

5. A topical preparation as claimed in claim 2, wherein said aliphatic alcohol is a higher alcohol having a branched chain and carbon atoms of 8 or more.

6. A topical preparation as claimed in claim 5, wherein said higher alcohol is 2-octyldodecanol.

7. A topical preparation as claimed in claim 1, wherein said organic solvent is a fatty acid monoester with a polyvalent alcohol, having liquid state at ambient temperature.

8. A topical preparation as claimed in claim 7, wherein said monoester is propyleneglycol monocaprate or propylene glycol monocaprylate.

9. A topical preparation as claimed in any one of claims 1 to 8, wherein said organic solvent is contained at a rate ranging from approximately 0.5 part to 10 parts by weight with respect to part by weight of said cyclosporin.

10. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is an ester of a monovalent fatty acid having carbon atoms of 8 or more.

11. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is a diester of a divalent fatty acid having carbon atoms of 4 or more.

12. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is a myristic acid ester and/or a sebacic acid diester.

13. A topical preparation as claimed in any one of claims 1 to 12, wherein said oily substance is at least one member selected from a group consisting of a fatty acid having a melting point of 25° C or higher, an alcohol, an ester and a triglyceride.

14. A topical preparation as claimed in claim 13, wherein said triglyceride is vegetable oil.

15. A topical preparation as claimed in any one of claims 1 to 14, wherein said surfactant is a non-ionic surfactant.

16. A topical preparation as claimed in any one of claims 1 to 15, further comprising a filler.

17. A topical preparation as claimed in any one of claims 1 to 16, further comprising an alkylene glycol and/or a polyalkylene glycol.

18. A topical preparation comprising (a) cyclosporin; (b) a lower alcohol; (c) an ester of a fatty acid with a monovalent alcohol having a total number of carbon atoms of 8 or more and/or an alkanol amine, each being in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant; wherein said cyclosporin is contained at a rate ranging from approximately 0.1% to 10% by weight, said lower alcohol is contained at a rate ranging from approximately 2% to 15% by weight, and said ester and/or said alkanol amine are/is contained at a rate ranging from approximately 1% to 15% by weight.

19. A topical preparation as claimed in claim 18, wherein said lower alcohol is selected from at least one member selected from a group consisting of ethanol, isopropanol, propanol and isobutanol.

20. A topical preparation as claimed in claim 18 or 19, wherein said fatty acid ester with said monovalent alcohol is an ester of a straight-chained or branched-chain fatty acid having from 8 to 24 carbon atoms.

21. A topical preparation as claimed in any one of claims 18 to 20, wherein said oily substance is vegetable oil.

22. A topical preparation as claimed in any one of claims 18 to 21, wherein said surfactant is a non-ionic surfactant.

23. A topical preparation as claimed in any one of claims 18 to 22, further comprising a filler at a rate ranging from approximately 5% to 10% by weight.

24. A topical preparation comprising 0.1% to 10% by weight of cyclosporin; 2% to 15% by weight of ethanol; 1% to 15% by weight of isopropyl myristate; 35% to 60% by weight of olive oil or camellia oil; 20% to 40% by weight of a surfactant; and 5% to 10% by weight of silica.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP92/00798

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

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II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K37/02, 9/06

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts 1967 - 1992

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 3-109332 (Shiseido Co., Ltd.), May 9, 1991 (09. 05. 91), Claim, lower left column, page 4	1-24
A	JP, A, 2-121929 (Sand AG.), May 9, 1990 (09. 05. 90), Claim, upper right column, page 6 & GB, A, 2222770 & DE, A, 3930928 & FR, A, 2636534 & AU, A, 8941400 & CH, A, 679118 & ZA, A, 8907066	1-24
A	JP, A, 2-17127 (Sand AG.), January 22, 1990 (22. 01. 90), Claim & GB, A, 2218334 & DE, A, 3915617 & FR, A, 2631235 & CH, A, 679119	1-24

* Special categories of cited documents: [10]

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

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International Searching Authority	**Signature of Authorized Officer**
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(54) Oil-in-water microemulsions

(57) Water-insoluble pharmaceutically active substances such as cyclosporin are formulated for administration in the form of an oil-in-water microemulsion, wherein the active substance is fully dissolved in the dispersed oil particles. The oil is C_8 to C_{20} fatty acid vegetable oil glycerides, and lecithin and another surfactant are included to form and stabilise the microemulsion in which the hydrophilic phase comprises propylene glycol. A preconcentrate comprising the above components but free from any hydrophilic phase can be utilised to make up the compositions, which are most suitably soft gelatine capsules or oral administration fluids. The glycerides are preferably from castor oil, coconut oil or peanut oil.

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Description

This invention relates to pharmaceutical compositions for the administration of water-insoluble pharmaceutically active substances.

There are a number of pharmaceutically active substances which are water-insoluble and which, as a result, present a number of problems for their safe administration and bioavailability. Among such substances are the cyclosporins, and water-insoluble peptides, antimicrobials and antineoplastics, for example. There have been many proposals of pharmaceutical formulations for the administration of the cyclosporins, some of which are described in the following patent specifications: WO92/09299, GB-A-2015339, GB-A-2270842, WO94/08610, WO92/18105, GB-A-2228198, US-A-4388307, GB-A-2222770, EP-A-0539319 and EP-A-0589843.

In general, because the cyclosporins are hydrophobic, pharmaceutical compositions containing them usually comprise lipophilic materials such as oils. GB-A-2228198 describes, for example, pharmaceutical compositions containing cyclosporin in a carrier medium of a fatty acid triglyceride, a glycerol fatty acid partial ester or propylene glycol or sorbitol complete or partial ester, and a surface active agent having an HLB of at least 10. These oil-based compositions are not intended to be emulsified in water but are used as such, and are preferably free of ethanol.

Other cyclosporin compositions are known which contain not only hydrophobic oils but also hydrophilic materials such as propylene glycol and ethanol in which cyclosporins are soluble. There compositions are in the form of emulsions. Emulsions have certain advantages over oil-based single phase compositions, and EP-A-0589843 describes some cyclosporin emulsion compositions containing, as the carrier medium, a hydrophilic organic solvent, a mixed mono-, di- and tri-glyceride or a transesterified and polyethoxylated vegetable oil, a polyoxyethylene sorbitan-fatty acid ester surfactant, and an aqueous phase. The carrier medium with the cyclosporin but without the aqueous phase is described as an emulsion preconcentrate.

In recent times, microemulsions have been developed for cyclosporin administration and these have provided provided further advantages over the prior known (coarse) emulsions, especially for oral administration formulations. It is also known to provide so-called "microemulsion preconcentrates". For example, GB-A-2222770 describes a pharmaceutical microemulsion preconcentrate composition comprising cyclosporin, a hydrophilic phase, a lipophilic phase and a surfactant. This preconcentrate is converted to a microemulsion by adding water or another suitable aqueous medium.

These and other microemulsions for cyclosporin are all made by dissolving the cyclosporin in a hydrophilic phase e.g. propylene glycol, and then mixing the solution with the oil and eventually with an aqueous phase. We have found that there can be a tendency with these microemulsions for solid microfine cyclosporin to be formed during their use, e.g. after administration. This is basically undesirable, and we have now found that microemulsions can be made in which this tendency is very much reduced or totally absent.

In particular, we have found that if the water-insoluble active substance is first dissolved directly in the lipophilic phase, rather than in a hydrophilic phase, and then the oil-in-water microemulsion produced therefrom, the substance remains in solution in the lipophilic (oil) phase. This phase is distributed throughout the aqueous phase of the microemulsions as very tiny particles, and it appears that in this way the substance can be taken up very easily and efficiently by the body. In addition, there is no precipitation of the substance out of the oil solution.

In one aspect the present invention provides a pharmaceutical composition in the form of a stable oil-in-water microemulsion, which composition comprises

a) a water-insoluble pharmaceutically active material;
b) C_8 to C_{20} fatty acid mono-, di, or tri-glycerides from a vegetable oil or any mixture of two or more thereof;
c) a phospholipid and another surfactant; and
d) a hydrophilic phase; wherein component (a) has been wholly directly dissolved in component (b), component (b) is dispersed as tiny particles in component (d), and the composition is free from ethanol.

The invention also provides a preconcentrate for mixture with a hydrophilic phase to form a microemulsion of the invention, the preconcentrate composition comprising:

a) a water-insoluble pharmaceutically active material;
b) a C_8 to C_{20} fatty acid mono-, di-, or tri-glyceride from a vegetable oil or any mixture of two or more thereof; and
c) a phospholipid and another surfactant;

wherein component (a) is directly dissolved in component (b), and component (c) is such that, upon mixing the composition with a hydrophilic phase, a stable oil-in-water microemulsion is formed in which component (a) is in solution in the micro dispersed oil particles, the said preconcentrate being free from a hydrophilic phase.

The invention also provides a process for making a preconcentrate or microemulsion of the invention, wherein component (a) is dissolved directly in component (b) and not in component (d). It is to be understood that component (a) is

dissolved directly in component (b) rather than first being dissolved in another liquid and the solution then mixed with component (b).

EP-A-327280 describes dissolving cyclosporin in a mono- or di-glyceride of a C_6 - C_{10} fatty acid. The solution can then be emulsified in an aqueous medium. However, these emulsions are not microemulsions and do not contain the mixture of lecithin and another surfactant which is especially used, together with the particular triglycerides component (b) all of which are necessary to obtain the significant advantages of the invention.

Microemulsions are transparent due to the very small particle size of the dispersed phase, typically less than 200nm. Such small droplets produce only weak scattering of visible light when compared with that from the coarse droplets (1-10 m) of normal emulsions. An essential difference between microemulsions and emulsions is that microemulsions form spontaneously and, unlike emulsions, require little mechanical work in their formulation. General reviews on microemulsions are provided by Attwood, D. et al, J. Colloid Interface Sci. 46:249 and Kahlweit, M. et al, J. Colloid Interface Sci. 118:436.

In the present invention, component (a) is a water-insoluble pharmaceutically active material. The invention is particularly useful with the cyclosporins, e.g. cyclosporin A, dihydrocyclosporin C, cyclosporin D and dihydrocyclosporin D. It is also useful with other water-insoluble substances such as, for example, taxol.

In the compositions of the invention, component (a) is in solution in component (b). Component (b) can be a single glyceride or any mixture of glycerides (mono- and/or di- and/or tri-) derived from vegetable oils and containing C_8 to C_{20} fatty acid residues. The preferred oils are coconut oil, peanut oil and castor oil. The whole oils can be used or the refined glycerides. The preferred glycerides are those containing C_{12} to C_{18} fatty acid residues, especially triglycerides, and these are the major components of the preferred oils.

The compositions of the three oils are as follows:

Castor Oil:

Tryglycerides of:	ricinoleic acid	87%
	oleic acid	7%
	linoleic acid	3%
	palmitic acid	2%
	stearic acid	1%

and dihydroxystearic acid in trace amounts

Coconut oil:

Tryglycerides of mainly lauric and myristic acids with smaller proportions of capric, caproic acid, caprylic acid, oleic acid, palmitic acid and stearic acid.

Peanut oil:

Glycerides of:	oleic acid	56%
	linoleic acid	26%
	palmitic acid	8.3%
	stearic acid	3.1%
	arachidic acid	2.4%
	behenic acid	3.1%
	lignoceric acid	1.1%

and capric and lauric acid in trace amounts.

Component (c) is a mixture of a phospholipid, preferably lecithin, and another surfactant to provide the stable microemulsion. Those skilled in the art will be aware of many surfactants which can be used, but we prefer to use polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate. These surfactants are extremely effective with lecithin and are highly preferred. Any lecithin can be used but we prefer soya lecithin and egg lecithin. Hydroxylated lecithins are particularly suitable, especially when component (a) is a clyclosporin, since lecithin per se can be lipophilic to an extent sufficient to affect the desired spontaneous formation of a microemulsion.

In the microemulsions of the invention, component (d) is a hydrophilic phase. The preferred material is propylene glycol, but other substances can be used. Ethanol cannot be present. Water can of course also be present but it is not preferred. Despite the use of propylene glycol, component (a) remains wholly dissolved in the oil phase (component (b)).

In use, the microemulsion preconcentrates of the invention are diluted with aqueous liquid (eg. water, fruit juice, milk, etc) to form an oil-in-water microemulsion, e.g. for oral administration. This aids in ready absorption as the surface area of the globules is largely increased. The role played by bile salts in the initial step of fragmentation of fat globules, essential for fat digestion, is circumvented.

In the compositions of the invention, the polar phospholipid lecithin aids in emulsification of the fat and absorption of triglycerides into the GIT. The combination of HLB values of the polar lecithin and for example, the polyoxyl-40-hydrogenated castor oil, is very suitable for forming a balanced microemulsion.

The rate determining factor for the absorption of drug in the vehicle is not the enzymatic metabolism of triglycerides but rests primarily in the breakdown of the fat globules into micro particles since the enzymes (lipases) act mainly at the surface of the fat globules.

In the preconcentrates of the invention, the amounts of the components, in percent by weight, are as follows:

Component		General	Usual	Preferred
(a)	active pharmaceutical	1-12%	2.5-10%	7-10%
(b)	oil phase	20-80%	30-60%	40-50%
(c)	phospholipid	1-10%	3-8%	5-6%
	other surfactant	10-60%	20-50%	35-40%

In the microemulsions, the weight percent of hydrophilic phase is generally up to about 75%, most usually from 15 to 50%, and preferably from 35 to 50%.

The compositions can consist only of the components described, or they can contain other substances. For example, in order to prevent oxidation/rancidification of the natural oils, an antioxidant, e.g. α-tocopherol can be used. Propyl gallate may be used as an alternative.

In order that the invention may be more fully understood, the following Examples are given by way of illustration only.

EXAMPLES 1-4

Microemulsion preconcentrates were made of the substances indicated, by simple mixing. The cyclosporin A was completely dissolved in the oil phase in each case.

Preconcentrate 1:	
Component	Parts
Castor oil	3.0700
Coconut oil	1.6050
Polyoxyl-40 Hydrogenated Castor oil	3.7500
Lecithin	0.5650
α- tocopherol	0.0100
Cyclosporin A	1.0000

Preconcentrate 2:	
Component	Parts
Castor oil	3.1450
Arachis oil	1.5425
Polysorbate-80 (Tween 80)	3.7500
Lecithin	0.5525
α- tocopherol	0.0100
Cyclosporine A	1.0000

Preconcentrate 3:	
Component	Parts
Castor oil	4.1484
Coconut oil	2.0416
Polyoxyl-40 Hydrogenated Castor oil	2.5000
Lecithin	0.3000
α- tocopherol	0.0100
Cyclosporine A	1.0000

Preconcentrate 4:	
Component	Parts
Castor oil	4.690
Coconut oil	1.500
Polysorbate-80 (Tween 80)	2.500
Lecithin	0.300
α- tocopherol	0.010
Cyclosporin A	1.000

When diluted with water or propylene glycol, or another hydrophilic substance, oil-in-water microemulsions formed spontaneously. There was no evidence of any insolubilisation of the cyclosporin.

The microemulsion preconcentrates were filled into bottles to be administered as a drink solution using a syringe or more preferably with the aid of a metered dose pump with a droper actuator. The formulations were also encapsulated in soft gelatin capsules.

The compositions described in Examples 1 to 4 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C), Ref, 40°C-80% RH and 45°C after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Sandimmun Neoral capsules containing 100 mg cyclosporin A per capsule was also examined.

The quantative determination of cyclosporin A was performed by using HPLC method under the following conditions of chromatography:

Pump	: Waters -510 HPLC Pump
Detector	: Waters -484 tunable absorbance detector
Injector	: Waters -715 ultra wisp sample processor
Column	: 4.6 mm x 25 cm column with L16 packing
Thermostat	: 70° - For capsules 50° - For oral solution
Eluant	: Filtered and degassed mixture of acetronitrile, water, methanol and phosphoric acid (550:400:50:0.5)
Flow rate	: 1 ml/min of the eluant
Integrator	: Waters -746

It was observed from the above examinations that the stability of solutions prepared according to the process of the invention did not differ from the stability of the commercially available composition.

Examples 5-9

Microemulsions of the invention were made of the compositions indicated, by dissolving the cyclosporin A in the oils and then forming the oil-in-water emulsions. The procedure was:

(a) dissolve the cyclosporin A in the mixture of oils with slight warming and under stirring to obtain a clear yellow liquid. Confirm the complete dissolution of the drug by microscopy.
(b) add the surfactant and hydroxylated lecithin in that order, with stirring.
(c) add the propylene glycol with stirring. Stirring was continued for an hour to ensure the formation of a homogeneous translucent to opalescent microemulsion.
(d) add the alpha tocopherol and mix thoroughly.

Example 5:

Preparation of W/O microemulsion for administration in Soft Gelatin capsules:

Component	Parts
Castor oil	1.7200
Coconut oil	0.8000
Polyoxyl-40 Hydrogenated Castor oil	3.3512
Lecithin	0.4200
α- tocopherol	0.0088
Propylene glycol	1.5000
Cyclosporin A	1.0000

Example 6:

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor Oil	1.2700
Arachis oil	0.6050
Polysorbate-80 (Tween 80)	3.7500
Lecithin	0.5525
α- tocopherol	2.0100
Propylene glycol	2.8125
Cyclosporin A	1.0000

Example 7:

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	1.3550
Coconut oil	0.6450
Polyoxyl-40 Hydrogenated Castor oil	3.7500
Lecithin	0.5525
α- tocopherol	0.0100
Propylene glycol	2.6875
Cyclosporin A	1.0000

Example 8

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	0.800
Coconut oil	0.200
Polysorbate-80 (Tween 80)	2.490
Lecithin	0.300
α- tocopherol	0.010
Propylene glycol	5.200
Cyclosporin A	1.000

Example 9

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	1.200
Coconut oil	0.300
Polyoxyl-40 Hydrogenated Castor oil	2.490
Lecithin	0.300
α- tocopherol	0.010
Propylene glycol	4.700
Cyclosporin A	1.000

The oral solution which is filled into bottles can be administered using a syringe or more preferably with the aid of a metered dose pump with a dropper actuator.

The compositions described in Examples 5 to 9 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C), Ref, 40°C-80% RH and 45°C after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Sandimmun Neoral capsules containing 100 mg cyclosporin A per capsule was also examined.

The quantitative determination of cyclosporin A was performed by using HPLC method under the conditions previously noted (Examples 1 to 4).

It was observed from the above examination that the stability of solutions prepared according to the process of invention did not differ from the stability of the commercially available composition.

Example 10

A drink formulation was made by taking an appropriate amount of the preconcentrate of Example 1 (to give the prescribed dose of cyclosporin A) and adding about 50 ml (or a glassful) of orange-flavoured cordial. The mixture was stirred and was then ready for oral consumption.

Claims

1. A pharmaceutical composition in the form of a preconcentrate for mixture with a hydrophilic phase to form a microemulsion, which composition comprises:

   a) a water-insoluble pharmaceutically active material;
   b) C_8 to C_{20} fatty acid mono-, di- or tri-glycerides from a vegetable oil or any mixture of two or more thereof; and
   c) a phospholipid and another surfactant;

   wherein component (a) is directly dissolved in component (b), and component (c) is such that, upon mixing the composition with a hydrophilic phase, a stable oil-in-water microemulsion is formed in which component (a) is in solution in the micro dispersed oil particles, the said preconcentrate being free from a hydrophilic phase.

2. A pharmaceutical composition in the form of a stable oil-in-water microemulsion, which composition comprises

   a) a water-insoluble pharmaceutically active material;
   b) C_8 to C_{20} fatty acid mono-, di-, or tri-glycerides from a vegetable oil, or any mixture of two or more thereof;
   c) a phospholipid and another surfactant; and
   d) a hydrophilic phase;

   wherein component (a) has been wholly directly dissolved in component (b), component (b) is dispersed as tiny particles in component (d), and the composition is free from ethanol.

3. A composition according to claim 1 or 2, wherein component (a) is a cyclosporin, or another water-insoluble peptide, or a water-insoluble antimicrobial or antineoplastic substance.

4. A composition according to claim 3, wherein component (a) is cyclosporin A, dihydrocyclosporin C, cyclosporin D or dihydrocyclosporin D, or desmopresin, calcitonin, insulin, leuprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol or etoposide.

5. A composition according to claim 1,2,3 or 4, wherein in component (b) the glycerides are of C_{12} to C_{18} fatty acids.

6. A composition according to claim 1,2,3,4 or 5, wherein component (b) is whole castor oil, peanut oil or coconut oil, or is derived therefrom.

7. A composition according to any of claims 1 to 6, wherein the phospholipid in component (c) is lecithin, preferably soya lecithin or egg lecithin.

8. A composition according to claim 7, wherein in component (c) the lecithin is hydroxylated lecithin.

9. A composition according to any of claims 1 to 8, wherein in component (c) said surfactant is polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate.

10. A composition according to any of claims 1 to 9, wherein the weight ratio of component (a) to component (b) is from 1:1 to 1:10.

11. A composition according to any of claims 1 to 10, wherein the weight ratio of component (a) to said phospholipid is from 1:0.5 to 1:5.0.

12. A composition according to any of claims 1 to 11, wherein the weight ratio of component (a) to said surfactant is from 1:1 to 1:5.0.

13. A process for making a composition according to claim 2, which comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution with component (d) and component (c) if not included earlier.

14. A process according to claim 13, wherein a preconcentrate composition as claimed in claim 1 is mixed with component (d).

15. A soft gelatin capsule which comprises a composition as claimed in claim 2, or as claimed in any of claims 3 to 12 when dependent on claim 2.

16. An oral administration fluid which comprises a composition as claimed in claim 2, or as claimed in any of claims 3 to 12 when dependent on claim 2.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 30 6022

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	DE-A-32 25 706 (A.NATTERMANN & CIE GMBH) * claims 1-13 * * page 7, line 13 - line 17 * ---	1,3,5,7, 13,14	A61K9/107 A61K38/13
X	WO-A-93 18752 (PHARMOS CORP.) * claims 1-15,22-24 * * page 8, line 10 - page 9, line 35 * * page 12, line 16 - line 26 * ---	2-9,13	
X	EP-A-0 521 799 (YISSUM RESEARCH DEVELOPMENT COMPANY.....) * claims 1-10 * * page 3, line 30 - line 41 * * page 4, line 43 - page 5, line 3 * ---	2,5-8, 13,14	
X	EP-A-0 429 248 (SHISEIDO COMPANY LIMITED) * claims 1-10 * ---	2-9,13, 14	
Y	EP-A-0 651 995 (DR. HANS DIETL) * claims 1-20 * * page 5, line 20 - line 23 * * example 1 * ---	2-11, 13-16	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
Y,D	EP-A-0 327 280 (SANKYO COMPANY LTD) * claims 1-22 * * page 8; examples 1-4 * ---	2-11, 13-16	
A,D	EP-A-0 589 843 (SANDOZ AG) * claims 1-10 * * page 6, line 2 - line 7 * ---	1-16	
A D	FR-A-2 636 534 (SANDOZ S.A.) * claims 1-30 * & GB-A-2 222 770 -----	1-16	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	7 March 1996	Siatou, E

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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E : earlier patent document, but published on, or after the filing date
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(54) Ophthalmic preparations.

(57) An ophthalmic preparation in uni-dose form comprises an aqueous solution of timolol maleate in the absence of any buffering agent or preservative. It may contain sodium chloride to increase the tonicity of the solution or make it isotonic, and may further contain sodium hydroxide to adjust the pH to the range 6.5 to 7.5. Preferably the preparation is supplied in plastics containers formed by the blow-fill-seal process, each container containing 0.1 to 0.3 ml of solution and 0.001 to 5.0 mg of timolol maleate.

EP 0 448 856 A1

The invention relates to ophthalmic preparations suitable for the treatment of glaucoma through lowering intra-ocular pressure in human beings and animals.

Patent Specification GB 1524405 relates to ophthalmic compositions comprising 1-t-butylamino-3-(4-morpholino-1,2,5-thiadiazol-3-yloxy)-2-propanol hydrogen maleate (timolol maleate) together with an ophthalmic carrier in the form of a solid, a vegetable oil, or a buffered isotonic liquid, or in which the carrier is a solid water-soluble polymer. In practice these compositions always contain buffering agents and preservatives. Suitable water-soluble buffering agents are alkali metal and alkaline-earth metal carbonates, phosphates, bicarbonates, citrates and borates, such as sodium phosphate, citrate, borate, acetate, bicarbonate and carbonate. These agents may be present in amounts sufficient to obtain a pH of the system of between 6.5 to 8.0. The suitable water-soluble preservatives that may be included are typically sodium bisulphate, sodium thiosulphate, ascorbate, benzalkonium chloride, chloro-butanol, thimerosal, phenylmercurate acetate, phenylmercuric borate, parabens, benzyl alcohol and phenyl ethanol. These agents may be present in amounts of from 0.001% to as much as 5% by weight.

The compositions are in multi-dose form. All ophthalmic preparations, whether drops, lotions or ointments, are required to be sterile; the purpose of the preservatives, which are typically benzalkonium chloride (0.01%) or phenylmercuric nitrate (0.002%) is to maintain sterility after the sealed lid or sealed screw cap of the multi-dose container has been breached. Contamination of the multi-dose container occurs easily, due both to the entry of atmospheric micro-organisms when the bottle is opened, and more typically due to contamination of the eye-dropper which invariably touches the eye surface or eyelids during application of the drops, such surfaces being typically non-sterile. The contaminated eye-dropper, which is attached to the screw cap, is then re-inserted into the multi-dose container for closure. In an attempt to avoid the growth of these contaminating micro-organisms, a high concentration of preservatives must be used.

The preservatives in use in ophthalmic solutions are powerful disinfectants, typically quaternary ammonium disinfectants such as benzalkonium chloride which may produce hypersensitivity after repeated applications and is also occasionally irritant. Additionally, since tear secretions drain into the nasal cavity, small amounts of the composition are generally absorbed into the systemic circulation from the conjunctival vessels or from the nasal mucosa. Ophthalmic solution entering the nasal cavity may itself then drain into the mouth and will eventually be absorbed through the buccal mucosa or swallowed and absorbed through the gastric mucosa.

Because the treatment of raised intra-ocular pressure needs long-term repeated administration the patient is exposed to the risk of adverse local or systemic effects from the preservatives, a situation which is clearly undesirable.

The invention provides an ophthalmic preparation comprising an aqueous or saline-aqueous solution of timolol maleate in uni-dose form in the absence of any preservative or buffering agent. The preparation may include sodium chloride or another reagent to increase the tonicity of the solution or to make it isotonic.

The method of preparation is simple and uncomplicated, requiring dissolution of timolol maleate and sodium chloride in water for injection or some similar sterile pyrogen-free water, and adjusting where necessary the pH to 7.0 (with limits of 6.5 - 7.5) with sodium hydroxide solution or some similar acceptable reagent, making to volume filtering and filling.

The preparation may be supplied in a plastics container, preferably of polypropylene or polyethylene, for topical application as single dose eye drops. The containers may be formed into a sheet by the blow-fill-seal process using a Rommelag (Trade Mark) or similar machine to make up a pack comprising a number of unit doses. The machine comprises a mould which is fed with thermoplastics material in tubular or granular form. The mould is closed, and a special mandrel is introduced to form a container by blowing. A metered amount of product solution, for example 0.1 to 0.3 ml, preferably 0.2 ml, is forced into the container. The mandrel is retracted, and sealing jaws close the container. A vacuum is formed, so the container is hermetically sealed. The sheet may be marked with dosage, lot number, expiry date and/or other information. A sheet or strip comprising say ten unit doses assists patient compliance.

Since the uni-dose container is filled and hermetically sealed under sterile conditions and remains so until the moment before use, the sterility of the ophthalmic solution when delivered to the patient, is assured. The undesirable preservatives, with the consequent potential risk of systemic or topical adverse effects, can therefore be safely omitted. Similarly, because the pH of the solution is adjusted by an acceptable simple reagent such as sodium hydroxide, the buffering agents may also be omitted with confidence that because the pH is at neutral and therefore physiological levels, no undue stinging, smarting or irritation of the eye will occur.

After instillation into the eye (or eyes) of the prescribed one or two drops the uni-dose container with any remaining unused ophthalmic solution

should be discarded. Typically, only a small amount of ophthalmic solution should be present in each uni-dose container so that any remaining unused portion is not so great as to tempt the patient to retain the remaining unused portion for use at a later date with all the risks of bacterial contamination in the meantime. A unit dose of from 0.001 to 5.0 mg, and preferably from 0.005 to 2.0 mg, and especially from 0.005 to 1.0 mg of timolol maleate in a sterile preservative-free and buffer-free solution is generally applied to the human eye for the treatment of reducing raised intra-ocular pressure.

Example 1

17.10g timolol maleate base and 17.50 g sodium chloride are dissolved in 4800 ml purified water. The pH is adjusted to 7.0 (+0.1) by addition of 0.1 M sodium hydroxide. The final volume is adjusted to 5000 ml by adding purified water at 20°C. The solution is sterilized by filtration, and kept protected from light. The tonicity of the solution is physiological or near-physiological, being approximately isotonic with normal saline, and has an active ingredient content of 2.5 mg per ml, 0.25% w/v. It is fed into polypropylene uni-dose containers with a fill volume of 0.2 ml.

Example 2

34.20 g timolol maleate base and 35.00 g sodium chloride are dissolved in 4800 ml purified water. The pH is adjusted to 7.0 (+0.1) by addition of 0.1 M sodium hydroxide. The final volume is adjusted to 5000 ml by adding purified water at 20°C. The solution is sterilized by filtration, and kept protected from light. The tonicity of the solution is physiological or near-physiological, being approximately isotonic with normal saline, and has an active ingredient content of 5.0 mg per ml, 0.5% w/v. It is fed into polypropylene uni-dose containers with a fill volume of 0.2 ml.

Claims

1. An ophthalmic composition comprising an aqueous solution of timolol maleate characterised in that the composition is in uni-dose form and is free from any buffering agent or preservative.

2. A composition according to claim 1 characterised in that it further includes sodium chloride to increase the tonicity of the solution or to make it isotonic.

3. A composition according to claim 1 or claim 2 characterised in that the pH of the timolol maleate aqueous solution is adjusted to from 6.5 to 7.5 by the addition of sodium hydroxide.

4. A composition according to any preceding claim characterised in that it is supplied in polypropylene or polyethylene uni-dose containers which are formed by the blow-fill-seal process using a Rommelag or like machine filled under sterile conditions and hermetically sealed at the moment of filling.

5. A composition according to claim 4 characterised in that each container contains from 0.1 to 0.3 ml of the solution.

6. A composition according to any preceding claim characterised in that it contains from 0.001 to 5.0 mg of timolol maleate.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 3231

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 014 842 (MERCK & CO.) * Page 9; page 9, lines 1-7 *	1,2,6	A 61 K 31/535 A 61 K 9/06
X	GB-A-1 256 502 (PHARMAX LTD) * Claims 1,11,13 *	4,5	
X	GB-A-1 465 383 (REMEDIA LTD) * Page 1, line 73; page 2, lines 60-61 *	4	
D,A	GB-A-1 524 405 (MERCK & CO.) * Example 1 *	3	
E	GB-A-2 225 237 (CHATFIELD PHARMACEUTICALS LTD) * Page 2, lines 3-5,13; example; claims 1,2 *	1-6	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	09 October 90	VENTURA AMAT A.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same category
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

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(54) Aqueous ophthalmic microemulsions of tepoxalin.

(57) The invention provides an ophthalmic composition suitable for topical application to the eye comprising an oil in water microemulsion wherein the microemulsion contains tepoxalin in an anti-inflammatory effective concentration.

EP 0 480 690 A1

The invention relates to aqueous ophthalmic microemulsions of tepoxalin.

Background Of The Invention

Tepoxalin, whose formal chemical name is 3-[5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-pyrazolyl]-N-hydroxy-N-methylpropanamide, is a nonsteroidal anti-inflammatory drug. It is a potent inhibitor of the cyclooxygenase and lipoxygenase pathways of the arachidonic acid metabolism when administered topically or parenterally. The drug is presently being developed as an ophthalmic topically administered anti-inflammatory composition. Tepoxalin is nearly insoluble in water. Many attempts have been made with various surfactants, cyclodextrins, etc., to enhance its aqueous solubility in order to achieve a homogenous solution rather than a suspension. These attempts have been largely unsuccessful. Tepoxalin, however, is much more soluble in oils than in water and a procedure to disperse the oil in an aqueous phase using surfactants has been discovered and forms the basis of this invention.

A dispersion of oil in water (o/w) can be defined as either a macroemulsion or a microemulsion. A macroemulsion is a cloudy turbid composition with an oil-droplet size of 0.5 to 100 μm. Macroemulsions are usually unstable. A microemulsion is a translucent to transparent composition having a droplet size of 0.005 to 0.5 μm. Microemulsions are usually stable (Stig E. Friberg and Pierre Bothorel, Microemulsions:1 Structure and Dynamics, CRC press, Inc., Boca Raton, Florida, 1987, page 154). The components to generate an emulsion include water, an organic solvent, a surfactant, and possibly a co-surfactant. The o/w system is titrated with the surfactant(s) to a hydrophilic-lipophilic balance (HLB) to obtain a "one-phase transparent o/w dispersion" (H.L. Rosano, J.L. Cavallo and G.B. Lyons, Chapter 16, Microemulsion Systems, vol. 24, ed. H.L. Rosano and M. Clause, Marcel Dekker, Inc., New York and Basel, 1987 page 271). Thermodynamically stable microemulsions can form upon mixing with the proper composition of water, oil, and surfactants. Other emulsions require a high input of energy by sonication, by homogenization, or by shear. (D.M. Lidgate, R.C. Fu and J.S. Fleitman, BioPharm, October, 1988, page 26).

The potential advantages of an emulsion formulation versus aqueous are enhanced solubility for hydrophobic drugs (A. El-Sayed and A. Repta, Int. J. Pharm., 13 (1903) 303), enhanced stability for hydrolytic drugs [P. Grover, Ph.D. Thesis, The University of Connecticut (1984)], and sustained-release characteristics of a drug out of the oil phase [S. Davis, Pharm. Technol., 71 (May 1981); P. Madan, Pharm. Manuf., 51 (June 1985)]. For the above reasons, many investigators have examined the pharmaceutical use of emulsions, as is illustrated by the following citations:

(a) ophthalmic flurbiprofen preparation, Mizushima, Y., Okamoto, H., Sugio, S., Yokoyama, K., Suyama, T., Tohmo, M., Ohumura, M., Konishi, Y., Ichikawa, K. (Kahen Pharmaceutical Co., LTD.), European patent Application, #87304334.3;

(b) dexamethasone acetate, O. Yoichi, S. Takashi, Y. Eiichiro (Shiesido Co., Ltd.) Jpn. Kokai Tokkyo Koho JP 63 10,717 (88 10,717) (CL A61k9/10), 18 Jan 1988, JP Appl. 86/50,219, 07 March 1986; pp. 17;

(c) indomethacin, M. Yu, M. Kawachi, H. Nakajima (Shiesido Co., Ltd.) Jpn. Kokai Tokkyo Koho JP 63,126,542 (88,126,542) (Cl. B01j13/00), 30 May 1988, Appl. 86/273,672, 17 Nov 1986, page 8; and

(d) tolnaftate (transdermal), Y. Ota, E. Yagi, M. Fukuda, T. Suzuki (Shiesido Co., Ltd.) Jpn. Kokai Tokkyo Koho JP 86, 291,518 A2, JP 61,291,518 22 December 86.

The solubility of tepoxalin was tested in several representative oils such as sesame oil, sunflower seed oil, and castor oil. Initial toxicity studies with neat castor oil indicated irritation to the eye (NZW rabbit). This provided an impetus to develop aqueous stable emulsions containing tepoxalin in the hope that they would not be irritating. Acute animal toxicity tests with the microemulsions made in accordance with this invention showed no eye irritation.

Brief Summary of the Invention

The invention provides an ophthalmic composition suitable for topical application to the eye comprising an oil in water microemulsion wherein the microemulsion contains tepoxalin in an anti-inflammatory effective concentration. The tepoxalin is contained in the oil phase, the oil/surfactant/water interface, and the water phase.

Detailed Description of the Invention

The pharmacologically active component of the ophthalmic compositions of the invention is tepoxalin, or 3-[5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-pyrazolyl]-N-hydroxy-N-methylpropanamide. Tepoxalin and its preparation are described in Wachter et al., U.S. Patent No. 4,826,868, the disclosure of which is incorporated herein by reference.

The oils that can be used in the microemulsions of the invention include castor oil (USP grade), sesame oil, sunflower seed oil, mineral oil, and other non-volatile oils. The preferred class of oils that are used in the invention are the fixed oils (i.e., non-volatile oils of vegetable origin). Castor oil is preferred because of tepoxalin's greater solubility therein, and because previous pharmaceutical uses of castor oil have demonstrated its safety and efficacy. In selecting the oil to be used, it is not necessary that the oil per se dissolve tepoxalin, because some oils in which tepoxalin is not soluble to any great degree will form an aqueous microemulsion with a surfactant such that the aqueous emulsion will dissolve sufficient tepoxalin for the microemulsion to be useful. Mineral oil is an illustrative example of such an oil.

The proportions of oil and water employed in the microemulsion are not narrowly critical. The oil is used in an amount sufficient to dissolve enough tepoxalin so that the final concentration of the tepoxalin in the microemulsion will be sufficient to impart anti-inflammatory properties to the unit dosage quantities (e.g., from one to three drops of the microemulsion). This concentration of tepoxalin is usually within the range of from about 0.05 weight per cent to about 1.0 weight percent of the total weight of the aqueous emulsion of the invention. The upper limit of oil concentration is that concentration that will begin to impart irritation properties to the eyes of patients, or that concentration that will be too high to form a microemulsion. Generally, oil/water proportions of from about 1/99 to about 49/51 (v/v) will prove to be useful. In particular cases, optimum proportions of oil and water can be determined by routine experimentation.

The microemulsions of the invention also contain surfactants, additives to impart the proper tonicity to the microemulsion, buffers, preservatives, and other such additives and adjuvants that are known in the art. Examples of surfactants that can be used include sorbitan mono-oleate, NF (Span 80), sorbitan monostearate (Span 60), which are sorbitan fatty acid esters, polyoxyethylene 20 sorbitan monostearate (Tween 60), polyoxyeithylene 20 sorbitan monooleate (Tween 80), which are polyoxyethylene sorbitan esters, Pluronic F127, which is a polyoxyethylene-polyoxypropylene block copolymer, sucrose stearate (Crodesta F-160), which is a sugar/fatty acid ester, and the like. The experimental section below gives illustrations of the types and proportions of surfactants and other additives that can be employed in the microemulsions of the invention.

The microemulsion employed in the invention can be prepared by first dissolving tepoxalin in the oil, mixing the oil/tepoxalin solution with a surfactant to form a homogeneous solution which is then mixed with water to form an oil/water mixture which is then subjected to appropriate energy such as sonication to form the microemulsion. If desired, additional tepoxalin can be added to the oil/water mixture after the microemulsion forming step. The following is a representative preparation procedure of a microemulsion of the invention wherein the energy applied to form the microemulsion is sonic energy:

General Procedure for Preparing a Tepoxalin Microemulsion

The oil such as castor oil is presaturated with tepoxalin (64.06:1 w/w, oil to drug) by stirring the mixture at 50°C until all the material is dissolved (about 16 hours).

The oil and surfactant (e.g., a polyoxyethylene sorbitan fatty acid ester such as Tween 80) are mixed together with a magnetic stir bar in a suitable container such as a plastic (e.g., polymethylpentene) beaker at ambient temperature until a homogenous solution is attained. The oil phase is then mixed with water and stirred until homogenous (cloudy emulsion).

The mixture is sonicated in a suitable container such as a 40 ml clear plastic beaker for 30 minutes with a sonicator such as a "VibraCell" sonicator set at maximum output with a 30% duty cycle (pulse rate); the pulser switch is set to the "on" position (turns the duty cycle on or initiates a discontinuous rate of sonication). The beaker is kept at a constant temperature via a water jacket controlled at a cool temperature such as about 5°C. A low temperature in the jacket is necessary in order to prevent the contents of the beaker from exceeding an optimum temperature needed for formation of the microemulsion (a higher or lower temperature in the beaker will prolong the time to form the microemulsion). The mixture is sonicated for abut 30 minutes or until the mixture becomes translucent, which is the indication that a microemulsion has formed. The temperature of the emulsion is not allowed to rise above a temperature within the range of from about 45°C to about 55°C. Additional tepoxalin (sufficient to bring the concentration of tepoxalin in the microemulsion up to about 0.1%, by weight) is added to the emulsion and the mixture is stirred until all the particles of the drug have dissolved. The emulsion is then filtered through a 0.2 μ membrane filter to remove any residual particles.

Disodium edetate (that is, the disodium salt of ethylenediaminetetraacetic acid) is slowly added to the emulsion while stirring until totally dissolved. Disodium edetate is a metal-chelator used to prevent the degradation of tepoxalin in the formulation. Aqueous BAK (Benzalkonium chloride - 50% w/v), NaCl, and buffer salts are then added to the microemulsion, in the following sequence:

The NaCl is added after BAK to adjust for tonicity in the range of 200 to 330 mOsm. The buffer is prepared in situ by adding and dissolving citric acid first and adjusting the final Ph with Na_2HPO_4 to 4.5 - 7.0. The final

formulation is then filtered through a 0.2 micron membrane filter into sterile containers. The clarity of the microemulsion is determined by percent transmittance at 520 nm (H.L. Rosano, J.L. Cavallo and G.B. Lyons, Chapter 16, Microemulsion Systems, edited by H.L. Rosano and M. Clausse, Marcel Dekker, Inc., new York and Basel, Copyright 1987). The transmittance is preferably greater than 70%.

The Examples set forth below further illustrate the invention. In the Examples, the following materials and equipment were used:

Materials and Equipment:

Drugs, chemicals, and reagents used for the preparation of the microemulsion formulation of tepoxalin are listed below with their sources.

1.	Distilled water	
2.	Tepoxalin	Ortho
3.	Castor oil (Gold Bond Oil, conforms to USP XIX)	CasChem, Inc
4.	Tween 60	Sigma Chemical
5.	Tween 80 (polysorbate 80 USP)	Fisher
6.	Sodium Chloride Crystals AR	Mallinckrodt
7.	Disodium Edetate USP	Ciba Geigy
8.	Benzalkonium chloride 97% USP (BAK)	Henkel
9.	Citric acid USP, powder anhy.	Pfizer
10.	Sodium Phosphate Dibasic USP (anhy.)	Mallinckrodt

The sonicator used in the Examples to form the microemulsions was a Sonics and Materials "VibraCell" model VC300. The sonicator probe is a 0.5 inch high-intensity threaded end type with a titanium tip. The microemulsion has to be kept at a constant temperature during sonication. The circulating system used is a MGW-/LAUDA type RSC 6 (Range -30° to 150°C) cooler/heater set to 5°C.

Modified Ussing type chambers (H.F. Edelhauser, J.R. Hoffert, P.O. Fromm, Invest. Ophthalmol. Vis. Sci., 4:290-296 (1965)) designed with 2.5 ml donor and 2.5 ml receiver cells (separated by the cornea which is mounted between two sealing rings) are used for the studies to determine penetration of the cornea by the microemulsions of the invention. The system is 2-chambered (in vitro cells) (see Figure 1) and is designed to monitor the penetration of drug entities across biological membranes. Usually the cells are temperature controlled by means of an exterior water jacket; the stirring of the dosing and receiving solutions in the cells is achieved by bubbling a gas through the cells. In the case of the cornea, the gas is a mixture of 5% CO_2 balanced with oxygen, to maintain physiological Ph.

Modified Glutathione Bicarbonate Ringer's solution (GBR) (R.D. Schoenwald and H.S. Huang, J.Pharm. Sci. 72(11) (1983) 1266-1271) is freshly prepared for the studies, by mixing equal volumes of two stock solutions which can be stored up to a week in a refrigerator prior to use. After reconstitution the solution should only be used for approximately 6 hours. During use, the pH of the mixture should be maintained at 7.4 by bubbling a 5% CO_2/O_2 mixture through it. The chemicals used for the preparation of GBR are listed below.

Stock Solution I		
NaCl	(14.2 g)	Fisher
KCl	(0.716 g)	Fisher
NaH_2PO_4	(0.50 g)	Fisher
$NaHCO_3$	(4.908 g)	Fisher
water	dilute to 1 liter.	

Stock Solution II		
$CaCl_2 \cdot 2H_2O$	(0.30 g)	Fisher
$MgCl_2 \cdot 6H_2O$	(0.318 g)	Fisher
D(+)-glucose	(1.80 g)	Sigma
Reduced glutathione	(0.184 g)	Sigma
water - dilute to 1 liter.		

The corneas are obtained from adult albino rabbits of both sexes, weighing approximately 3 Kg.

The analysis of tepoxalin for the in vitro corneal penetration studies uses the following equipment:

1. Pump - Model 600E System Controller from Waters Associates;
2. Autosampler - 712 WISP from Waters Associates;
3. Integrator - SP4270 from Spectra Physics;
4. Detector - Perkin Elmer LS-5B Luminescence Spectrophotometer or a Waters 990 Photodiode Array Detector; and
5. Column - μBONDPAK C18 (30 cm L x 3. 9 mm I.D.) from Waters Associates.

The methodology to quantify tepoxalin in GBR was developed using reversed-phase high performance liquid chromatography (HPLC) and fluorescent detection. A GBR sample containing tepoxalin is quantified by adding the internal standard 4,5-diphenylimidazole to the sample, mixing and injecting an aliquot into the HPLC. A ratio of peaks heights of tepoxalin to internal standard is plotted against tepoxalin's standard concentrations to obtain a calibration curve.

The chemicals used are 2-propanol for the mobile phase and 4,5-diphenylimidazole 98% for the internal standard. The internal standard is a reference compound to tepoxalin used in the assay to compensate for any variations that might occur in the measurement of tepoxalin from sample to sample. The mobile phase partitions the analytes in and out of the stationary phase (in the analytical column).

Preparation of a tepoxalin microemulsion by sonication

The castor oil is presaturated with tepoxalin (64.06:1, w/w, oil to drug) by stirring the mixture at 50°C until all the tepoxalin is dissolved (about 16 hours).

The oil and surfactant (e.g., Tween 60) are mixed in a ratio of 1:2 v/v with a magnetic stir bar in a plastic (e.g., polymethylpentene) beaker at ambient temperature until a homogenous solution is attained. The oil phase is then mixed with water and stirred until homogenous (cloudy emulsion).

The mixture is sonicated in a 40 ml clear plastic beaker for 30 minutes with the "VibraCell" sonicator set at maximum output with a 30% duty cycle; the pulser is set to the "on" position. The beaker is kept at a constant temperature via a water jacket controlled at 5°C. The mixture is sonicated for 30 minutes or until the mixture becomes translucent (a microemulsion). The temperature of the emulsion is not allowed to rise above 52°C. Tepoxalin is added to the microemulsion and stirred until all the particles of the drug have dissolved. The emulsion is then filtered through a 0. 2 μ Tuffryn Membrane filter to remove any residual particles.

Disodium Edetate is slowly added to the emulsion while stirring (until totally dissolved). BAK (50% w/w), NaCl and buffer salts are then added, in this sequence. The NaCl is added after the BAK to adjust for tonicity in the range of 200 to 330 mOsm. The buffer is prepared in situ by adding and dissolving citric acid first and adjusting the final pH with Na_2HPO_3 to 4.4 - 7.0. The final formulation is then filtered through 0.2 micron Tuffryn Membrane into sterile containers. The clarity of the microemulsion is determined by percent transmittance at 520 nm (H.L. Rosano, J.L. Cavallo and G.B. Lyons, Chapter 16, Microemulsion Systems, edited by H.L. Rosano and M. Clausse, Marcel Dekker, Inc., New York and Basel, Copyright 1987). The transmittance should be gre-

ater than 70%.

Preparation of Microemulsion Using Tween 60 as Surfactant

Procedure A -- (flexible procedure that allows for changes in pH, tonicity, etc.) -- Castor oil is presaturated with tepoxalin by mixing 100 ml of the oil with 1.5 g of tepoxalin for 16 hours at 50°C. An aliquot of 0.3 ml of the oil and 0.6 ml of Tween 60 are mixed together to a homogenous solution (as described above) and then are mixed with 28.82 ml of water giving a cloudy emulsion.

The emulsion is then sonicated as previously described to a translucent solution. The microemulsion is stirred with 0.03 g of tepoxalin for about 1 hour until there are no particles remaining. The solution is filtered, and 0.009 g of disodium Edetate, 3-6 µl of BAK (50%), 0.20 g of NaCl, 0.001 g of citric acid and 0.005 g of Na_2HPO_4 are added/titrated into the microemulsion to give the tonicity and pH described previously.

Procedure B -- (Less flexible procedure for making adjustments, but is easier to use for the preparation of the microemulsion) -- 0.3 ml of castor oil is mixed with 0.6 ml of Tween 60 and stirred at ambient temperature until homogenous. The temperature of the oil mixture is raised to 50°C and 0.03 g of tepoxalin is added while stirring. The oil continues to be stirred at 50°C until all the tepoxalin is completely dissolved (approximately 1 hour).

The aqueous phase that will be mixed with the oil mixture in order to form the emulsion is made by dissolving 0.2 g NaCl, 0.009 g of disodium Edetate, 0.001 g citric acid, 0.005 g dibasic sodium phosphate, and 12 µl of BAK (50%) in 25 ml water. The solution is stirred until all of the excipients dissolve. The oil mixture is then added to the aqueous phase and mixed well until a cloudy emulsion is formed. The solution is diluted further with 5 ml of water to 30 ml while stirring.

The crude emulsion is sonicated to a microemulsion as described in Procedure A.

The following table displays a summary of solubility studies carried out on tepoxalin in various vehicles:

Table 1 : Solubility of Tepoxalin in Various Oils, Surfactants, HP-β-Cyclodextrin, and Combinations Thereof.

Matrix		Aqueous Concentration (Percent)	Tepoxalin Solubility (Percent)
Water		Neat	0.00044
Oils	sesame	Neat	0.4
	castor	Neat	1.6
	mineral	Neat	0.0
	sunflower Seed	Neat	0.4
	Cottonseed	Neat	0.06
	perfluoro-decalin	Neat	0.0
Surfactants	Pluronic P-105	6% w/w	0.099
	Pluronic F-127	6%	0.035
	Tween 60	6%	0.120
	Tween 80	3%	0.099
	Tween 80	2%	0.089
	Tween 80	1%	0.030
	Tween 40	2%	0.066
	Tween 20	2%	0.053
	Tween 60	2%	0.079
Surfactant and HP-β-CD Solution	HP-β-CD,	5%	
	Tween 80	2%	0.097
	HP-β-CD,	10%	
	Tween 80	1%	0.113
Oil, Surfactant Microemulsion	castor oil,	3%	0.108
	Pluronic P-105	7%	
	castor oil,	1%	
	Tween 80 (tepoxalin is added after microemulsion)	2%	0.085
	castor oil,	2%	
	Tween 80	2%	0.106
	castor oil,	1%	
	Tween 80 (tepoxalin is added before emulsion is prepared)	2%	0.147
	castor oil,	1%	
	Tween 60	2%	0.15

Preparation of a Tepoxalin Microemulsion using Tween 80 as the Surfactant and Span 80 as the Cosurfactant

The procedure to prepare a microemulsion with Tween 80/Span 80 is identical to the Tween 60 emulsion except Span 80 is added just after formation of the translucent solution with Tween 80. An oil/water (o/w) system is titrated with the surfactant and cosurfactant to a hydrophilic-lipophilic balance (HLB) to obtain a "one-phase transparent o/w dispersion" (H.L. Rosano, J.L. Cavallo, and G.B. Lyons, Chapter 16, Microemulsion Systems, vol. 24, editor H.L. Rosano and M. Clause, Marcel Dekker, Inc., New York and Basel, 1987, page 271).

An aliquot of 0.3 ml of castor oil (containing tepoxalin) and 0.55 ml of Tween 80 are premixed to a homogenous solution. The solution is prepared as described previously to a microemulsion containing tepoxalin. An aliquot of 0.05 ml of Span 80 is then added to the microemulsion, and the mixture is sonicated further for 10 minutes. The microemulsion is treated with disodium Edetate, BAK, NaCl, and buffer salts as described above.

In vitro Corneal Transport of Tepoxalin Via a 0.1% Suspension or a 0.1% Microemulsion

The eyes were enucleated with the conjunctival sac and lids attached. The cornea was excised and mounted using the Dikstein and Maurice Technique (S. Dikstein and D.M. Maurice, J. Physiol., 221: 29-41 (1972)).

The chambers were prepared by equilibrating them at 35°C, by means of a water jacket. Both the receiver and donor sides of the chamber were aerated and mixed with an oxygen:carbon dioxide (95:5) mixture; this maintains the pH of the modified glutathione bicarbonate Ringer's solution (GBR) at pH 7.4 and provides mixing of the volumes. The mounted corneas were assembled in the chamber; the receiver side filled with 35°C aerated GBR and the donor side filled with the test compound in GBR.

100 µl Aliquots were sampled from the receiver side over a 4-hour period (0, 15, 30, 45, 60, 90, 120, 180 and 240 minutes). The volume of the chamber was maintained by replenishing with GBR (kept at 35°C during the study), after each sample. The samples were immediately frozen in dry ice, so as to prevent any further metabolism occurring. At the end of the study, the bulk donor and receiver volumes were collected and also frozen. The mounted corneas were removed from the cells and the percentage hydration calculated by weighing the isolated tissue before and after drying (the corneas were placed in an oven overnight at 45°C). This hydration value is indicative of the condition of tissue (preferable range 75 to 83%). Untreated corneas were assessed as controls.

The samples were maintained at -70°C until analysis by HPLC.

Bioanalytical Assay for Tepoxalin in GBR

The assay involves reversed-phase high performance liquid chromatography (HPLC) using fluorescent detection. The pump is set at a flow rate of 1.0 ml/min., the autosampler is set with an injection volume of 10 µl, the integrator is set with an attenuation of 8 millivolts (full-scale) and the fluorometer is set with a fix scale of 30, an excitation wavelength of 282 nm and an emission wavelength at 418 nm. The C18 column is kept at ambient temperature during analysis. The mobile phase is prepared by mixing 400 ml of isopropanol and 2 ml of H_3PO_4 with water to 900 ml. The pH is then adjusted to 3.0 with 1 M NaOH, and the final volume is a dilution with water to 1 liter. The mobile phase is degassed by filtration through a 0.45 µm Nylon-66 filter (from Rainin Instruments Co., Inc.) under vacuum.

Standards for the calibration curve are prepared in balanced salt solution (BSS) at 0.030, 0.060, 0.150, 030 and 0.60 µg/ml. The standards and samples are prepared for HPLC analysis by transferring to autosampler vials (limited volume inserts) 100 µl of the sample and 100 µl of the internal standard 4,5-diphenylimidazole (at 9 µg/ml). The vials are capped and mixed by a Vortex mixer (American Scientific Products). The mixer spins the contents of a vial rapidly or causes a vortex (circular motion).

The chromatography involves µBONDPAK C18 column with 10 µm particles and the mobile phase consists of 40% isopropanol (IPA) and a phosphate buffer adjusted to pH 3.0. A C18 column with a heavier carbon-load or a mobile phase containing a more polar organic solvent (e.g. methanol) will not elute the drug off the C18 column. The drug can be detected by ultra-violet absorbance (254 nm) or by fluorescence (excitation wavelength at 282 nm and an emission at 518 nm). Fluorescent detection is about 10 times more sensitive than ultra-violet.

A GBR sample is prepared for HPLC analysis by transferring an aliquot to an autosampler vial, adding the internal standard 4,5-diphenylimidazole, capping and mixing the vial. A flow rate of 1.0 ml/min will elute the internal standard in about 4.0 minutes and the drug in about 11.6 minutes. A calibration curve with standards between 0.030 and 0.60 µg/ml will give a linear curve using fluorescence detection with a correlation coefficient of r=0.9996 (r^2=0.9997), a Y-intercept = -0.0059 and a slope = 1.240. Minimum sensitivity is about 0.025 µg/ml.

Solubility Experiments with Tepoxalin

The low aqueous solubility of tepoxalin prompted solubility experiments with oils, surfactants, hydroxypropyl-beta-cyclodextrin, and combinations thereof. These experiments are summarized above in Table 1. A tepoxalin content of greater than 0.1% could be attained with a microemulsion using 1% castor oil and 2% of a Tween (polysorbate). An oil:surfactant ratio of 1:1 does not form a translucent emulsion (cloudy). The sequence of adding, mixing, and sonicating the drug and excipients is important in attaining a concentration greater than 0.1%.

Two representative microemulsions containing 0.1% tepoxalin have been prepared and tested for physical stability, drug stability, acute toxicity, and/or efficacy. The composition of the two emulsions are listed below:

With Tween 60		
tepoxalin	0.030 g	0.1%
castor oil	0.300 ml	1.0%
Tween 60	0.600 ml	2.0%
NaCl	0.200 g	0.67%
disodium Edetate	0.009 g	0.03%
BAK (50% w/v)	0.003 ml	0.01%
citric acid	0.001 g	0.003%
Na_2HPO_4	0.005 g	0.0167%

The above is diluted to 30 ml with purified water.

With Tween 80/Span 80		
tepoxalin	0.03 g	0.1%
castor oil	0.300 ml	1.0%
Tween 80	0.55 ml	1.833%
Span 80	0.05 ml	0.166%

The remaining excipients and diluent are as listed above.

Acute Multiple-Dose Toxicity Studies in Rabbits

(Ocular Irritancy Testing)

The acute irritancy of the Tween 80/Span 80 and the Tween 60 microemulsions were evaluated by subjecting the rabbits to multiple doses (1 drop/20 minutes for 6 hours/day) of the two formulations and other appropriate controls. The irritancy of the formulation was determined by the number of rabbits (3 rabbits/formulation) that passed a grading system (e.g. redness, swelling, tearing, etc.) for one day, two days, or three days. The degree of irritancy was scored between 1 and 10: a score of 1 was least irritating and a score of 10 was the most irritating.

Neat castor oil gave a score of 10. All other formulations and the control 10% Pluronic F127 (poloxamer 407, excipient in Vasocidin® at 0.1%) gave a score of 1 (see Table 2, below).

TABLE 2 : Summary of Multidose Irritancy Studies in Rabbits. There are three rabbits/formulation that can pass the grading system for the first day, the second day, and the third day.

	Day (1)	Day (2)	Day (3)	Score
castor oil, USP	0	0	0	10
10% Tween 80	3	3	3	1
10% Pluronic F127	3	3	3	1
0.1% tepoxalin (Tween 80 Micro-emulsion)	3	3	3	1
0.1% tepoxalin (Tween 80 Micro-emulsion)	2	2	2	(?)*1
Microemulsion w/o drug, (Tween 80) (no BAK)	3	3	3	1

(*Animal removed from test; broke its back in retainer)

	Day (1)	Day (2)	Day (3)	Score
0.1% Microemulsion (Tween 80/Span 80)	3	3	3	1
Tween 80/Span 80 Microemulsion (w/o drug)	3	3	3	1
0.1% Microemulsion (Tween 80)	3	3	3	1
10% Solution of Pluronic F127 (No BAK in formulations)	3	3	3	1

	Day (1)	Day (2)	Day (3)	Score
10% Pluronic F127	3	3	3	1
0.1% tepoxalin μ emulsion (Tween 60, BAK & EDTA)	3	3	3	1
Placebo μ emulsion (Tween 60, BAK & EDTA)	3	3	3	1
0.1% tepoxalin μ emulsion (Tween 80, Span 80, BAK & EDTA)	3	3	3	1
0.1% tepoxalin μ emulsion (Tween 80, Span 80)	3	3	3	1
0.1% tepoxalin μ emulsion (Tween 60)	3	3	3	1

In Vitro Cytotoxicity Testing of Tepoxalin Microemulsions

Four microemulsion formulations containing tepoxalin and three controls were tested for cytotoxicity to 3T3-L1 cells (embryo, mouse). A dose-response curve was constructed to determine the ID20, ID50 and ID80 of each formulation that "reduced the final total cellular protein by 20, 50 and 80% in comparison with that of the control wells" (from a report issued to D.G. Musson on March 23, 1990 from Jane Greeves, Sandra Reading and Clive Wilson, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH). The data showed (a) enhanced toxicity with formulations containing BAK, (b) all the formulations were more toxic than the control Pluronic F127, (c) the microemulsion with Tween 80/Span 80 appears slightly less toxic than Tween 60, and (d) emulsions with tepoxalin are more toxic than those without.

Stability Studies of Tepoxalin and the Microemulsion:

Two stability studies have been performed with the second still on-going. The first study involved three formulations:

(1) 0.1% tepoxalin/Tween 60/castor oil/BAK/NaCl

(2) 0.1% tepoxalin/Tween 60/Span 60/castor oil/BAK/NaCl

(3) 0.1% tepoxalin/Tween 80/Span 80/castor oil/BAK/NaCl

The study demonstrated several problems with the formulations and differences between the formulations. Emulsion stability and drug stability seemed to favor the Tween 60 surfactant without a co-surfactant. The rate of degradation of tepoxalin at 45°C seemed to be much greater than at room temperature and 35°C. Similarly, the cracking of the emulsion seemed to occur much faster at 45°C over room temperature and 35°C.

The results of the first stability study prompted an investigation for a way to reformulate. Tepoxalin appears to hydrolyze at the amide group to form a carboxylic acid. An accelerated procedure has been developed to observe this degradation within 16 hours. The sample is analyzed by reversed-phase HPLC using a diode array for detection. A series of experiments (following section) suggested the use of 0.03% disodium Edetate in the formulation. With disodium Edetate in the microemulsion, the drug degraded at and above pH 7.0; degradation was minimal at pH's 5.0, 5.5 and 6.0. Thus, a second stability study was begun with 0.03% disodium Edetate in the formulation and the pH adjusted to 5.9 - 6.1 with a citric acid/sodium phosphate dibasic buffer.

The second stability study employed two formulations:

(4) 0.1% tepoxalin/Tween 60/castor oil/BAK/NaCl/disodium Edetate/citric acid/Na_2HPO_4/water

(5) 0.1% tepoxalin/Tween 80/Span 80/castor oil/BAK/NaCl/disodium Edetate/citric acid/Na_2HPO_4/water.

There is three months of data. The drug stability data is acceptable at room temperature and 35°C for formulation (4). The latter formulation (5) with Tween80/Span80 resulted in tepoxalin concentrations below the acceptable 90% level at 12 weeks at 35°C; the Tepoxalin concentration at 12 weeks at room temperature was 94.4%. The pH's for both formulations have dropped from 6.0 to 4.85 - 5.75, depending on the temperature. The percent transmittance appear to have dropped slightly at room temperature and 35°C for both formulations; at 45°C, the drop has been significant.

Experiments to Enhance Stability of Tepoxalin Microemulsion

A number of experiments were performed in succession in order to elucidate a mechanism for the degradation of tepoxalin in water and in the microemulsion and to determine optimum conditions for the tepoxalin microemulsion. The drug is suspected of vulnerability to acid and base catalysis of its N,N-hydroxymethyl amide group forming a carboxylic acid. The experiments and results:

(a) 0.1% tepoxalin was dissolved in 50:50 water: ethanol and aliquots were pH adjusted with 1M NaOH to 5.0, 6.0, 7.0, 8.0 and 12.6. The aliquots were heated in capped reaction vials for 16 hours at 100°C.

The HPLC chromatograms show a degradation peak eluting after tepoxalin in samples at pH 8, 7, 8, 12.6 but not at pH 5.0.

(b) 0.1% tepoxalin was prepared with castor oil, Tween 60 and a citrate/phosphate buffer (1 M) in the form of a microemulsion. Two drops of 1M citric acid solution were added to an aliquot (1 ml) and the pH was adjusted with 1.0 M sodium phosphate dibasic to pH 5.0, 6.0, 7.0, and 7.6. The samples were heated as before.

The chromatograms show two major degradation products: one eluting before tepoxalin and another after. The degradation occurs greatest at pH 5.0 and 7.6.

(c) The previous experiment (b) was repeated narrowing the pH range between 6.0 and 7.0 (6.0, 6.2, 6.4, 6.6, 6.8, and 7.0).

The optimum pH appears between pH 6.2 and 6.4, but there is still significant degradation.

(d) The previous experiment (c) was repeated with disodium Edetate 0.03% in each sample and with the same pH range.

Degradation is minimal at pH 6.0 and increases with pH to 7.0. The presence of the second degradate that elutes before tepoxalin is also minimal.

(e) Experiment (d) was repeated with a broader pH range: 5.0, 5.5, 6.0, and 7.0.

Degradation was minimal at pH 5.0, 5.5, and 6.0. Peak areas and heights for tepoxalin at these pH's were:

pH	Peak Areas	Peak Heights
5.0	0.50144	0.7807
5.5	0.55158	0.8312
6.0	0.56796	0.8390
7.0	0.31217	0.5556

and reflect optimum stability between 5.5 and 6.0. The degradation product was not clearly evident except at pH 7.0.

In Vitro Corneal Penetration Studies:

The penetration and metabolism of tepoxalin across the rabbit cornea as a suspension at 0.1% and as a microemulsion at 0.1% were compared by an in vitro procedure using the modified Ussing Chambers (H.F. Edelhauser, J.R. Hoffert, P.O. Fromm, Invest. Ophthalmol. Vis. Sci., 4 (1965) 290-296). The samples were removed at appropriate time intervals from 0 up to 240 minutes and frozen at -70°C until analysis by HPLC-fluorescence.

A plot of the amount of tepoxalin crossing the cornea against time showed the drug penetrating the cornea from both formulations. The chromatograms of the samples taken from the receiver cells also show other metabolites/-degradates present via both formulations. The metabolites/-degradates could be chemical and enzymatic hydrolysis of the N,N-hydroxymethylamide group to a carboxylic acid. The tepoxalin fluxes for both formulations are listed below:

	Flux ($\mu g/cm^2$-min)
Suspension	0.00384
Microemulsion	0.00959

The flux was calculated using the following equation (C. Fleeker, O. Wong, and J.A. Rytting) Pharm. Res., 6 (6) (1989) 443-448:

Flux = $(\Delta q/\Delta t)$ (1/A), where

$(\Delta q/\Delta t)$ = slope, and

A = corneal surface exposed to the drug, 1.039 cm^2.

For the Suspension, the slope was determined between 45 and 180 minutes; for the microemulsion, between 60 and 240 minutes.

Efficacy Studies in Cats:

Eight cats with heavily pigmented irides are pretreated with the 0.1% tepoxalin microemulsion containing the surfactants Tween 80/Span 80 in the right eye and control saline in the left eye. The cats are anesthetized fifteen minutes before completion of the dosage regimen and both irides are subjected to an argon laser. The effects of the drug and control solutions on the irides are observed by slit-lamp. The animal model is most informative for drug effect between 4 and 8 hours.

For iris hyperemia, there was a significant statistical effect in the treated eyes over controls at 4, 6, and 8 hours, there was no difference in the treated eyes and control eyes at 24 hours.

For iris edema, there was a significant statistical effect in the treated eyes at 6 hours.

An ophthalmic microemulsion formulation with tepoxalin would contain (concentrations are w/w):

(1) an oil immiscible with water at 0.1 to 2%;
(2) a non-ionic surfactant at 0.1 to 4%;
(3) a non-ionic co-surfactant at 0.0 to 4%;
(4) tepoxalin at 0.05 to 1%;
(5) tonicity agent at 0.25 to 2%;
(6) a buffering system to adjust the pH between 4.0 and 7.0 (above pH 7.0, the drug degrades). The concentration should vary from 0 to 3.0 millimolar;
(7) one or more preservatives at 0.02 to 0.7%; and
(8) a stabilizer for the microemulsion and/or the drug at 0.0 to 1.0%.

The above materials are mixed in such a manner by sonication, by homogenization, or by a technique using a microfluidizer, to obtain a translucent to transparent microemulsion (transmittance at 520 nm is >=70%).

Two illustrative formulations are the following:

Formulation A

1.	tepoxalin	0.1%
2.	castor oil	1.0
3.	Tween 60	2.0
4.	NaCl	0.67
5.	disodium edetate	0.03
6.	BAK	0.03
7.	citric acid	0.003
8.	Na_2HPO_4 1.18 millimolar,	0.0167
9.	water	diluted to 1 liter;

Formulation B

1.	tepoxalin	0.1%
2.	castor oil	1.0
3.	Tween 80	1.83
4.	Span 80	0.166

The remaining excipients and diluent are as listed above for Formulation A.

One or both formulations have been tested for drug stability, emulsion stability, toxicity (irritation), in vitro corneal penetration, and efficacy.

The materials used for the above two illustrative emulsions can be replaced with the following substitute materials, which are listed for illustrative purposes:

OILS

sesame oil
castor oil
mineral oil
sunflower seed oil
perfluoro-decalin
almond oil NF
apricot kernel oil
avocado oil
coconut oil
cross-essential EPO
menhaden oil
mink oil

olive oil
orange roughy oil
safflower oil USP
wheat germ oil

SURFACTANTS and CO-SURFACTANTS

polysorbates (polyoxyethylene sorbitan fatty acid esters:
Tweens 20, 21, 40, 60, 61, 65, 80, 81, 85);
sorbitan esters (sorbitan fatty acid esters: Span 20, 40, 60, 65, 80, 85);
Pluronics (P84, P105, F127);
Tetronics;
Crodestras (Combination of sucrose stearate and sucrose distearate, sucrose stearate);
lecithin,

TONICITY AGENTS

sodium chloride,
potassium chloride,
dextrose, glycerin, mannitol

BUFFERS

acetic acid,
boric acid,
hydrochloric acid,
citric acid,
phosphoric acid,
potassium carbonate,
potassium citrate,
potassium phosphates,
sodium acetate,
sodium bicarbonate,
sodium biphosphate,
sodium borate,
sodium carbonate,
sodium citrate,
sodium hydroxide,
sodium phosphate.

PRESERVATIVES

benzalkonium chloride,
benzethonium chloride,
chlorobutanol,
phenylmercuric acetate,
phenylmercuric nitrate,
thimerosal,
methylparaben,
propylparaben,
sodium benzoate,
sorbic acid,
phenylethyl alcohol,
boric acid,
gentamicin sulfate,
Bacitracin,
polymixin B sulfate,
Neomycin,

tetracycline hydrochloride,
erythromycin,
sulfacetamide sodium,
tobramycin,
Prednisolone acetate,
Prednisolone,
Prednisolone phosphate,
dexamethasone,
and combinations of the above.

STABILIZERS

disodium edetate (metal-chelating agent),
citric acid (reducing agent),
sodium metabisulfite (reducing agent),
ascorbic acid (reducing agent),
acetyl cysteine (reducing agent),
butylated hydroxyanisole (radical scavenger),
2,6-di-tert-butyl-p-cresol (radical scavenger),
vitamin E (radical scavenger).

Solubility experiments with formulations A and B suggest that much of the drug is located in the interface between the oil droplet, surfactant(s), and water. Maximum tepoxalin solubility for castor oil is 1.6%, and 1% of the oil is dispersed in the emulsion. Thus, 0.134% of the 0.15% drug in the emulsion resides in the interface.

The first stability study involved three formulations that contained different surfactants, were not buffered, and had no stabilizers. At 45°C versus room temperature and 35°C, both the concentrations of tepoxalin and the transmittances of the emulsions declined dramatically. All of the formulations tested for accelerated tepoxalin stability failed within two weeks at 45°C (below 90% of initial concentrations) and all failed within 8 weeks at 35°C. A method, therefore, was developed to investigate the degradation of tepoxalin in order to reformulate a stable product.

Initial stability experiments seemed to indicate that the emulsion system is accelerating the degradation of tepoxalin, forming two major by-products across the pH range of 6 to 7.6; minimum degradation occurs at 6.2 - 6.4. Degradation of tepoxalin in water/alcohol gave one major by-product at pH 6.0 or higher. Edetate in the emulsion stabilized tepoxalin in the pH range of 5.0 - 6.0 and only one major by-product was observed.

A second stability study is being conducted with two formulations, both containing edetate and a buffer, pH 5.9 - 6.1. As before, tepoxalin is degrading significantly faster at 45°C than at room temperature and 35°C. After three months of data collection, the percent remaining of tepoxalin is significantly higher compared to the previous study. The concentration change for tepoxalin at 35°C is acceptable at 90.3% using Tween 60 and at room temperature, at 98.3%; percent remaining at 45°C is 70.7%. Shelf-life for the two formulations will be determined at temperatures lower than 45°C.

The stability of tepoxalin and the physical stability of the emulsion appear to be related and dependent on temperature. The data from both stability studies and the experimental investigations indicate that at higher temperatures the emulsions are cracking faster and the drug is degrading. The formulations with Tween 60 as compared to formulations with Tween 80 in both studies are more stable in both aspects. In addition, reformulation with edetate and buffer does stabilize both the drug and the emulsion.

The acute multiple-dose toxicity tests basically indicate that the formulations containing Tween 60 or Tween 80/Span 80 are non-irritating. The in vitro cytotoxicity tests are more discriminating, showing that the microemulsions with tepoxalin and/or BAK are more cyto-toxic (cellular death) than without. The difference between the formulations containing Tween 60 and Tween 80/Span 80 is not significant considering the standard deviations.

The efficacy studies basically show that the 0.1% tepoxalin emulsion is effective for the reduction in iris swelling at 6 hours and in hyperemia at 4, 6, and 8 hours in eyes traumatized by an argon laser.

Claims

1. An ophthalmic composition suitable for topical application to the eye comprising an oil in water microemulsion containing tepoxalin in an anti-inflammatory effective concentration.

2. The ophthalmic composition of Claim 1 wherein the oil is a fixed oil.

3. The ophthalmic composition of Claim 1 wherein the microemulsion additionally contains a non-ionic surfactant in an amount effective to stabilize the microemulsion.

4. The ophthalmic composition of Claim 1 wherein the microemulsion additionally contains tonicity agents, buffers, and stabilizers.

5. The ophthalmic composition of Claim 2 wherein the oil is castor oil.

6. The ophthalmic composition of Claim 3 wherein the non-ionic surfactant is a polysorbate.

7. The ophthalmic composition of Claim 4 wherein the microemulsion contains sodium chloride in an amount sufficient to adjust tonicity between 200 and 330 mOsm.

8. The ophthalmic composition of Claim 4 wherein the microemulsion contains disodium edetate in an amount sufficient to enhance the stability of the tepoxalin contained in said composition.

9. The ophthalmic composition of Claim 5 wherein the microemulsion contains a phosphate buffer to adjust the pH between about 4.0 and 7.0.

Claims for the following Contracting State : ES

1. A method for preparing an ophthalmic composition suitable for topical application to the eye, which method comprises the step of forming an oil in water microemulsion containing tepoxalin in an anti-inflammatory effective concentration.

2. A method according to claim 1 wherein the oil is a fixed oil.

3. A method according to claim 1 or 2 wherein the microemulsion additionally contains a non-ionic surfactant in an amount effective to stabilize the microemulsion.

4. A method according to claim 1, 2 or 3 wherein the microemulsion additionally contains tonicity agents, buffers, and stabilizers.

5. A method according to any preceding claim wherein the oil is castor oil.

6. A method according to claim 3 wherein the non-ionic surfactant is a polysorbate.

7. A method according to any preceding claim wherein the microemulsion contains sodium chloride in an amount sufficient to adjust tonicity between 200 and 330 mOsm.

8. A method according to any preceding claim wherein the microemulsion contains disodium edetate in an amount sufficient to enhance the stability of the tepoxalin contained in said composition.

9. A method according to any preceding claim wherein the microemulsion contains a phosphate buffer to adjust the pH between about 4.0 and 7.0.

Corneal Penetration Apparatus

(Modified Ussing Chamber)

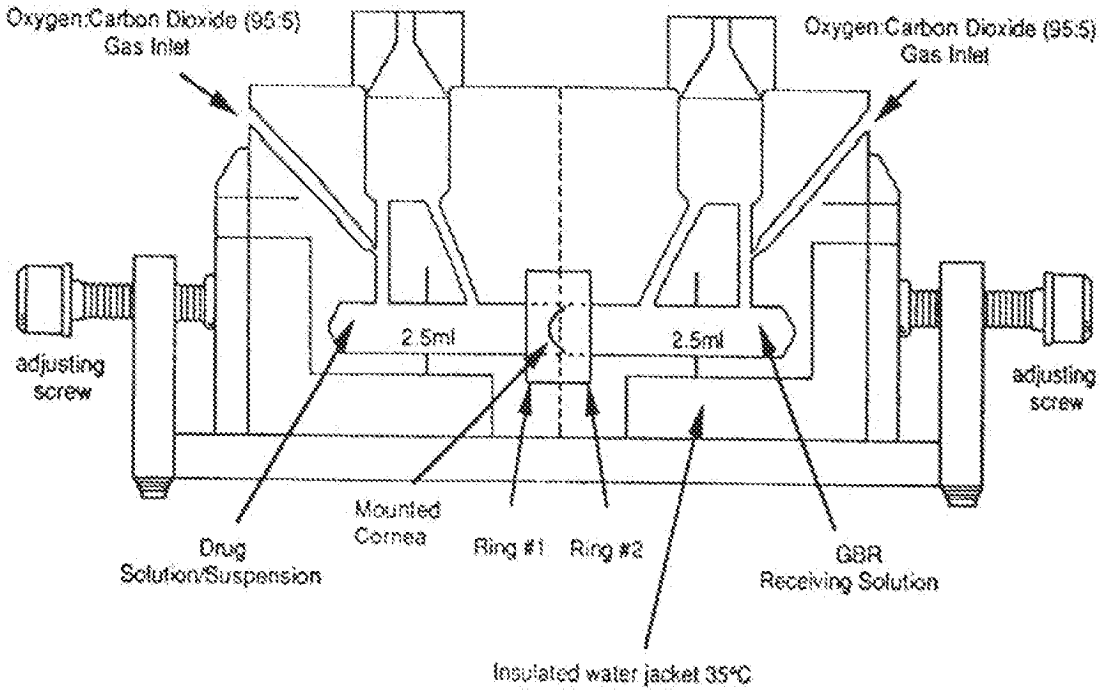

Figure 1.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 30 9243

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,A	EP-A-0 248 594 (ORTHO PHARM. CORP.) * Claims 1,10-11; page 6, lines 55-57; page 7, lines 20-25,33-37,50-52 *	1-4	A 61 K 9/107 A 61 K 9/06 A 61 K 31/415
D,A	EP-A-0 253 472 (GREEN CROSS) * Claims 1,3-8,11,15; page 4, lines 40-56; page 5, lines 1-14 *	1-4	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	25-11-1991	SCARPONI U.

EPO FORM 1503 03.82 (P0401)

CATEGORY OF CITED DOCUMENTS

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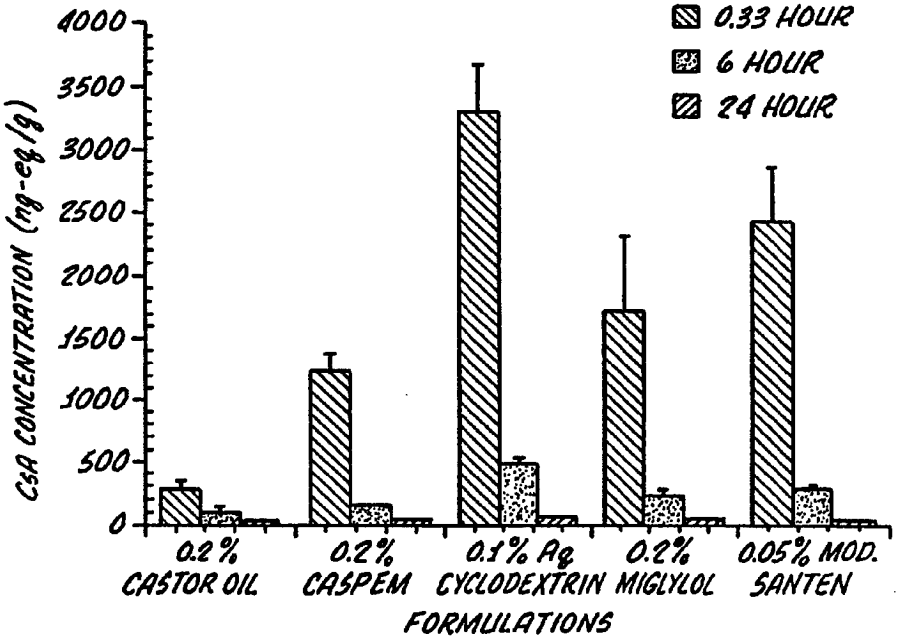

(57) Abstract

A pharmaceutical composition is disclosed in the form of a nonirrtating emulsion which includes at least one cyclosporin in admixture with a higher fatty acid glyceride and polysorbate 80. More particularly, the cyclosporin may be cyclosporing A and the higher fatty acid glyceride may be castor oil. Composition has been found to be of a high comfort level and low irritation potential suitable for delivery of medications to sensitive areas such as ocular tissues with enhanced absorption in the lacrimal gland. In addition, the composition has stability for up to nine months without crystallization of cyclosporin.

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LACRIMAL GLAND SPECIFIC EMULSIONS FOR TOPICAL APPLICATION TO OCULAR TISSUE

This application is a continuation-in-part of pending US patent application SN 08/243,279 filed May 17, 1994.

The present invention generally relates to novel pharmaceutical compositions incorporating chemicals which are poorly soluble in water and is more particularly related to a novel ophthalmic emulsion including cyclosporin in admixture with castor oil and polysorbate 80 with high comfort level and low irritation potential.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. In addition, as set forth in U.S. Patent No. 4,839,342, cyclosporin (sometimes referred to in the literature as "cyclosporine") has been found as effective in treating immune medicated keratoconjunctivitis sicca (KCS or dry eye disease) in a patient suffering therefrom.

As hereinabove noted, cyclosporin comprises a group of cyclic oligopeptides and the major component thereof is cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$)which has been identified along with several other minor metabolites, cyclosporin B through I. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different

substituents or configurations of some of the amino acids.

It should be appreciated that reference to the term "cyclosporin" or "cyclosporins" is used throughout the present specification in order to designate the cyclosporin component in the composition of the present invention.

However, this specific reference is intended to include any individual member of the cyclosporin group as well as admixtures of two or more individual cyclosporins, whether natural or synthetic.

The activity of cyclosporins, as hereinabove noted, is as an immunosuppressant and in the enhancement or restoring of lacrimal gland tearing.

This activity can be enhanced if it is possible to enhance the absorption of the cyclosporin in the lacrimal gland. The present invention provides for a formulation and method that produces optimal cyclosporin A concentrations in the lacrimal gland and other ocular surface tissues.

Unfortunately, the solubility of cyclosporin in water is extremely low and as elaborated in U.S. Patent No. 5,051,402, it has been considered not merely difficult but practically impossible to prepare a pharmaceutical composition containing cyclosporin dissolved in an aqueous medium.

As reported, the solubility of cyclosporin in water is between about 20 μg/ml to 30 μg/ml for cyclosporin A. Hence, heretofore prepared formulations incorporating cyclosporin have been prepared as oily solutions containing ethanol. However, these

preparations limit the bioavailability to oral preparations and this is believed to be due to the separation of cyclosporin as a solid immediately after it comes into contact with water, such as in the mouth or eye of a patient.

In the case of injectable preparations of cyclosporin, they first must be diluted with physiological saline before intravenous administration but this is likely to result in the precipitation of cyclosporin and therefore may be considered undesirable for intravenous administration.

Surface active agents such as polyoxyethylated castor oil have been utilized as solubilizers to inject preparations in order to prevent cyclosporin from separating. However, this also may give rise to safety problems (see U.S. Patent No. 5,051,402).

The practical usefulness of cyclosporin would be greatly enhanced if administration thereof could be effective; for example, cyclosporin's effectiveness in the treatment of ocular symptoms of Behcet's Syndrome. However, if it is administered orally for the treatment of these symptoms, the accompanying side effects due to systemic circulation may cause adverse reactions such as hypertrichosis or renal dysfunction.

On the other hand, if oily preparations containing cyclosporin are applied directly to the eyes, irritation or a clouding of visual field may result. This plus the difficulty in formulating cyclosporin limits its use in formulations that would be useful during keratoplasty as well in the treatment of herpetic keratitis and spring catarrh.

Heretofore, as for example in U.S. Patent No. 5,051,402, attempts have been made to dissolve sufficient cyclosporin in an aqueous solvent system so as to reach an effective concentration for treatment. Importantly, this solvent system does not contain any surface active agent such as polyoxyethylated castor oil.

Conceptually, the purpose of dissolving the cyclosporin in an aqueous solvent system is to enable contact with body fluids which would merely constitute dilution of the aqueous solvent system which hopefully would eliminate the immediate precipitation of cyclosporin when contacted with the water content of the body fluids.

For direct use in the eye, cyclosporin has been formulated with a number of pharmaceutically acceptable excipients, for example, animal oil, vegetable oil, an appropriate organic or aqueous solvent, an artificial tear solution, a natural or synthetic polymer or an appropriate membrane.

Specific examples of these pharmaceutically acceptable excipients, which may be used solely or in combination, are olive oil, arachis oil, castor oil, mineral oil, petroleum jelly, dimethyl sulfoxide, chremophor, liposomes, or liposome-like products or a silicone fluid, among others.

In summary, a great deal of effort has been expended in order to prepare a pharmaceutical composition containing cyclosporin dissolved in an aqueous medium or cyclosporin prepared as an oily solution. However, successful formulations have yet to be accomplished as evidenced by the lack of commercial products.

As hereinabove noted, it has been reported that cyclosporin has demonstrated some solubility in oily preparations containing higher fatty acid glycerides such as olive oil, peanut oil, and/or castor oil. These formulations frequently produce an unpleasant sensation when applied to the eye because of stimulation or the viscousness which is characteristic of these oils.

Another drawback of these formulations is that they contain a high concentration of oils, and oils exacerbate the symptoms of certain ocular surface diseases such as dry eyes, indicated by cyclosporin. Therefore, these oily formulations may not be clinically acceptable. Additionally, these formulations often suffer from physical instability due to cyclosporin's propensity to undergo conformational change and crystallize out. The crystallization problem has been noticed in formulations containing corn oil or medium chain triglycerides. Lastly, these formulations often have a low thermodynamic activity (degree of saturation) of cyclosporin which leads to a poorer drug bioavailability.

It may be possible to minimize the problems related to unpleasant sensation and syndrome exacerbation by reducing the oil content and dispersing the oil phase in water into an emulsion. However, it is not an easy task to formulate an ophthalmic emulsion because one indispensable class of ingredients in an emulsion system is emulsifiers, and the majority of emulsifiers is highly irritating to the eyes.

The present invention is directed to an emulsion system which utilizes higher fatty acid glycerides but in combination with polysorbate 80 which results in an emulsion with a high comfort level and low irritation

potential suitable for delivery of medications to sensitive areas such as ocular tissues. Further, the present invention provides a pharmaceutical composition and method for causing preferential absorption of cyclosporin in the lacrimal gland. That is, for a given instillation of the composition into an eye, a greater amount of absorption occurs in the lacrimal gland for formulations made in accordance with the present invention than heretofore utilized formulations.

SUMMARY OF THE INVENTION

In accordance with the present invention, a non-irritating pharmaceutical composition with high comfort level and low irritation potential suitable for delivery to sensitive areas such as ocular tissues comprises cyclosporin in admixture with an emulsifying amount of a higher fatty acid glycerol and polysorbate 80. More particularly, the composition may comprise cyclosporin A and the higher fatty acid glyceride may comprise castor oil.

Preferably, the weight ratio of the castor oil to the polysorbate 80 is between about 0.3 to about 30 and a weight ratio of the cyclosporin to castor oil is below 0.16. More preferably, the weight ratio of castor oil to polysorbate 80 is between 0.5 and 12.5, and the weight ratio of cyclosporin to castor oil is between 0.12 and 0.02.

When cyclosporin is dissolved in the oil phase in accordance with the present invention, the emulsion is found to be physically stable upon long term storage. No crystallization of cyclosporin was noticed after nine months at room temperature. Moreover, the cyclosporin emulsion is formulated in such a way that

the drug has reasonably high thermodynamic activity, yet without the crystallization problem.

Importantly, the composition of the present invention provides for enhanced absorption of the cyclosporin in the lacrimal gland of the eye. In this manner, the activity of the cyclosporin in restoring lacrimal gland tearing is increased. That is, since a greater amount of cyclosporin is absorbed into the lacrimal gland, more of the cyclosporin is effective in producing lacrimal gland tearing than heretofore possible.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and features of the present invention will be better understood by the following description when considered in conjunction with the accompanying drawings in which:

Figure 1 is a bar chart of conjunctival concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye;

Figure 2 is a bar chart of cornea concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye;

Figure 3 is a bar chart of ciliary body concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye; and

Figure 4 is a bar chart of lacrimal gland concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye.

DETAILED DESCRIPTION

As hereinabove noted, cyclosporin is available as a mixture in which the principal ingredient is cyclo-

sporin A with significant, but smaller, quantities of other cyclosporins such as cyclosporin B through I. However, as also hereinabove noted, the present invention may be applied to either a pure cyclosporin or to a mixture of individual cyclosporins.

The discovery on which the present invention is founded relates to a combination of a higher fatty acid glyceride and an emulsifier and dispersing agent, polysorbate 80. The selection of these components could not have been anticipated on the basis of conventional thinking.

For example, although it is well known that cyclosporin may be used in combination with castor oil, this combination is irritating to sensitive tissues such as the eye. Thus, conventional teaching in the art is away from a formulation which utilizes a higher fatty acid glyceride, such as castor oil, and cyclosporin.

Stated another way, there is no way of deducing that the use of an emulsifier and dispersing agent such as polysorbate 80 will reduce the irritation potential of an emulsion utilizing castor oil. There are no examples of polysorbate in combination with castor oil which, when admixed to cyclosporin, produces an emulsion with a high comfort level and low irritation potential suitable for the delivery of medication to sensitive areas such as ocular tissues.

The present invention achieves a stable solution state of cyclosporin. This stable solution state is another important performance characteristic differentiating the present invention from the conventional oil systems. Cyclosporin is notorious for its ten-

dency to precipitate out in conventional oil systems in which it is fully dissolved initially.

In accordance with the present invention, the emulsions can be further stabilized using a polyelectrolyte, or polyelectrolytes if more than one, from the family of cross-linked polyacrylates, such as carbomers and Pemulen®.

Pemulen® is a polymeric emulsifier having a CTFA name of Acrylates/C10-30 Alkyl Acrylate Cross-Polymer and is discrived in th "Carbomer 1342" monograph in the USPXXII/NFXVII.

In addition, the tonicity of the emulsions can be further adjusted using glycerine, mannitol, or sorbitol if desired. The Ph of the emulsions can be adjusted in a conventional manner using sodium hydroxide to a near physiological pH level and while buffering agents are not required, suitable buffers may include phosphates, citrates, acetates and borates.

While the preferable medications in accordance with the present invention include cyclosporin, other chemicals which are poorly soluble in water such as indomethacin and steroids such as androgens, prednisolone, prednisolone acetate, fluorometholone, and dexamethasones, may be emulsified with castor oil and polysorbate 80 resulting in a composition with similar low irritation potential.

The invention is further illustrated by the following examples with all parts and percentages expressed by weight. The cyclosporin used in the examples was supplied by Sandoz.

Example 1

	A	B	C	D	E
Cyclosporin A	0.40%	0.20%	0.20%	0.10%	0.05%
Castor oil	5.00%	5.00%	2.50%	1.25%	0.625%
Polysorbate 80	1.00%	1.00%	1.00%	1.00%	1.00%
Pemulen®	0.05%	0.05%	0.05%	0.05%	0.05%
Glycerine	2.20%	2.20%	2.20%	2.20%	2.20%
NaOH	qs	qs	qs	qs	qs
Purified water	qs	qs	qs	qs	qs
pH	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6

Example 2

	A	B	C	D
Castor oil	5.00%	2.50%	1.25%	0.625%
Polysorbate 80	1.00%	1.00%	1.00%	1.00%
Pemulen®	0.05%	0.05%	0.05%	0.05%
Glycerine	2.20%	2.20%	2.20%	2.20%
NaOH	qs	qs	qs	qs
Purified water	qs	qs	qs	qs
pH	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6

Example 3

	A
Castor oil	2.50%
Polysorbate 80	0.75%
Carbomer 1382	0.05%
Glycerine	2.20%
NaOH	qs
Purified water	qs
pH	7.2-7.6

Example 4

	A
Castor oil	5.00%
Polysorbate 80	0.75%
Carbomer 981	0.05%
Glycerine	2.20%
NaOH	qs
Purified water	qs
pH	7.2-7.6

The formulations set forth in Examples 1-4 were made for treatment of keratoconjunctivitis sicca (dry eye) syndrome with Examples 2, 3 and 4 without the active ingredient cyclosporin utilized to determine the toxicity of the emulsified components.

The formulations in Examples 1-4 were applied to rabbit eyes eight times a day for seven days and were found to cause only slight to mild discomfort and slight hyperemia in the rabbit eyes. Slit lamp examination revealed no changes in the surface tissue. In addition, the cyclosporin containing castor oil emulsion, as hereinabove set forth in Examples 1A-1D, was also tested for ocular bioavailability in rabbits; and the therapeutic level of cyclosporin was found in the tissues of interest after dosage. This substantiates that cyclosporin in an ophthalmic delivery system is useful for treating dry eye as set forth in U.S. Patent No. 4,839,342.

In addition, no difference in toxicity was found between formulations with cyclosporin (Examples 1A-1D) and formulations without cyclosporin (Examples 2-4).

The formulations set forth in Examples 1-4 were found to be physically stable upon long term storage.

With regard to formulations 1A-1D, no crystallization of cyclosporin was noticed after nine months at room temperature.

Further, other higher fatty acid glycerides such as olive oil, peanut oil and the like may also be utilized with the polysorbate 80 with similar results regarding biotoxicity.

The following examples demonstrate the activity of the composition in accordance with the present invention for enhanced absorption of cyclosporin A in the lacrimal gland.

Materials

The [Mebmt-^{3}H]-cyclosporin-A (lot #TRQ6553) was prepared by Amersham International (Buckinghamshire, England) with radiochemical purity of -98% (by reversed phase HPLC) and specific activity of 2.6 Ci/mmol (2.16 mCi/mg). The ^{3}H-label is a metabolically stable position as shown by the asterisk. The radiolabeled CsA was supplied as an ethanol solution (1 mCi/ml). All organic solvents used in the procedures described in this study were "HPLC grade". all other chemicals and reagents were analytical grade unless otherwise noted.

The compositions of the six formulations tested are listed in Table A.

TABLE A

Ingredients	Castor Oil	Castor Oil-in-Water Emulsion	Aqueous-α Cyclo-dextrin	Miglyol Oil-in-Water Emulsion	Polyoxyl 40	Polyoxyl 40 with Edetate
Cyclosporin-A	0.20	0.20	0.10	0.20	0.05	0.05
Cyclodextrin			14			
Castor Oil	99.8	1.25				
Miglyol Oil				20		
Pluronic L121+P123				0.75		
Tween 80		1.00				
Glycerin		2.20		2.20		
Pemulen® TR-2		0.05				
Carbopol 981				0.05		
Polyoxyl 40 Stearate (mg)					20	20
HPMC					0.3	0.3
Butylated Hydroxytoluene					0.001	0.001
Ethanol(9200 proof)						0.1
Sodium Chloride					0.73	0.73
Sodium Monophosphate					0.2	0.2
Disodium Edetate						0.1
Water		QS	QS	QS	QS	QS
Batch Size	1 g	5 g	1 g	5 g	1 g	1 g

The radiolabeled formulations were formulated to ensure that the radioactivity was homogeneous throughout the vehicle. The expected radioactivity concentrations of the radiolabeled drug formulations were 1-2 mCi/ml. The expected specific activity of radiolabeled cyclosporin A (CsA) formulations was 0.5-2 mCi/mg. All test articles were stored at ambient temperature.

Analysis of Test Drug Formulations

The test formulations were analyzed in triplicate by HPLC to determine the concentration of CsA and radiochemical purity of the CsA dosing solutions (>93%) before dosing. The radioactive concentrations of the test formulations were quantified by liquid scintillation counting (LSC).

Chromatographic Conditions

Pump:	Beckman Model 126 (Beckman Instruments, San Ramon, CA)
Mobile phase:	Acetonitrile: 0.03% H_3PO_4 in water, pH 3 (65:35 v/v)
Flow rate:	1.0 ml/min
Column:	Supercosil C8, 7.5 cm x 4.6 mm, 3 μm (Supelco, Bellefonte, PA) Superguard LC-8 (Supelco) Column heater (Bio-rad, Richmond, CA) at 60-70°C
Injector:	WISP 712B (Waters Associates, Milford, MA)
^{14}C detector:	Radio Isotope 171 Detector (Beckman Instruments)
Scintillant:	Ready Flow III (Beckman Instruments), Flow Rate of ~4 ml/min
UV detector:	Model 166 (Beckman Instruments), 202 nm
Data processor:	Beckman System Gold (Beckman Instruments)
Run Time:	15 min
Retention Time:	6 min (cyclosporin A)

Animals

Female New Female New Zealand albino rabbits were obtained and quarantined for at least five days before procedures. Animals were housed in temperature- and humidity-controlled rooms. Food and tap water were provided *ad libitum*. Fifty-eight rabbits (2-3 kg) were selected from the colony to minimize bias. They were individually identified by ear tags and appeared to be healthy.

Dosing

The animals were divided into six groups of nine rabbits; each group was treated with one of the six CsA formulations. During dosing, the lower eyelid of each rabbit was gently pulled away from the eye and 35 μl of the formulation were administered in the lower conjunctival cul-de-sac of each eye. After dosing, the upper and lower eyelid were handheld closed for ~5 seconds and released. The animals were observed visually for any signs of tearing or ocular discomfort.

Sampling

Tissues were collected at 20-min., 6-hr. and 24-hr. post-dose for each group. Three rabbits (six eyes) were used at each time point. At the specific sampling times, the animals were euthanized by an intravenous injection of 0.5-1 ml Eutha-6 (Western Supply Co., Arcadia, California) via marginal ear vein. Each eye was then rinsed with normal saline. The aqueous humor (~200 μl) was removed by means of a 0.5 ml tuberculin syringe. The orbital lacrimal gland (~400 mg), upper and lower bulbar conjunctivae (~50 mg each), corneal (~50 mg) and iris-ciliary body (~50 mg)

were dissected. The tissues dissected were blotted dry and weighed. Ocular tissue and aqueous humor samples from both eyes were collected from four untreated animals to be used as blank samples.

Analysis of Radioactivity

An aliquot of aqueous humor (50-175 μl) was counted directly in 10 ml of Ready-Solv HP by LSC. Tissue and blood samples were weighed into combustion cones prior to combustion in a Model 307 Packard Tissue Oxuduzer (Packard Co., Downers Grove, Illinois). After combustion of the tissue samples, ^3H_2O was trapped in the Monophase-S solution (Packard) and the radioactivity of the samples was determined by LSC in a Beckman Model 1801 or 3801 scintillation counter (Beckman Instruments, San Ramon, California).

Data Analyses

Excel software (version 4.0, Microsoft Corp, Redmond, Washington) was used for data analysis. concentrations of total radioactivity in the tissue samples were expressed as dpm/g or dpm/ml and converted to ng equivalents (eq) of CsA/g or ml, using the specific activity of the dosing formulations. Mean, standard deviation (SD) or standard error of the mean (SEM) was calculated according to standard methods. Radioactivity levels were not considered significant unless the dpm was greater than twice that of background b=(blanks).

Comparisons of tissue drug concentrations at each time point for the formulations were determined by one-factor ANOVA. All statistical comparisons were made using StatView® (version 1.03, Abacus Concepts, Inc., Berkeley, California). the Fisher and Scheffe

F tests were used to determine significant differences between formulations at the 95% level ($\alpha = 0.05$). The rejection criteria for excluding any outlier data was based on standard outlier tests. No more than one outlier was eliminated from any data set.

Results and Discussion

The radioactivity concentrations in ocular tissues at 20 minutes, 6 hours, and 24 hours after a single topical application of various formulations are depicted in Figures 1-4. In general, the concentrations in the ocular tissues were greatest at the earliest time point of 20 minutes as reported in previous single dose studies (2, 3). The radioactivity concentration was highest in the conjunctiva and cornea for each formulation. The relatively low aqueous humor and iris-ciliary body concentrations suggest low intraocular absorption of CsA, consistent with the low CsA corneal permeability of -1.0×10^{-6} cm/sec (6). The decline of radioactivity concentrations from the cornea was slower than those from the conjunctiva, lacrimal gland, and aqueous humor. The observed blood radioactivity concentrations (<3 ng-eq/ml) were much lower than trough plasma CsA concentrations of 80-250 ng/ml observed after oral dosing to humans (1).

The dependence of CsA corneal and conjunctival penetration on the formulation was interpreted in terms of CsA concentration in formulation and the release rate of CsA from formulation into tear film. The aqueous formulations demonstrated a greater propensity to release CsA for diffusion across the surface tissue epithelia. The 0.2% straight castor oil was formulated below the CsA solubility and therefore the release rate could be hampered by the less than maximal CsA thermodynamic activity (5).

The ocular surface tissues contained a higher fraction of the CsA dose than the other tissues and was used to discriminate among the aqueous, emulsion and the straight castor oil formulations. The polyoxyl 40 formulation produced higher ocular surface tissue concentrations than the emulsions and straight castor oil. The emulsions were also effective in delivery of CsA to the tissues of interest, lacrimal gland, cornea, and conjunctiva. The castor oil emulsion showed higher lacrimal gland concentrations than the modified Santen and the miglyol emulsion. The straight castor oil showed the lowest concentrations in surface ocular tissues. Apparently, the factors influencing CsA penetration into the lacrimal gland and the surface tissues are different.

Although there has been hereinabove described a particular pharmaceutical composition in the form of a nonirritating emulsion for the purpose of illustrating the manner in which the invention may be used to advantage, it should be appreciated that the invention is not limited thereto. Accordingly, any and all modifications, variations, or equivalent arrangements, which may occur to those skilled in the art, should be considered to be within the scope of the present invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a nonirritating emulsion of at least one cyclosporin in admixture with a higher fatty acid glyceride, polysorbate 80 and an emulsion-stabilizing amount of Pemulen® in water suitable for topical application to ocular tissue.

2. The pharmaceutical composition according to claim 1 wherein the cyclosporin comprises cyclosporin A.

3. The pharmaceutical composition according to claim 2 wherein the weight ratio of the higher fatty acid glyceride to the polysorbate 80 is between about 0.3 and about 30.

4. The pharmaceutical composition according to claim 3 wherein the higher fatty acid glyceride comprises castor oil and the weight ratio of cyclosporin to castor oil is below about 0.16.

5. A pharmaceutical composition comprising a nonirritating emulsion of at least one cyclosporin in admixture with castor oil and polysorbate 80 in water suitable for topical application to ocular tissue.

6. The pharmaceutical composition according to claim 5 wherein the cyclosporin comprises cyclosporin A.

7. The pharmaceutical composition according to claim 6 wherein the weight ratio of castor oil to the polysorbate 80 is between about 0.3 and about 30.

8. The pharmaceutical composition according to claim 7 wherein the weight ratio of cyclosporin to castor oil is below about 0.16.

9. The composition according to claim 1 wherein the higher fatty acid glyceride and polysorbate 80 are present in amounts sufficient to prevent crystallization of cyclosporin for a period of up to about nine months.

10. A stable, nonirritating ophthalmic composition comprising cyclosporin in admixture with an emulsifying amount of a higher fatty acid glyceride and polysorbate 80.

11. A pharmaceutical emulsion comprising cyclosporin A, castor oil, Pemulen®, glyceride and water in amounts sufficient to prevent crystallization of cyclosporin A for a period of up to about nine months, said pharmaceutical emulsion being suitable for topical application to ocular tissue.

12. The pharmaceutical emulsion according to claim 11 wherein the cyclosporin A is present in an amount of between about 0.05 to about 0.40%, by weight, the castor oil is present in ana mount of between about 0.625%, by weight, the polysorbate 80 is present in an amount of about 1.0%, by weight, the Pemulen® is present in an amount of about 0.05%, by weight, and the glyceride is present in an amount of about 2.2%, by weight.

13. A pharmaceutical emulsion consisting of between about 0.05% and about 0.40%, by weight, cyclosporin A, between about 0.625% and about 5.0%, by weight, castor oil, about 1.0%, by weight, polysorbate 80, about 0.05%, by weight, Pemulen®, and about 2.2%,

by weight, glycerine in water with a pH of between about 7.2 and 7.6 suitable for topical application to ocular tissue.

14. A pharmaceutical composition suitable for instillation into an eye, said pharmaceutical composition comprising a nonirritating emulsion of at least one cyclosporin and castor oil in an amount causing enhanced lacrimal gland absorption.

15. The pharmaceutical composition according to claim 14 wherein the cyclosporin comprises cyclosporin A.

16. The pharmaceutical composition according to claim 15 wherein the cyclosporin is present in an amount of between about 0.20 and about 5.0% by weight.

17. The pharmaceutical composition according to claim 15 further comprising an emulsion-stabilizing amount of Pemulen® in water suitable for topical application in the eye.

18. The pharmaceutical composition according to claim 17 wherein the cyclosporin is present in an amount of about 0.20% by weight, the castor oil is present in an amount of about 1.25% by weight, and the Pemulen® is present in an amount of about 0.05% by weight.

19. The pharmaceutical composition according to claim 18 further comprising Tween 80 in an amount of about 1.0% by weight, and glycerin in an amount of about 2.20% by weight.

20. A pharmaceutical composition suitable for instillation into an eye, said pharmaceutical

composition comprising a nonirritating admixture of at least one cyclosporin and castor oil in an amount causing enhanced lacrimal gland absorption.

21. The pharmaceutical composition according to claim 20 wherein the cyclosporin comprises cyclosporin A.

22. A method of causing enhanced absorption of cyclosporin A in the lacrimal gland of an eye, said method comprising the steps of:

admixing cyclosporin A with castor oil; and

instilling the admixture into the eye.

23. The method according to claim 22 wherein the step of admixing includes forming an emulsion of cyclosporin A, castor oil and water.

24. A method of causing enhanced absorption of cyclosporin A in the lacrimal gland of an eye, said method comprising the steps of:

forming an emulsion of cyclosporin A, castor oil, Pemulen® and water; and

instilling the emulsion into the eye.

25. The method according to claim 24 wherein the cyclosporin is present in an amount of about 0.20% by weight, the castor oil is present in an amount of about 1.25% by weight, and the Pemulen® is present in an amount of about 0.05% by weight.

26. The method according to claim 24 wherein the emulsion further comprises Tween 80 in an amount of about 1.0% by weight, and glycerin in an amount of about 2.20% by weight.

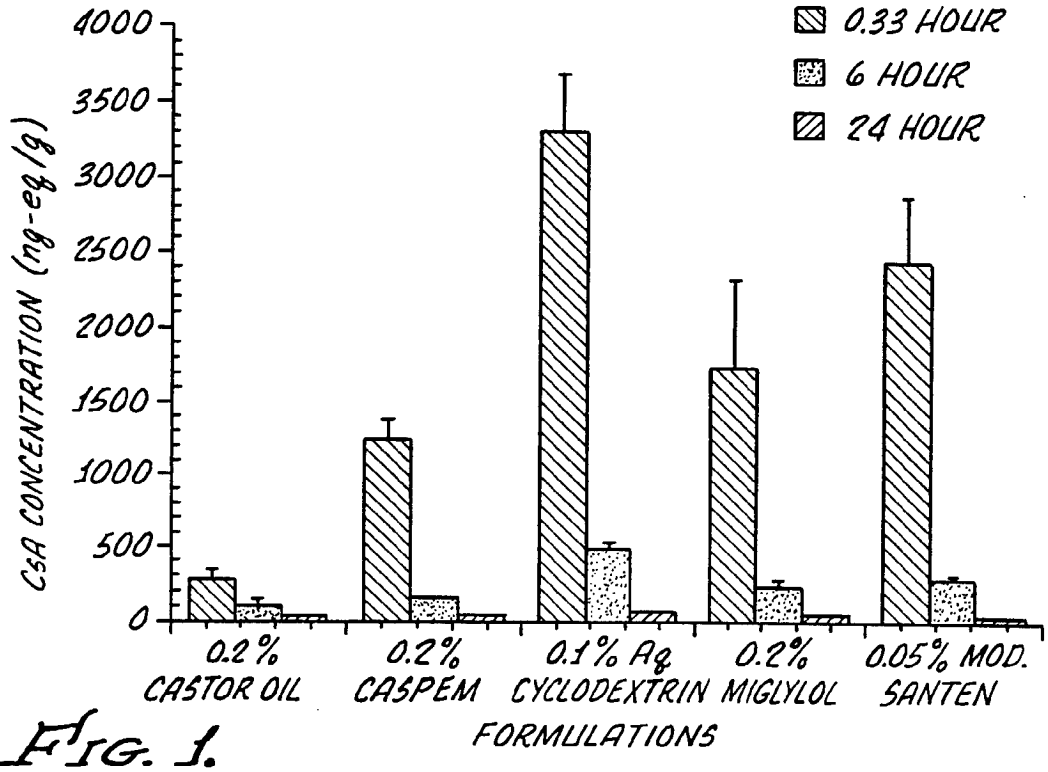
0.33 HOUR
6 HOUR
24 HOUR
CsA CONCENTRATION (ng-eq/g)
4000
3500
3000
2500
2000
1500
1000
500
0
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
FORMULATIONS

FIG. 1.

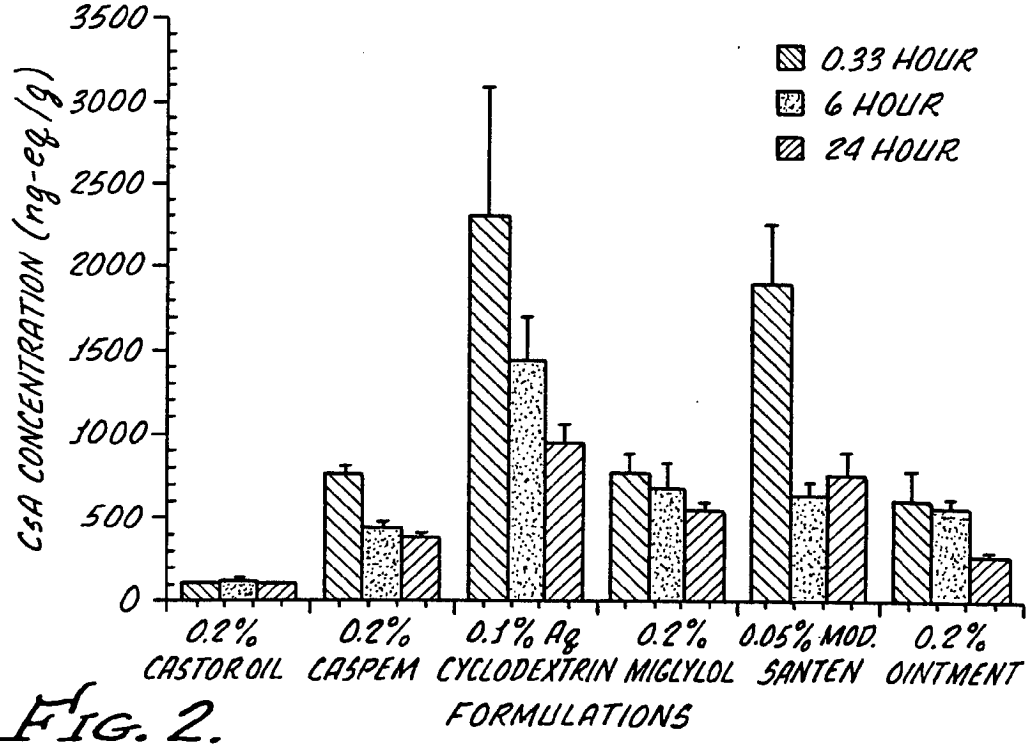
0.33 HOUR
6 HOUR
24 HOUR
CsA CONCENTRATION (ng-eq/g)
3500
3000
2500
2000
1500
1000
500
0
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

FIG. 2.

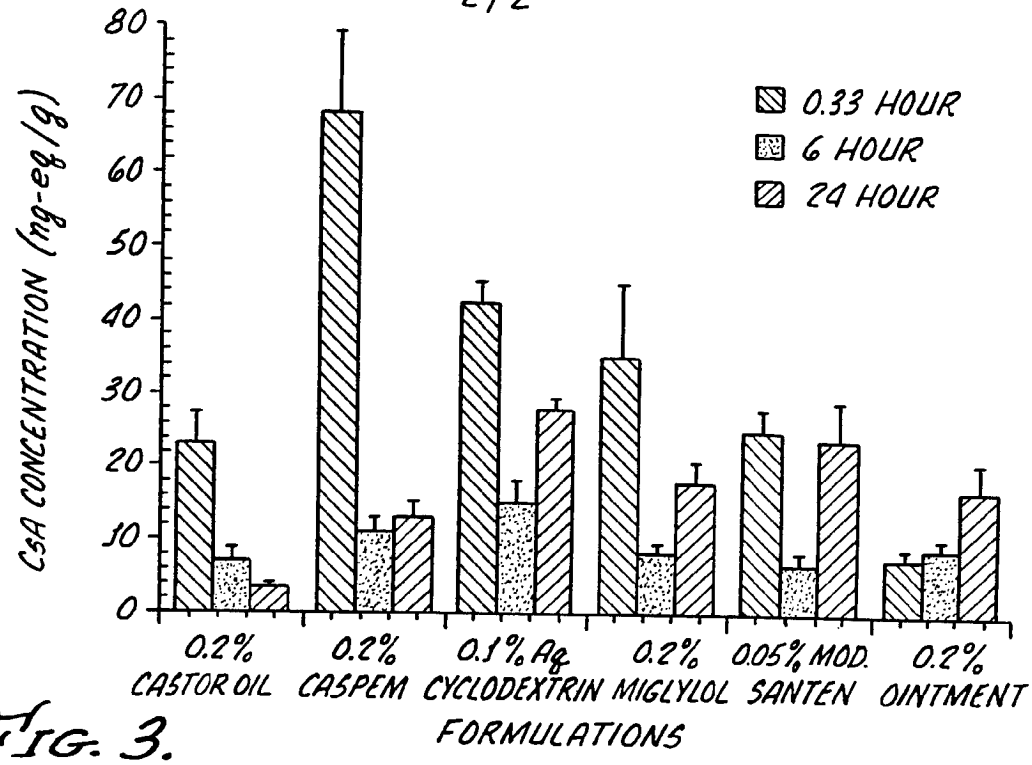

FIG. 3.

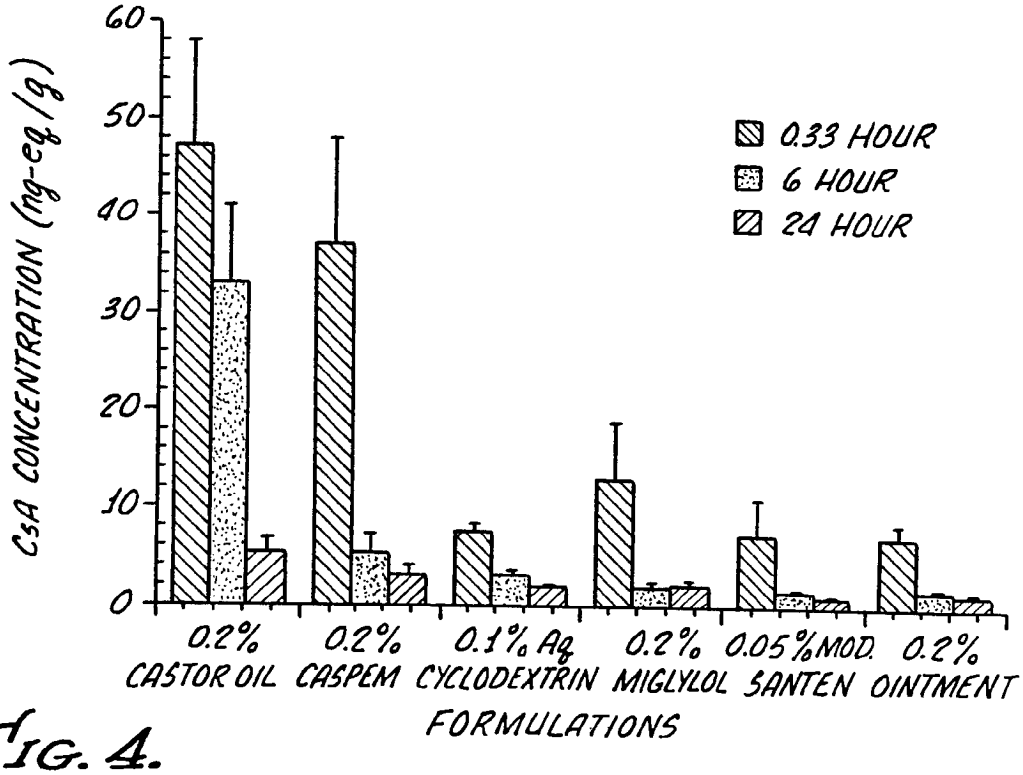

FIG. 4.

INTERNATIONAL SEARCH REPORT

Inten ıal Application No
PCT/US 95/06302

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/13 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 228 198 (SANDOZ LTD.) 22 August 1990	10
Y	see claim 1 see page 13, paragraph 2 see page 27, paragraph 3	5-8
X	WO,A,89 01772 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION INC.) 9 March 1989	14-16, 20,23
Y	see claims 11-13,15 see page 10, line 17 - line 31 see page 11, line 11 - line 17	5-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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1

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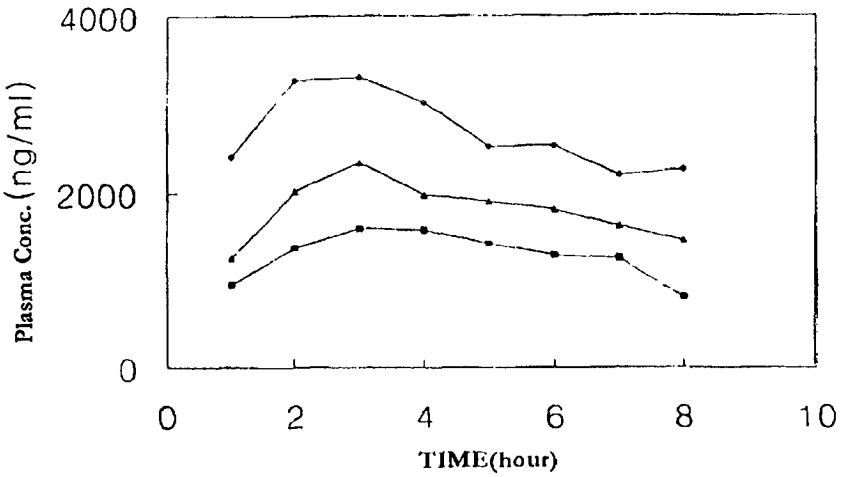

(57) Abstract

Disclosed is a solid dispersed preparation for poorly water–soluble drugs, which is prepared by dissolving or dispersing the poorly water–soluble drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water–soluble polyol matrix and drying the mixture. The solid dispersed preparation can be formulated into a power formulation or a granule formulation. The solid dispersed preparation is improved in the solubility of poorly water–soluble drugs in the gastro–intestinal tract, resulting in a great increase in the bioavailability of the drugs. In addition, the solid dispersed preparation gives the pharmaceutical solutions to the problems that the conventional semi–solid or liquid preparations possess, enabling medicinally effective, poorly water–soluble compounds to be formulated, molded and processed, quickly and in an economically favorable manner without use of any organic solvent.

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SOLID DISPERSED PREPARATION OF POORLY WATER-SOLUBLE DRUG CONTAINING OIL, FATTY ACID OR MIXTURES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a solid dispersed preparation for poorly water-soluble drugs or biologically active substances. More particularly, this invention relates to a solid dispersed preparation which allows poorly water-soluble drugs to be increased in the uptake efficiency in the gastro-intestinal track and is convenient to make in a pharmaceutical formulation.

Description of the Prior Art

A good many drugs poorly dissolve in water. When being administered to a body, these poorly water-soluble drugs have so low solubility and releasing rate in digestive juices as to retard their absorption, resulting the bioavailability decreased. In order to solve this problem, various preparation methods were developed with the aim of solubilizing these poorly water-soluble drugs and increasing their releasing rates. For instance, there have been reported many methods for improving the bioavailability of drugs, including micronization, formation of micelles by use of surfactant, solvent deposition, utilization of dry elixirs, co-precipitation

by use of inert water-soluble carriers, solid-dispersion and formation of inclusion complexes using cyclodextrins. In conducting these methods, however, the drugs to be administered do not show a constant increase in solubility. Thus, they are problematic in terms of preparation, commercialization, and efficiency.

For the poorly water-soluble drugs, which are also poor in internal uptake, there have been made attempts to enhance their bioavailability upon administration. However, the dosage forms developed thus far, are of semi-solid or liquid form, giving disadvantages in pharmaceutics, especially in formulating, molding and processing.

SUMMARY OF THE INVENTION

We, the inventors made the intensive and thorough research on the formulation of poorly water-soluble drugs, to improve the bioavailability of the drugs upon administration. As a result, we found that the dispersion or solution of the poorly water-soluble drugs in oils, fatty acids or mixtures thereof, followed by mixing with a water-soluble polymer matrix allowed the drugs to efficiently release in the gastro-intestinal tract and the mixture can be formed into a solid form.

Therefore, it is an object of the present invention to provide a solid dispersed preparation which improves the

bioavailability of poorly water-soluble drugs by enhancing the release of the drugs in the gastro-intestinal tract.

It is another object of the present invention to provide a solid dispersed preparation which can be prepared by simple and convenient process with an economical benefit.

According to the present invention, a solid dispersed preparation for poorly water-soluble drugs is prepared by dissolving or dispersing the drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water-soluble polyol matrix and drying the mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph in which the plasma concentration of cyclosporine is plotted against the times after administrating the solid dispersed preparations of the present invention (closed rectangle and closed triangle) and a commercially available preparation (Neoral, closed lozenge);

Fig. 2 is a graph in which the plasma concentration of aceclofenac is plotted against the times after orally administrating aceclofenac powder (closed circle) and the solid dispersed preparation of the present invention (open circle, oleic acid 5%) to rats;

Fig. 3 is a graph in which the plasma concentration of cyclosporine is plotted against the times after

administrating the solid dispersed preparation of the present invention (closed circle, capsule containing 100 mg of the preparation) and a commercially available preparation (open circle, Airtal capsule 100 mg) to bealgle dogs;

Fig. 4 is a graph in which the plasma concentration of aceclofenac is plotted against the times after orally administrating the solid dispersed preparation of the present invention (closed circle, capsule containing 100 mg of the preparation) and a commercially available preparation (open circle, Airtal capsule 100 mg) to humans; and

Fig. 5 is a graph in which the plasma concentration of cisapride is plotted against the times after orally administrating the solid granular preparations of the present invention (open circle, bead 10 mg) and a commercially available preparation (closed circle, prepulsid 10 mg) to humans.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in detail.

In accordance with the present invention, there is provided a solid dispersed preparation for poorly water-soluble drugs, which is prepared by dispersing or dissolving the drugs in an oil, a fatty acid or a mixture

thereof, incorporating the dispersion or solution into a water-soluble polymer matrix and drying this mixture.

In particular, this invention provides two types of fomulation, i.e., the solid powdery preparation and the solid granular preparation.

The preparation method of the solid dispersed powders comprises the following steps; Dissolving or dispersing the poorly water-soluble drugs in an oil, a fatty acid or the mixture thereof; mixing with the water-soluble polymer matrix; drying the mixture; and grinding the pellet into powders.

In addition, the preparation method of the solid dispersed granules comprises the following steps; Dissolving or dispersing the poorly water-soluble drugs in an oil, a fatty acid or the mixture thereof; mixing with the water-soluble polymer matrix; spraying onto a pharmaceutically acceptable nucleus, resulting the granules. In a preferred embodiment, the pharmaceutically acceptable nucleus may be a sugar sphere.

The solid dispersed powdery preparation or the solid dispersed granular preparation of this invention can be formulated into the pharmaceutically acceptable medicines for internal use such as powders, granules, tablets and capsules.

Hereinafter, the word "solid dispersed preparation" means "solid dispersed powdery preparation", "solid dispersed granular preparation" or the both.

In this regard, the oil, the fatty acid or the mixture thereof may be used alone or in a form of an emulsion or microemulsion inclusive of itself. When dispersing or dissolving poorly water-soluble drugs in the oil, fatty acid or mixture thereof, a surfactant may be added together. Further, the water-soluble polymer matrix may be used alone or in combination with another water-soluble matrix.

Illustrative examples of the oil that can be used in the preparation of the present invention include lipid additives, such as α-bisabolol, stearyl glycerrhetinate, salicylic acid, tocopheryl acetate, a mixture of water, alcohol and Perilla extract, sodium hyaluronate, panthenol, propylene glycol and apple(*Pirus Malus*), propylene glycol and pineapple, ivy (*Hedera halix*) extract and 1,3-B.G, peach (*Prums persica*) leaf extract, hydrolyzed soy flour, wheat (*Triticum Vulgare*) protein, birch (*Betula alba*) extract and 1,3-B.G, burdock (Arctium majus)extract and 1,3-B.G; liposomes; phosphatidylcholines; esters, such as glyceryl stearate, captylic/capric triglyceride, cetyl octanolate, isopropyl myristate, 2-ethylene isopelagonate, di-C12-13 alkyl malate, ceteatyl octanoate, butylene glycol dicaptylate/dicaprate, isononyl isostearate, isostearyl isostearate, coco-captylate/caprate, cetyl octanoate, octyldodecyl myristate, cetyl esters, C10-30 cholesterol/lanosterol ester, hydrogenated castor oil, monoglycerides, diglycerides, and triglycerides; hydrocarbons, such as beeswax, canauba wax, suctose

distearate, PEG-8 beeswax and candelilla (*euphorbia cerifera*) wax; mineral oils such as ceresin and ozokerite; vegetable oils such as macadamia ternifolia nut oil, hydrogenated hi-erucic acid rape seed oil, olive oil, jojoba oil, hybridsunflower (*Helian thus annuus*) oil, neen (*melia azadirachta*) seed oil, dog rose (*rosa canina*) lips oil with preference to mineral oils, squalene, squalane, monoglycerides, diglycerides, triglycerides, medium-chain glyceride, myglyol, cremophor, hydrogenated caster oil, corn oil, Perilla oil, cotton seed oil and lipid-soluble vitamins.

As for the fatty acid, it is preferable to use oleic acid, cetyl alcohol, stearyl alcohol, stearic acid, myristic acid, linoleic acid or lauric acid. More preferable is to use oleic acid, linoleic acid, or isopropyl myristate.

As the water-soluble matrix, polyethylene glycol (PEG), carbowax or polyvinyl pyrrolidone (PVP) is available. Aforementioned water-soluble matrix may be used in combination with other matrixces, examples of which include water-soluble matrices such as gelatin, gum, carbohydrates, celluloses, polyvinyl alcohol, polyacrylic acid, inorganic compounds and mixtures thereof; and enteric matrices such as hydroxypropylmethylcellulose acetyl succinate (HPMCAS), cellulose acetate phthalate, shellac, zein, polyvinyl acetate phthalate, Eudragit L100, Eudragit S100, sodium arginate and poly-L-lysine.

In order to enhance the dispersion or dissolution of poorly water-soluble drugs in the oil, fatty acid or their mixture, a surfactant may be added, which is selected from the group comprising glyceryl stearate, polysorbate 60, polysorbate 80, sorbitan trioleate, sorbitan sesquioleate, sorbitan stearate, PEG-20 glyceryl isostearate, ceteth-25, PEG-60 hydrogenated castor oil, nonoxynol-15, PEG-6-decyltetradeceth-20, dimethicone copolyol, glyceryl diisostearate, ceteth-24, cetearyl alcohol, polyoxylethylene nonyphenyl ether, PEG-40 hydrogenated castor oil, cetyl dimethicone copolyol, polyglyceryl-3-methylglucose distearate, PEG-100 stearate, sorbitan isostearate, sodium lauryl glutamate, disodium cocoamphodiacetate, lauric acid diethanolamide, coconut fatty acid diethanolamide, N,N-bis-(2-hydroxy ethyl)-cocomide, and cocoamidopropyl betain.

The solid dispersed preparation of the present invention can be applied for all the poorly water-soluble drugs and preferably for ketoconazole; itraconazole and its derivatives; cyclosporine; cisapride; acetaminophen; aspirin; acetylsalicylic acid; indomethacin; naproxen; warfarin; papaverine; thiabendazole; miconazole; cinnarizine; doxorubicin; omeprazole; cholecalciferol; melphalan; nifedipine; digoxin; benzoic acid; tryptophan; tyrosine; phenylalanine; aztreonam; ibuprofen; phenoxymethylpenicillin; thalidomide; methyltestosterone; prochlorperazine; hydrocortisone; dideoxypurine

nucleoside; vitamin D_2; sulfonamide; sulfonylurea; p-aminobenzoic acid; melatonin; benzylpenicillin; chlorambucil; diazepin; digitoxin; hydrocortisone butyrate; metronidazole benzoate; tolbutamide; prostaglandin E1(PGE_1); fludrocortisone; griseofulvin; miconazole nitrate; leukotriene B_4 antagonist; propranolol; thephylline; flubiprofen; sodium benzoate; benzoic acid; riboflavin; benzodiazepine; phenobardital; glyburide; sulfadiazine; sulfaethylthiadiazole; sodium diclofenac; aceclofenac; phyniroin; hioridazinehydrochloride; bropirimine; hydrochlorothiazide; fluconazole; acyclovir; bucillamine; ciprofluoxacin; acetyl-L-carnitine; baclofen; sodium alendronate; lovocarnitine; nimodipine or nimodifine; atenolol; provastatin sodium; lovastatin; isotretinoin; etidronate disodium; doxifluridine; fosfomycin calcium; sotepine; epinastine hydrochloride; carvedilol; epinastine hydrochloride; carvedilol; fosinopril; trandolapril; etretinate cap; metergoline; mercaptopurine; vancomycin hydrochloride; cefixime; cefuroxim axetil; dirithramycin; and dadanosin and more preferably for ketoconazole, itraconazole and its derivatives, cisapride, cyclosporine and nifedipine.

Over conventional methods, the present invention has an advantage, in that, the solid dispersed preparation can be prepared with ease and show high efficiency in absorption and release.

First, a poorly water-soluble medicine is homogeneously mixed and dispersed in an oil, fatty acid or their mixture and added in water-soluble polymer matrices molten at room temperature or about 60-80 °C, after which the resulting mixture is cooled rapidly to room temperature and dried in an oven for 12 hours or more. The dried pellet is powdered in a mortar and passed through a sieve to give powder which is uniform in particle size. As aforementioned, when the drug is dispersed or dissolved in the oil, fatty acid or their mixture, the oil, fatty acid or their mixture may be emulsified or micro-emulsified. In this case, a surfactant may be added to the solution.

Alternatively, after the homogeneous dispersion of the poorly water-soluble drug is added in the water-soluble polymer matrix molten at about 60-80 °C, it may be sprayed to pharmaceutically acceptable nucleus to give a granule.

As a consequence of an examination which was made on the solubility of the solid dispersed preparation in distilled water, artificial intestinal juice and artificial gastric juice, the solubility of the solid dispersed preparation is found to be better than those of poorly water-soluble drugs themselves. Particularly, a great advance can be brought into the solubility of poorly water-soluble drugs when they are incorporated into a solid dispersed preparation containing oleic acid or micro-emulsified oleic acid.

The data obtained from the experiments in which the

solid dispersed preparations of the present invention are eluted in artificial gastric juice and artificial intestinal juice, show that the solid dispersed preparations of the present invention are superior to the poorly water-soluble drugs themselves in releasing rate. A significant improvement in releasing rate is observed when a solid dispersed preparation containing oleic acid or microemulsified oleic acid is used. In the artificial intestinal juice, a severer condition in which for drugs to dissolve, rather than in the artificial gastric juice, the improvement in the releasing rate by virtue of the solid dispersed preparation is more apparent.

Through an experiment which is conducted for examining the uptake efficiency of poorly water-soluble drugs in the gastro-intestinal tract, the superiority of the solid dispersed preparation according to the present invention is also demonstrated. Even when only a water-soluble matrix is used, the uptake efficiency of the drugs is minutely increased. In particular, the uptake efficiency of drugs in the gastro-intestinal tract is remarkably improved when they are incorporated in a solid dispersed preparation using oleic acid-containing microemulsions.

In addition, comparison of the plasma concentration of target drug molecule after oral administration between the solid dispersed preparation and conventional preparations, is helpful in understanding the present invention. As a result, similar levels are observed, suggesting that the

solid dispersed preparation of the present invention can substitute for conventional preparations when account is taken of pharmaceutical aspects.

A better understanding of the present invention may be obtained in light of the following examples which are set forth to illustrate, but are not to be construed to limit the present invention.

Following are the compositions of emulsions and microemulsions used in Examples.

EMULSIONS

PREPARATION EXAMPLE I

Waxes	Composition (%)
KALCHOL 6870	1.800
EMERSOL 132	1.000
Multi-Wax W-445	1.700
Emulsifiers	
ATLAS G-144	1.800
ATLAS G-610	1.900
ATMOS 370	0.800
KM-105	2.000
Oils	
CRODALAN SWL	1.500

LEXOL GT 865	4.000
NIKKOL CIO	4.000
SEPERIOR JOJOBA OIL	1.000
SF 1202	0.200
KF-96(100CS)	0.300
DRAKEOL 7	5.000
Squalane	2.000
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.200

Aqueous Phase

DI-WATER	60.852
glycerin	2.000
P.G	7.000
NATURAL EXT.AP	0.500
LUBRAGEL CG	0.200
Carbopo 1940	0.100
KELTROL F	0.020
NaOH	0.028

PREPARATION EXAMPLE II

Waxes

KALCHOL 6870	1.800
EMERSOL 132	1.000
Multi-wax W-445	1.700

Emulsifiers	
RHEODOL AO-15	0.800
RHEODOL MS-162	2.000
RHEODOL TW-S120	1.900
KM-105	2.000
Oils	
CRODALAN SWL	1.500
LEXOL GT 865	5.000
NIKKOL CIO	2.500
Macadamia ternifolia nut oil	1.000
SF 1202	0.300
KF-96(100CS)	0.300
DRAKEOL 7	7.000
Squalane	0.500
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.100
Aqueous phase	
DI-WATER	61.780
glycerin	2.000
1.3-B.G	6.000
NATURAL EXT.AP	0.300
LUBRAGEL CG	0.200
Carbopol 940	0.100
KELTROL F	0.020
TEA	0.100

PREPARATION EXAMPLE III

Waxes	
KALCHOL 6870	0.500
EMERSOL 132	0.500
Beeswax	0.400
Emulsifiers	
ATLAS G-114	2.200
ATLAS G-610	0.800
ATMOS 370	0.800
KM-105	0.700
Oils	
CRODALAN SWL	0.500
LEXOL GT 865	3.000
NIKKOL CIO	3.000
SUPERIOR JOJOGA OIL	0.500
SR 1202	0.200
KF-96(100CS)	0.100
DRAKEOL 7	3.000
Squalane	0.500
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.200
Aqueous phase	
DI-WATER	74.146

Glycerin	2.000
P.G	6.000
NATURAL EXT.AP	0.500
LUBRAGEL CG	0.200
Carbopol 940	0.100
KELTROL F	0.020
NaOH	0.0336

PREPARATION EXAMPLE IV

Waxes

KALCHOL 6870	0.400
EMERSOL 132	0.500
Multi-Wax W-445	0.400

Emulsifiers

RHEODOL AO-15	0.800
RHEODOL MS-165	2.200
RHEODOL TW-S120	0.800
KM-105	0.600

Oils

CRODALAN SWL	0.500
LEXOL GT 865	3.000
NIKKOL CIO	2.000
Macadamia ternifolia nut oil	1.000
SF 1202	0.400

DRAKEOL 7	4.500
Squalane	0.500
dl-a-tocopheryl acetate	0.100
POLYOLPERPOLYMER-2	0.100

Aqueous phase

DI-WATER	73.480
glycerin	2.000
1,3-B.G	6.000
NATURAL EXT.AP	0.300
LUBRAGEL CG	0.200
Cabopol	0.100
KELTROL F	0.020
TEA	0.100

MICROEMULSIONS

PREPARATION EXAMPLE V

Waxes

Cetyl Alcohol	3.000

Emulsifiers

NIKKOL HCO-60	5.000
RHEODOL TW-0120	5.000
Cremophor EL	20.000

Oils	
I.P.M	5.000
CAPTEX	5.000
Aqueous phase	
DI-WATER	52.000
Ethanol	5.000

PREPARATION EXAMPLE VI

Emulsifiers	
NIKKOL HCO-60	5.000
RHEODOL TW-0120	5.000
Cremophor EL	5.000
Oils	
I.P.M	5.000
Lanolin oil	5.000
CAPTEX	5.000
Aqueous phase	
DI-WATER	50.000

PREPARATION EXAMPLE VII

Surfactant	
LABRASOL	15.000

Surfactant Aid	
Polyglyceryl oleate	5.000
PLURL OLEIQUE	5.000
Oil phase	
LABRAFIL M1994CS	4.500
Sub-Solvent	
Transcutol	5.000
Aqueous phase	
Phosphate buffer(pH 6)	64.500

PREPARATION EXAMPLE VIII

Oil phase	
GELUCIRE 44/14	11.429
GELUCIRE 48/09	11.429
Surfactant	
LABRAFAC CM 10	10.714
Surfactant Aid	
LAUROGLYCOL	7.143
Transcutol	59.285

PREPARATION EXAMPLE IX

Aqueous Phase	
Water (Buffer)	57,050
Physiological Saline Solution	4,000
Glucose	1,000
Propylene Glycol PEG 300,400	5,000
Glycerol	5,000
Oil Phase	
Fatty Acid Esters	5,000
Modified Vegetable Oil	0.500
Silicon Oil	0.500
Surfactant Aid	
Long Chain Alcohol	3,750
Glycol Derivative	2,500
Propylene Glycol Derivative	1,200
Polyglycerol Derivative	4,500
Surfactant	
Non-ionic Surfactant	10,000

PREPARATION EXAMPLE X

Oil Phase	
Oleic Acid	6,250

Surfactant	
Tween 80	12,500
Surfactant Aid	
Transcutol	8,750
Aqueous Phase	
Water	72,500

PREPARATION EXAMPLE XI

Oil Phase	
Captex	5,000
Surfactant	
Cremophor	12,500
Surfactant Aid	
Transcutol	6,250
Aqueous Phase	
Water	76,250

COMPARATIVE EXAMPLE I

After being melted at about 70 °C, 90 g of PEG 6000 was added with 10 g of ketoconazole, cooled rapidly to room

temperature and dried in an oven for 12 hours or more. The dried solid dispersed preparation was milled in a mortar and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE I

In 5 g of oleic acid were homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added into 85 g of PEG 6000 which was molten at about 70 °C. After being cooled rapidly to room temperature and dried in an oven for 12 hours or more, the dried solid dispersed preparation was milled in a mortar and passed through a sieve to give a powder which was uniform in particular size.

EXAMPLE II

In 5 g of oleic acid and 5 g of Tween 80 were homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE III

In 5 g of isopropyl myristate was homogeneously mixed

and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE IV

In 5 g of liquid paraffin was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE V

In 5 g of cremophor was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE VI

In 5 g of cremophor and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was

obtained in the same procedure as in Example I.

EXAMPLE VII

In 5 g of isopropyl myristate and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE VIII

In 5 g of liquid paraffin and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE IX

In a microemulsion containing 5 g of cremophor, 5 g of oleic acid, 35 g of alcohol and 1 g of transcutol was homogeneously dissolved and dispersed 10 g of ketoconazole, followed by evaporating the alcohol. The solid residue was, then, added in 43 g of PEG 6000 molten at about 70 °C.

Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE X

In a microemulsion containing 5 g of cremophor, 5 g of oleic acid and 1 g of transcutol was dissolved 10 g of ketoconazole which was, then, dispersed in 35 g of distilled water, followed by evaporating the distilled water in an oven. The solid residue was added in 43 g of PEG 6000 molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XI

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of itraconazole which was, then, added in 80

g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of itraconazole. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 which was molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIV

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of an itraconazole derivative (Dong-A Pharmacy Co., Ltd., Korea) which was, then, added in 80 g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XV

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cyclosporine which was, then, added in 80

g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVI

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cyclosporine. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 which was molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cisapride which was, then, added in 80 g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVIII

In 5 g of oleic acid and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of cisapride which was, then, added in 80 g of PEG 6000 which was molten at 70

°C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIX

In a microemulsion containing 10 g of cremophor, 5 g of oleic acid and 7 g of transcutol was homogeneously dissolved and dispersed 10 g of itraconazole, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XX

In a microemulsion containing 10 g of cremophor, 4 g of captex and 5 g of transcutol was homogeneously dissolved and dispersed 10 g of cyclosporine, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXI

In a microemulsion containing 10 g of cremophor, 5 g of oleic acid and 7 g of transcutol was homogeneously

dissolved and dispersed 10 g of cisapride, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of molten PVP. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXIII

In a microemulsion containing 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of molten PVP. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

COMPARATIVE EXAMPLE II

After being melted at about 70 °C, 2.5 g of molten PEG 6000 was added with 1.75 g of aceclofenac, cooled rapidly to room temperature and dried in a freeze-drier for 24 hours or

more. The dried solid dispersed preparation was finely milled in a grinder and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE XXIV

In 0.25 g of oleic acid and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac, and then, the solution was added in 2.5 g of PEG 6000 which was molten at about 75 °C. After being cooled rapidly to room temperature

And dried in a freeze-drier for 24 hours or more, the dried solid dispersed preparation was finely milled in a grinder and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE XXV

In 0.25 g of cemophor and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 which was molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVI

In 0.25 g of labrasol and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVII

In 0.25 g of transcutol and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVIII

A mixture of 10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween 80, 5 g of talc and 10 g of PEG 6000 was heated at about 80 °C and homogeneously dispersed in 150 ml of an alcohol. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting solution was sprayed at a rate of 4 ml/min onto 35 g of sugar spheres to give a solid dispersed granule.

EXAMPLE XXIX

10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween

80, 3 g of talc, 25 g of Eudragit (Rhompharm, Germany) RS30D and 25 g of Eudragit L30D were homogeneously mixed. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting solution was sprayed at a rate of 4 ml/min onto 35 g of the sugar spheres prepared in Example XXVIII.

EXAMPLE XXX

10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween 80, 3 g of talc and 50 g of Eudragit RS30D were homogeneously mixed. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto the sugar spheres prepared in Example XXVIII.

EXAMPLE XXXI

A mixture of 2.5 g of cisapride, 2.5 g of oleic acid, 2.5 g of Tween 80, 5 g of talc and 23 g of PEG 6000 was heated at about 80 °C and added with 150ml of a mixture of acetone and water(acetone:water, 1:1). With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto 100 g of sugar spheres.

EXAMPLE XXXII

2.5 g of cisapride, 2.5 g of oleic acid, 2.5 g of Tween 80, 3 g of talc, 25 g of Eudragit RS30D and 25 g of Eudragit

L30D were homogeneously mixed in 150ml of acetone. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto 70 g of the sugar spheres prepared in Example XXXI.

EXAMPLE XXXIII

Aceclofenac, lactose, starch and talc were mixed to give a tablet in accordance with the established method. 2.5g of aceclofenac, 2.5g of oleic acid, 2.5g of tween 80, 3g of talc, 25g of Eudragit RS30D and 25g of Eudragit L30D were homogeniously mixed. With the aid of a fluid bed-coating machine (nozzle;0.8mm), the resulting mixture was sprayed at a rate of 4ml/min onto the said tablets to obtain a solid dispersed tablet.

EXAMPLE XXXIV

Cisapride, lactose, starch and talc were mixed to give a tablet in accordance with the established method. 2.5g of cisapride, 2.5g of oleic acid, 2.5g of tween 80, 25g of Eudragit RS30D and 25g of Eudragit L30D were homogeniously mixed. With the aid of a fluid bed-coating machine (nozzle;0.8mm), the resulting mixture was sprayed at a rate of 4ml/min onto the said tablets to obtain a solid dispersed tablet.

EXPERIMENT I : The Drug Solubility of Solid Dispersed Preparation In Water and Artificial Intestinal Juice

In this experiment, the solubility of poorly water-soluble drugs in water and artificial intestinal juice was investigated for the solid preparations obtained in Comparative Example and Examples. In this regard, suspensions of 2 g of the solid dispersion preparations of this invention in water or artificial intestinal juice were filtrated through a 0.2 μm filter paper (Millipore, Waters, Milford, MA, USA) and the filtrate was diluted for the convenient quantification of the drugs. The solubility results are given in Table 1.

TABLE 1

Solubility of Ketoconazole in distilled water and artificial intestinal juice

Solid Dispersed Preparatio	Solubility (μg/mP)	
	DI-Water	Artificial Intestinal Juice
Ketoconazole Powder	0.10	2.08
Comparative Example	3.77	-
Example I	41.4	44.8
Example II	73.9	-
Example III	2.47	-
Example IV	2.28	-
Example V	8.02	-

Example VI	12.0	-
Example VII	6.31	-
Example VIII	12.2	-
Example IX	72.8	50.7
Example X	63.6	37.8

As apparent from the data of Table 1, the solubility of the drugs in distilled water was significantly improved when they were incorporated in solid dispersed preparations containing oleic acid. Particularly, the drugs in the solid dispersed preparations prepared from microemulsions containing oleic acid showed a great advance in the solubility in water as well as in artificial intestinal juice.

EXPERIMENT II: The drug-releasing Rate of Solid Dispersed Preparations in Artificial Gastric and Intestinal Juices

The solid dispersed preparations comprising ketoconazole or cisapride, respectively, obtained in Examples, were tested for releasing rates in artificial gastric juice and artificial intestinal juice.

According to the paddle process described in Korean Pharmacopoeia VI(KP VI), this releasing test was carried out in artificial gastric juice and artificial intestinal juice at 37±0.5 °C while the paddle was rotated at 50 rpm.

At an interval of a predetermined period of time, samples were taken from the artificial juices and filtered through 0.2 μm Millipore paper and the filtrates were measured for plasma concentration of drug. The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Tables 2 and 3.

TABLE 2

Releasing Level (μg/ml) and Percentage (%) of Poorly water-soluble Drugs in Artificial Gastric Juice

Prep.	Time (hours)								
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0
Keto. Powder	432 (72.0)	437 (72.8)	436 (72.7)	436 (72.6)	434 (72.4)	439 (73.2)	437 (72.7)	435 (72.5)	437 (72.7)
Exmp. I	46.7 (95.9)	49.4 (101.5)	50.5 (103.8)	50.8 (104.3)	50.8 (104.3)	50.6 (104.1)	50.4 (103.6)	50.5 (103.7)	50.5 (103.8)
Exmp. II	49.5 (108.9)	51.6 (113.5)	52.7 (115.9)	53.1 (116.9)	53.5 (117.7)	53.4 (117.4)	52.9 (116.4)	82.9 (116.4)	53.0 (116.6)
Exmp. III	51.6 (107.5)	51.6 (107.6)	52.7 (109.9)	53.1 (110.8)	53.5 (111.6)	53.8 (112.2)	53.0 (110.6)	53.4 (111.3)	53.0 (110.7)
Exmp. IV	51.7 (112.3)	51.4 (111.8)	51.4 (111.7)	51.2 (111.3)	51.6 (112.2)	52.0 (113.0)	51.5 (111.9)	50.9 (110.6)	51.8 (112.6)
Exmp. V	50.3 (111.5)	50.9 (112.7)	50.4 (111.7)	50.7 (112.3)	50.9 (112.7)	50.8 (112.4)	50.8 (112.4)	60.0 (112.9)	50.7 (112.3)
Exmp. VI	45.8 (99.0)	46.3 (100.0)	46.2 (99.8)	46.2 (99.9)	46.2 (99.9)	45.8 (98.9)	45.6 (98.5)	45.1 (97.5)	45.8 (99.1)
Exmp. VII	48.8 (100.4)	48.8 (100.4)	48.9 (100.4)	48.9 (100.6)	49.0 (100.9)	49.9 (102.5)	49.9 (102.7)	50.2 (103.2)	50.1 (102.9)
Exmp. VIII	46.5 (104.3)	45.8 (102.8)	45.9 (102.9)	45.5 (102.1)	46.4 (104.2)	46.4 (104.1)	45.8 (102.8)	45.3 (101.7)	45.6 (102.3)
Cisa-pride Powder	-	5.249 (51.57)	-	5.492 (54.51)	5.914 (58.63)	6.243 (61.81)	6.173 (61.22)	-	6.446 (65.80)

Exmp. XVII	-	8.83 (85.27)	-	8.74 (84.09)	9.12 (87.9)	8.79 (84.54)	9.13 (87.81)	-	9.30 (89.47)
Exmp. XVIII	-	9.84 (103.2)	-	9.74 (102.1)	10.03 (105.2)	9.93 (104.3)	9.76 (102.4)	-	9.68 (101.5)

As shown in Table 2, ketoconazole, although it can be released in the artificial gastric juice to an extent because of its acidic property, is relatively further improved in the releasing level and percentage when it is incorporated in the oleic acid-containing solid dispersed preparations. Therefore, these data are consistent with those of Experiment I. In the meanwhile, cisapride was released to an extent by virtue of its solubility, but also considerably increased in the releasing properties when it was used in the solid dispersed preparations of the present invention.

TABLE 3

Releasing Level (μg/ml) and Percentage (%) of Poorly water-soluble Drugs in Artificial Intestinal Juice

Prep.	Time (hours)								
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0
Ketoconazole Powder	1.84 (0.092)	1.89 (0.095)	1.91 (0.096)	1.92 (0.096)	1.94 (0.97)	1.99 (0.099)	1.98 (0.099)	2.05 (0.103)	2.08 0.104
C.Exmp	3.05 (4.99)	3.38 (5.53)	3.69 (6.05)	3.71 (6.08)	3.75 (6.14)	3.84 (6.29)	3.87 (6.33)	3.87 (6.34)	4.32 (7.08)
Exmp. I	4.89 (10.05)	5.14 (10.56)	5.68 (11.67)	5.80 (11.91)	5.17 (10.61)	5.2 (10.68)	5.2 (10.68)	5.32 (0.92)	6.00 (12.33
Exmp.	3.55	3.61	3.71	3.98	3.7	3.97	4.09	4.11	4.29

II	(7.82)	(7.94)	(8.15)	(8.75)	(8.13)	(8.73)	(8.98)	(9.04)	(9.42)
Exmp. III	1.44 (3.00)	1.45 (3.04)	1.46 (3.05)	1.67 (3.47)	1.77 (3.69)	1.92 (3.99)	1.95 (4.06)	2.14 (4.47)	2.36 (4.92)
Exmp. IV	1.03 (2.23)	1.27 (2.76)	1.31 (2.84)	1.36 (2.95)	1.45 (3.15)	1.48 (3.21)	1.57 (3.41)	1.63 (3.54)	1.69 (3.67)
Exmp. V	2.21 (4.89)	2.23 (4.94)	2.21 (5.03)	2.27 (5.10)	2.31 (5.15)	2.33 (5.46)	2.47 (5.26)	2.38 (5.34)	2.40 (5.30)
Exmp. VI	2.78 (6.00)	2.53 (5.47)	2.42 (5.23)	2.54 (5.49)	2.19 (4.72)	2.41 (5.21)	2.3 (4.97)	2.34 (5.06)	2.45 (5.29)
Exmp. VII	2.09 (4.28)	2.03 (4.16)	2.1 (4.31)	2.20 (4.51)	2.07 (4.26)	2.2 (4.52)	2.16 (4.43)	2.08 (4.26)	2.08 (4.26)
Exmp. VIII	2.26 (5.07)	2.51 (5.61)	2.42 (5.42)	2.64 (5.92)	2.58 (5.77)	2.57 (5.76)	2.42 (5.41)	2.52 (5.64)	2.59 (5.81)
Exmp. IX	3.55 (10.70)	3.95 (11.89)	4.12 (12.41)	4.27 (12.86)	4.28 (12.88)	4.34 (13.08)	4.38 (13.19)	4.38 (13.20)	4.36 (13.13
Exmp. X	2.37 (6.75)	2.48 (7.05)	2.39 (6.79)	2.14 (6.08)	2.78 (7.92)	2.67 (7.60)	3.42 (9.72)	3.61 (10.27)	3.63 (10.33
Cisapride Powder	-	0 (0)	-	0 (0)	0 (0)	0 (0)	0.005 (0.047)	0.028 (0.27)	0.0745 (0.618
Exmp. XVII	-	2.43 (20.15)	-	3.22 (26.7)	2.70 (22.42)	2.66 (22.1)	2.64 (21.94)	3.10 (25.73)	3.99 (33.12
Exmp. XVIII	-	6.34 (63.0)	-	6.75 (67.01)	6.56 (65.15)	6.55 (65.05)	6.69 (66.46)	6.74 (66.9)	6.96 (69.05

The effect of the solid dispersed preparations on improving the releasing rates of the two drugs is more apparent in the artificial intestinal juice, a more difficult condition in which for the two drugs to dissolve. As shown in Table 3, the releasing properties of drugs are better when they are incorporated in the solid dispersed preparations using fatty acid and oil than when they are used alone. A better improvement effect was obtained from the solid dispersed preparations containing oleic acid. Further, the use of microemulsified oleic acid brought

about a great advance in the releasing properties.

In a ddition, the solid dispersed preparations containing itraconazole, its derivatives, and cyclosporine, respectively, was tested for the releasing properties in the artificial gastric and intestinal juices. The results are given in Table 4. Also, the data of Table 4 demonstrate that the drugs in the solid dispersed preparations are superior to the drugs alone in the releasing properties.

TABLE 4

Releasing Level (μg/ml) of Poorly water-soluble Drugs in Artificial Gastric and Intestinal Juice

Prep.	Time (Min)											
	Artificial Gastric Juice						Artificial Intestinal Juice					
	5	15	30	60	90	120	5	15	30	60	90	120
Itra[1]	14.4	16.4	17.4	16.7			0.05	0.05	0.05	0.05	-	-
Exmp.XII	292	293	321	331			130	95.0	146	102	-	-
Exmp.XIII	138	160	179	192			246	214	204	203	-	-
Itra Drv.[2]	96.9	156.2	189.2	211.0	216.7		-	-	-	-	-	-
Exmp.XIV	204.5	198.6	232.6	252.9	259.8		-	-	-	-	-	-
Cyclo.[3]	-	-	1.8	1.7	-	1.9	-	-	2.1	2.3	-	2.5
Exmp.XV	-	-	111.4	94.8	-	71.7	-	-	102.8	99.4	-	91.0
Exmp.XVI	-	-	11.1	10.8	-	9.8			595.0	66.3	-	56.7

[1] Itrakonazole powder

[2] Itrakonazole derivative

[3] Cyclosporine

EXPERIMENT III: Uptake of Poorly water-soluble Drugs in

Rabbit 's Gastrointestinal tract

The solid dispersed preparations containing ketoconazole, prepared from Examples, were tested for the uptake in rabbit's gastrointestinal tract. The results are given in Table 5.

In this regard, first, a rabbit was killed by introducing air in its ear vein and its stomach, duodenum, jejunum, ileum, colon and rectum were excised and washed with physiological saline solution at 37 °C. These organs were fixed between the receptor and donor of a Franz diffusion cell. In the receptor, a physiological saline solution warmed to 37 °C was poured, and stirred with a magnetic stirrer while the solid dispersed preparations obtained in Examples were added in the donor. Samples were harvested from the receptor at predetermined times for 6 hours while the receptor was supplemented with a fresh physiological saline solution in order to constantly maintain the total volume in the receptor. The samples taken were measured for their plasma concentrations of drugs.

TABLE 5

Uptake ($\mu g/cm^2$) of Ketoconazole in Rabbit's GI tract

Prep.	Time (hours)							
	0.3	0.67	1	1.5	2	3	4	6
C. Exmp	0	0.92	2.45	4.50	4.67	5.15	5.56	5.95

Exmp. I	0	1.72	3.44	5.46	9.10	10.2	11.6	13.2
Exmp. II	0.50	2.78	5.50	9.83	15.5	16.3	18.6	22.4

It is apparent from the data of Table 5 that the uptake of the drug in the GI tract is much better when it is incorporated in the solid dispersed preparation using oleic acid than when it is incorporated in the conventional dispersed preparation which uses a water-soluble matrix merely. Particularly, a significant improvement in the uptake of ketoconazole in the GI tract was brought about by the use of the solid dispersed preparations obtained from microemulsions containing oleic acid. These results are consistent with those of Experiment I and II.

EXPERIMENT IV: Comparison of the Plasma Concentration of Drugs Formulated into a Solid Dispersed Preparation and Commercially Available Ones

Before an experiment, male mice (Sprague-Dawley lineage) weighing 250-310 g, purchased from the Korea National Institute of Health, were adapted to new circumstances for 1-2 weeks. After the mice, which were starved from one day before the experiment, were etherized, their left femoral arteries were inserted with cannulas connected to syringes containing 80 IU/ml of heparin. After 2 hours, the mice came out of the ether and were administered with a suspension of the cyclosporine-

containing solid dispersed preparation of the present invention and a commercially available preparation with the aid of a sonde. At an interval of a predetermined period of time, blood was taken from the left femoral arteries and measured for the plasma concentration of drug.

With reference to Fig. 1, the cyclosporine level in blood are plotted against the times after administration for the solid dispersed preparations of the present invention and a commercially available preparation. As shown in the graph, the plasma concentration of the solid dispersed preparations according to the present invention are similar to that of the commercially available preparation, Neoral. Although being a little bit lower concentration than that of Neoral as a whole, the solid dispersed preparations according to the present invention are thought to have the initiatives to substitute for the conventional preparations which contain liquid drugs, in a pharmaceutical aspect.

Solid dispersed preparations according to the present invention and a commercial available preparation, all containing itraconazole as a medicinally effective ingredient, were administered to beagle dogs in an oral route. After a blood sample was taken from their veins at an interval of a predetermined period of time, the plasma concentration of drug was measured. The results are given in Table 6.

TABLE 6

Itraconazole Level (μg/ml) in Blood According to Times

Prep.		Time (hours)							
		1	2	3	4	6	8	10	24
Starved Dog	Exmp. XII	0	0.03	0.03	0.04	0.03	0.02	0.02	0.02
	Exmp. XIII	0.02	0.06	0.07	0.08	0.10	0.07	0.04	0.03
	Purchased	0	0.06	0.04	0.03	0.09	0.03	0.06	0.03
Non-starved	Exmp. XIII	0.12	0.41	0.38	0.44	0.43	0.43	0.42	0.36
	Purchased	0.30	0.60	0.79	0.58	0.54	0.44	0.41	0.30

As apparent from Table 6, a similar pharmacokinetic pattern was observed between the plasma concentration of itraconazole from the solid dispersed preparations and from the conventional preparation (itazol) when starved beagle dogs were administered therewith, while the lower value shown in case the preparation of Examp. XII was administrated. In non-starved beagle dogs, the drug reached a high maximal level in blood within a fast period of time when the commercially available preparation was administered whereas the preparation of Example XIII maintained the plasma concentration of drug constantly, owing to its solubilization in the gastro-intestinal tract.

EXPERIMENT V: Solubility of Aceclofenac in Various Vehicles

Excess aceclofenac was added in 5 ml of a vehicle in a test tube, which was then voltexed to an extent that the drug

was not dissolved further, and incubated for 3 days in a 37 °C water bath. The resulting solution was filtrated through a 0.2 μm filter paper (Millipore, Waters, Milford, MA, USA) and the filtrate was diluted for the convenient quantification of the drug. The solubility results are given in Table 7.

Table 7

Solubility of Aceclofenac in Vehicles

Vehicles	Solubility (mg/mP)
Transcutol	149.34
Labrasol	114.83
Tween 80	98.70
Tween 20	85.71
Cremophor EL	40.92
Cremophor RH40	23.34
Oleic acid	4.59
Linoleic acid	5.44
Triacetin	18.01
Castor oil	13.21
Sesame oil	2.83
Corn oil	2.20
Mineral oil	0.34

As apparent from the data of Table 7, large values are found in the solubility of aceclofenac in fatty acids, triacetin, castor oil and cremophor. Particularly, the drug is dissolved at great amounts in transcutol, labrasol and Tweens.

EXPERIMENT VI: Releasing of Aceclofenac in Solid Dispersed Preparations Against Artificial Gastric and Intestinal

Juices

The solid dispersed preparations comprising aceclofenac, obtained in Examples XXIV to XXVII, were tested for releasing properties against artificial gastric juice and artificial intestinal juice in a similar manner to that of Experimental Example II.

The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Tables 8 and 9.

Table 8

Releasing Level (μg/ml) and Percentage (%) of Aceclofenac in Artificial Gastric Juice

Prep.	Time (hours)						
	0.25	0.5	0.75	1.0	1.5	2.0	3.0
Aceclofenac Powder	0.46 (0.23)	0.53 (0.26)	0.57 (0.28)	0.60 (0.30)	0.61 (0.31)	0.69 (0.34)	0.73 (0.37)
Exmp. XXIV	1.01 (0.51)	1.16 (0.58)	1.29 (0.65)	1.33 (0.67)	1.35 (0.67)	1.43 (0.72)	1.38 (0.69)
Exmp. XXV	1.68 (0.84)	2.38 (1.19)	2.43 (1.22)	2.51 (1.25)	2.68 (1.34)	2.65 (1.33)	2.70 (1.35)
Exmp. XXVI	1.61 (0.80)	1.88 (0.94)	1.96 (0.98)	1.98 (0.99)	1.99 (1.10)	1.95 (0.98)	2.08 (1.04)
Exmp. XXVII	1.76 (0.88)	2.04 (1.02)	2.36 (1.18)	2.51 (1.26)	2.61 (1.30)	2.70 (1.35)	2.63 (1.31)
Airtal	0.93 (0.46)	1.02 (0.51)	1.18 (0.59)	1.23 (0.61)	1.32 (0.66)	1.34 (0.67)	1.39 (0.70)

As shown in Table 8, the releasing of aceclofenac in the artificial gastric juice was much improved when it was

in the solid dispersed preparations of the present invention relatively to the other preparations.

TABLE 9

Releasing Level (μg/ml) and Percentage (%) of Aceclofenac in Artificial Intestinal Juice

Prep.	Time (hours)							
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	5.0
Aceclo. Powder	88.37 (44.19)	117.34 (58.67)	121.65 60.82	126.64 (63.32)	128.10 (64.05)	131.70 (65.85)	136.55 (68.27)	136.55 (68.28)
Exmp. XXIV	152.97 (76.49)	157.43 (78.72)	161.90 80.95	160.40 (80.20)	162.66 (81.33)	164.09 (82.05)	165.27 (82.63)	166.71 (83.35)
Exmp. XXV	151.72 (75.86)	163.33 (81.67)	161.72 80.86	163.11 (81.55)	162.26 (81.13)	165.57 (82.79)	166.16 (83.08)	166.16 (83.08)
Exmp. XXVI	148.21 (74.10)	152.40 (76.20)	154.58 77.29	154.95 (77.47)	154.49 (77.24)	155.48 (77.74)	157.97 (78.99)	159.74 (79.87)
Exmp. XXVII	138.83 (69.41)	150.41 (75.21)	155.85 77.92	161.51 (80.75)	161.63 (80.81)	163.29 (81.64)	164.22 (82.11)	167.36 (83.68)
Airtal	133.76 (66.88)	136.54 (68.27)	136.62 68.31	137.70 (68.85)	142.55 (71.28)	145.72 (72.86)	143.66 (71.83)	142.34 (71.17)

As known from Table 9, aceclofenac, although it can be released in the artificial gastric juice to an extent because of its basic property, is relatively further improved in the releasing level and percentage when it is formulated into the solid dispersed preparation.

EXPERIMENT VII: Comparison of Plasma Concentration of Aceclofenac Between Solid Dispersed Preparation and Conventional Ones

Before an experiment, male mice (Sprague-Dawley lineage) weighing 250-310 g, purchased from the Korea National Institute of Health, were adapted to new circumstances for 1-2 weeks. After the mice, which were starved from one day before the experiment, were etherized, their left femoral arteries were inserted with cannulas connected to syringes containing 50 IU/ml of heparin. After 2 hours, the mice came out of the ether and were administered with a suspension of the aceclofenac-containing solid dispersed preparation of the present invention and a aceclofenac powder with the aid of a sonde. At an interval of a predetermined period of time, blood was taken from the left femoral arteries and measured for the plasma concentration of the drug.

In the meanwhile, the aceclofenac-carrying solid dispersed preparations of the present invention and a commercial available preparation were orally administered to beagle dogs and volunteers. At predetermined times after oral administration, blood was taken from the beagle dogs and the volunteers and measured for the drug levels.

After the oral administration of the aceclofenac-carrying solid dispersed preparations of the present invention, an aceclofenac powder and a commercial available preparation to mice, beagles dogs and volunteers, the plasma concentration of the drug with time were compared and are plotted in Figs. 2 to 4.

As shown in the graphs, the solid dispersed preparations of

the present invention maintain higher levels of aceclofenac in blood for all of the testees than the commercially available preparation. In addition, the use of the solid dispersed preparation according to the present invention was affirmed to increase the maximal value of plasma concentration and area under the curve, which are pharmacokinetic parameters, by 1.5-6 times.

After the oral administration of the aceclofenac-carrying solid dispersed preparation of the present invention, a commercially available preparation and an aceclofenac powder, the plasma concentration of aceclofenac was monitored with time and the results are given in Tables 10 to 12, below.

TABLE 10

Aceclofenac Level (μg/ml) in Blood of Rat

Prep.	Time (hours)								
	0.25	0.5	0.75	1	1.5	2	3	4	6
Exmp. XXIV	11.11	14.30	12.96	8.01	4.45	3.38	2.60	0.70	0.85
Aceclo. Powder	1.85	0.71	0.44	0.15	0.03	0.16	0.21	0.27	0.13

It is apparent from the data of Table 10 that the aceclofenac level in blood is significantly improved when the drug is administered by use of the preparation of the present invention relative to when aceclofenac is administered alone.

TABLE 11

Plasma Concentration (μg/ml) of Aceclofenac in Beagle Dogs

Prep.	Time (hours)											
	0.25	0.5	0.75	1	1.5	2	3	5	7	9	12	24
Exmp. XXIV	4.9	41.1	74.1	81.8	93.0	96.5	71.1	49.4	32.2	21.6	11.1	1.3
Airtal	4.6	15.1	28.9	35.7	50.8	43.2	31.9	16.6	8.8	6.4	3.9	0.8

The data of Table 11 demonstrate that the aceclofenac-carrying solid dispersed preparation of the present invention is superior to the conventional preparation in the plasma concentration.

TABLE 12

Plasma Concentration (μg/ml) of Aceclofenac in Human Blood

Prep.	Time (hours)										
	0.25	0.5	0.75	1	1.5	2	3	5	7	9	12
Exmp. XXIV	0.16	4.67	10.98	18.12	12.99	6.93	2.97	1.02	0.67	0.56	0.42
Airtal	0.85	1.75	3.84	5.51	5.48	8.34	3.44	0.48	0.29	0.14	0.10

As apparent from the data of Table 12, higher levels of aceclofenac in blood are maintained when the solid dispersed preparation of the present invention is administered than when the commercially available preparation is used.

EXPERIMENT VIII: Releasing of Cisapride From Solid

Dispersed Preparations Against Artificial Gastric and Intestinal Juices

The solid dispersed preparation comprising cisapride, obtained in Examples XXXI, was tested for releasing properties against artificial gastric juice and artificial intestinal juice in a similar manner to that of Experimental Example II.

The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Table 13.

TABLE 13

Releasing Level (μg/ml) and Percentage (%) of Cisapride in Artificial Gastric and Intestinal Juice

Juice	Time (hour)									
	0.5	1.0	1.5	2.0	3.0	4	5	6	8	12
Gastric	14.9	26.2	32.0	43.4	58.5	-	84.7	-	-	-
Intestina	8.1	16.5	25.2	38.1	48.5	62.1	-	72.7	87.8	98.0

The amount of the drug released from the solid dispersed preparation was increased almost linearly in both artificial gastric and intestinal juices, showing a zero order-like kinetics.

EXPERIMENT IX: Comparison of Plasma Concentration of

Cisapride Between Solid Dispersed Preparation and Commercially Available Ones

At an interval of a predetermined period of time after the oral administration of beagle dogs with the cisapride-carrying solid dispersed preparation obtained in Example XXXII and a commercially available preparation, blood was taken from the testees and measured for plasma concentration of drug.

With reference to Fig. 5, the cisapride levels in blood are plotted against the times after administration for the solid dispersed preparations of the present invention and a commercially available preparation, Prepulsid tablet. As shown in the graph, the plasma concentration of the solid dispersed preparations according to the present invention are greatly improved relative to that of the commercially available preparation. These drug concentrations are numerically shown in Table 14, below.

TABLE 14

Plasma Concentration of Cisapride (μg/ml) in Beagle Dog

Prep.	Time (hours)											
	0.5	0.75	1	1.25	1.5	2	3	5	7	9	12	24
Exmp. XXXII	48	80	68	82	97	151	271	284	152	104	83	63
Prepulsid	49	61	70	83	102	184	201	134	75	58	46	41

As shown in Table 14, the plasma concentration of cisapride is maintained at higher levels when the solid dispersed preparation of the present invention is used than when the conventional preparation.

As described hereinbefore, the solid dispersed preparations of the present invention are improved in the solubility of poorly water-soluble drugs in the gastro-intestinal tract, in detail, the releasing of the drugs against the gastric and intestinal juices, resulting in a great increase in the bioavailability of the drugs. In addition, the solid dispersed preparations of the present invention give the pharmaceutical solutions to the problems that the conventional semi-solid or liquid preparations possess, enabling medicinally effective, poorly water-soluble compounds to be formulated, molded and processed, quickly and in an economically favorable manner without use of any organic solvent.

The present invention has been described in an illustrative manner, and it is to be understood the terminology used is intended to be in the nature of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

WHAT IS CLAIMED IS:

1. A solid dispersed preparation for poorly water-soluble drugs, prepared by dissolving or dispersing the drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water-soluble polyol matrix and drying the mixture.

2. The solid dispersed preparation as set forth in claim 1, wherein the solid dispersed preparation is obtained by pulverizing the dried mixture to give a dispersed powdery preparation.

3. The solid dispersed preparation as set forth in claim 1, wherein the mixture is dried in such a way that the mixture is sprayed to pharmaceutically acceptable nuclei to give a dispersed granular preparation.

4. The solid dispersed preparation as set forth in claim 1, wherein the oil, the fatty acid or the mixture thereof is used in a form of an emulsion or a micro emulsion.

5. The solid dispersed preparation as set forth in claim 1, wherein the oil is selected from the group comprising α-bisabolol, stearyl glycerrhetinate, salicylic acid, tocopheryl acetate, sodium hyaluronate, panthenol, propylene glycol and apple (*Pirus Malus*), propylene

glycol and pineapple, ivy (*Hedera halix*) extract and 1,3-B.G, peach (*Prums persica*) leaf extract, hydrolyzed soy flour, wheat (*Triticum Vulgare*) protein, birch (*Betula alba*) extract and 1,3-B.G, burdock (*Arctium majus*) extract and 1,3-B.G, liposomes, phosphatidylcholines, glyceryl stearate, captylic/capric triglyceride, cetyl octanolate, isopropyl myristate, 2-ethylene isopelagonate, di-C12-13 alkyl malate, ceteatyl octanoate, butylene glycol dicaptylate/dicaprate, isononyl isostearate, isostearyl isostearate, coco-captylate/caprate, cetyl octanoate, octyldodecyl myristate, cetyl esters, C10-30 cholesterol/lanosterol ester, hydrogenated castor oil, monoglycerides, diglycerides, triglycerides, beeswax, canauba wax, suctose distearate, PEG-8 beeswax, ceresin, ozokerite, macadamia ternifolia nut oil, hydrogenated hi-erucic acid rape seed oil, olive oil, jojoba oil, hybridsunflower (Helian thus annuus) oil, and dog rose (rosa canina) lips oil.

6. The solid dispersed preparation as set forth in claim 5, wherein the oil is selected from the group comprising mineral oils, squalene, squalane, monoglycerides, diglycerides, triglycerides, medium chain glycerides, myglyol, cremophor, hydrogenated caster oil, corn oil, perilla oil, cotton seed oil and lipid-soluble vitamins.

7. The solid dispersed preparation as set forth in claim 1, wherein the fatty acid is selected from the group comprising oleic acid, cetyl alcohol, stearyl alcohol, stearic acid, myristic acid, linoleic acid and lauric acid.

8. The solid dispersed preparation as set forth in claim 7, wherein the fatty acid is selected from the group comprising oleic acid, linoleic acid, and isopropyl myristate.

9. The solid dispersed preparation as set forth in claim 1, wherein the water-soluble polymer matrix is selected from the group comprising polyethylene glycol (PEG), carbowax and polyvinyl pyrrolidone (PVP).

10. The solid dispersed preparation as set forth in claim 1, wherein the poorly water-soluble drugs are dissolved and dispersed in the oil, fatty acid or their mixture in the presence of a surfactant.

11. The solid dispersed preparation as set forth in claim 10, wherein the surfactant is selected from the group comprising glyceryl stearate, polysorbate 60, polysorbate 80, sorbitan trioleate, sorbitan sesquioleate, sorbitan stearate, PEG-20 glyceryl isostearate, ceteth-25, PEG-60 , PEG-60 hydrogenated

castor oil, nonoxynol-15, PEG-6-decyltetradeceth-20, dimethicone copolyol, glyceryl diisostearate, ceteth-24, cetearyl alcohol, polyoxylethylene nonyphenyl ether, PEG-40 hydrogenated castor oil, cetyl dimethicone copolyol, polyglyceryl-3-methylglucose distearate, PEG-100 stearate, sorbitan isostearate, sodium lauryl glutamate, disodium cocoamphodiacetate, lauric acid diethanolamide, coconut fatty acid diethanolamide, N,N-bis-(2-hydroxy ethyl)-cocomide, and cocoamidopropyl betain.

12. The solid dispersed preparation as set forth in claim 1, wherein the water-soluble polymeric matrix is used alone or in combination with other water-soluble matrices.

13. The solid dispersed preparation as set forth in claim 12, wherein the other water-soluble matrix is selected from the group comprising gelatin, gum, carbohydrates, celluloses, polyvinyl alcohol, polyacrylic acid, inorganic compounds and their mixtures, hydroxypropylmethylcellulose acetyl succinate, shellac, zein, polyvinyl acetate phthalate, Eudragit L100, Eudragit S100, sodium arginate, and poly-L-lysine.

14. The solid dispersed preparation as set forth in claim 1, wherein the poorly water-soluble drugs are selected

from the group comprising ketoconazole, itraconazole and its derivatives, cyclosporine, cisapride, acetaminophen, aspirin, acetylsalicylic acid, indomethacin, naproxen, warfarin, papaverine, thiabendazole, miconazole, cinnarizine, doxorubicin, omeprazole, cholecalciferol, melphalan, nifedipine, digoxin, benzoic acid, tryptophan, tyrosine, phenylalanine, aztreonam, ibuprofen, phenoxymethylpenicillin, thalidomide, methyltestosterone, prochlorperazine, hydrocortisone, dideoxypurine nucleoside, vitamin D_2, sulfonamide, sulfonylurea, p-aminobenzoic acid, melatonin, benzylpenicillin, chlorambucil, diazepin, digitoxin, hydrocortisone butyrate, metronidazole benzoate, tolbutamide, prostaglandin E1(PGE_1), fludrocortisone, griseofulvin, miconazole nitrate, leukotriene B_4 antagonist, propranolol, thephylline, flubiprofen, sodium benzoate, benzoic acid, riboflavin, benzodiazepine, phenobardital, glyburide, sulfadiazine, sulfaethylthiadiazole, sodium diclofenac, aceclofenac, phyniroin, hioridazinehydrochloride, bropirimine, hydrochlorothiazide, fluconazole, acyclovir, bucillamine, ciprofluoxacin, acetyl-L-carnitine, baclofen, sodium alendronate, lovocarnitine, nimodipine or nimodifine, atenolol, provastatin sodium, lovastatin, isotretinoin, etidronate disodium, doxifluridine, fosfomycin calcium, sotepine,

epinastine hydrochloride, carvedilol, epinastine hydrochloride, carvedilol, fosinopril, trandolapril, etretinate cap, metergoline, mercaptopurine, vancomycin hydrochloride, cefixime, cefuroxim axetil, dirithramycin, and dadanosin.

15. The solid dispersed preparation as set forth in claim 13, wherein the poorly water-soluble drugs are selected from the group comprising ketoconazole, itraconazole and its derivatives, cisapride, cyclosporine, nifedipine and aceclofenac.

16. Medicines for internal use such as powders, granules, tablets and capsules, prepared using the solid dispersed preparation of claim 1.

FIG. 1

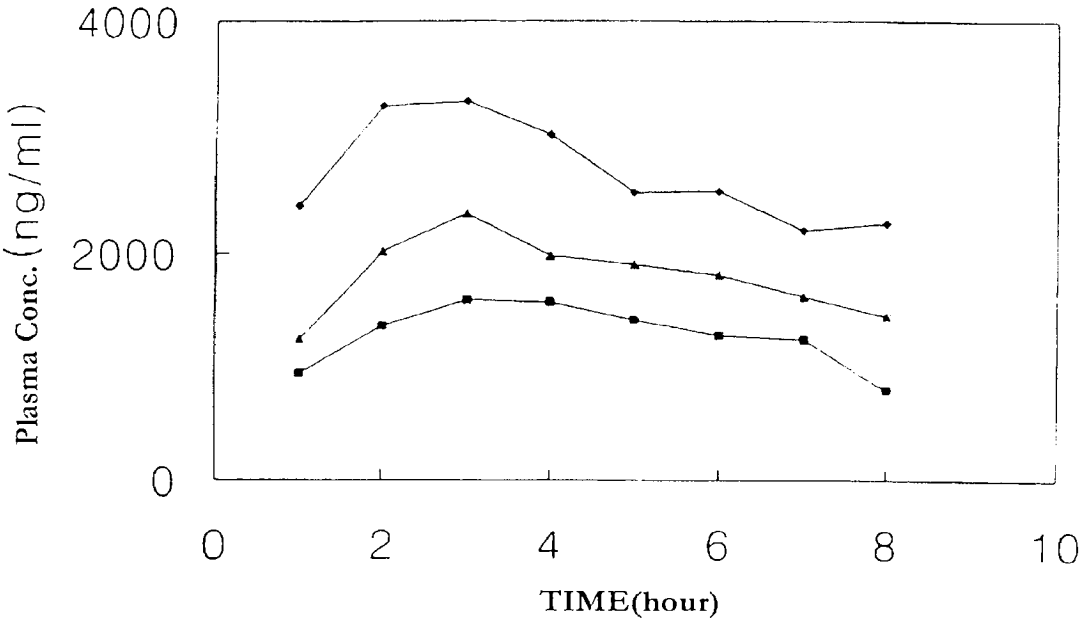


FIG. 2

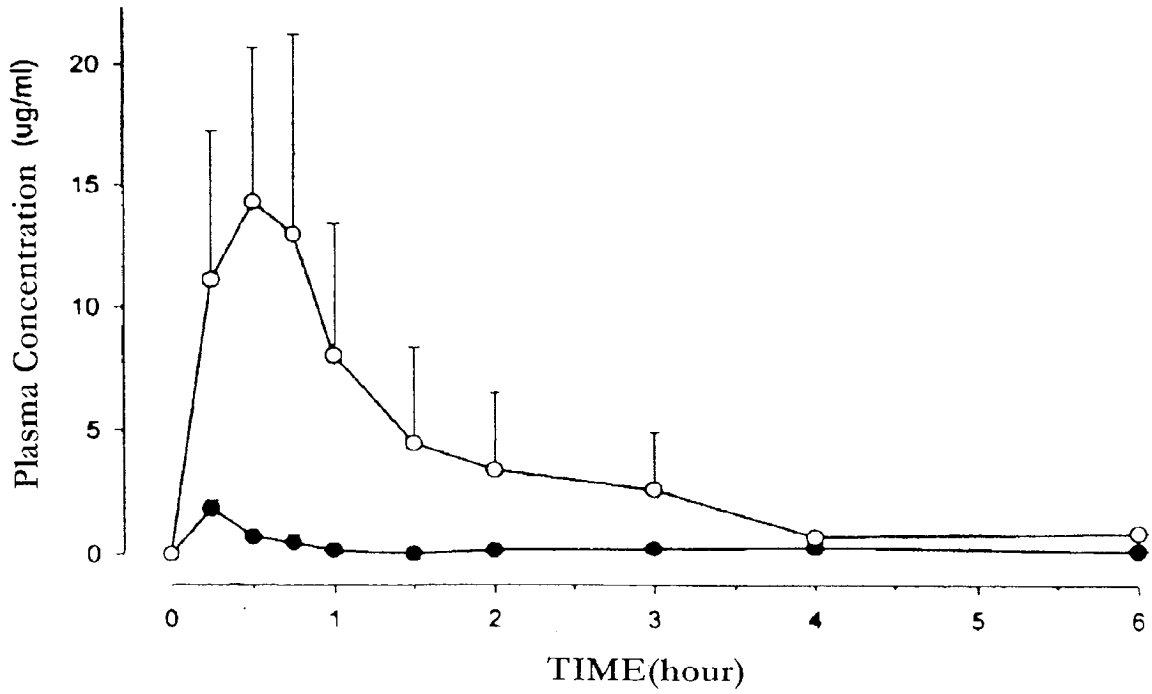


FIG. 3

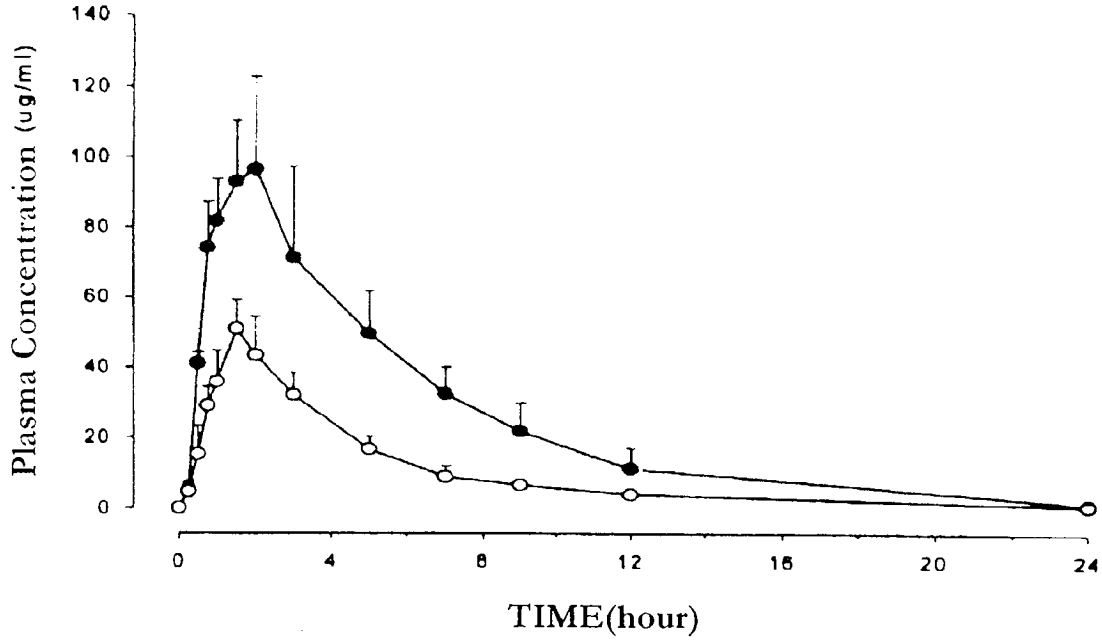


FIG. 4

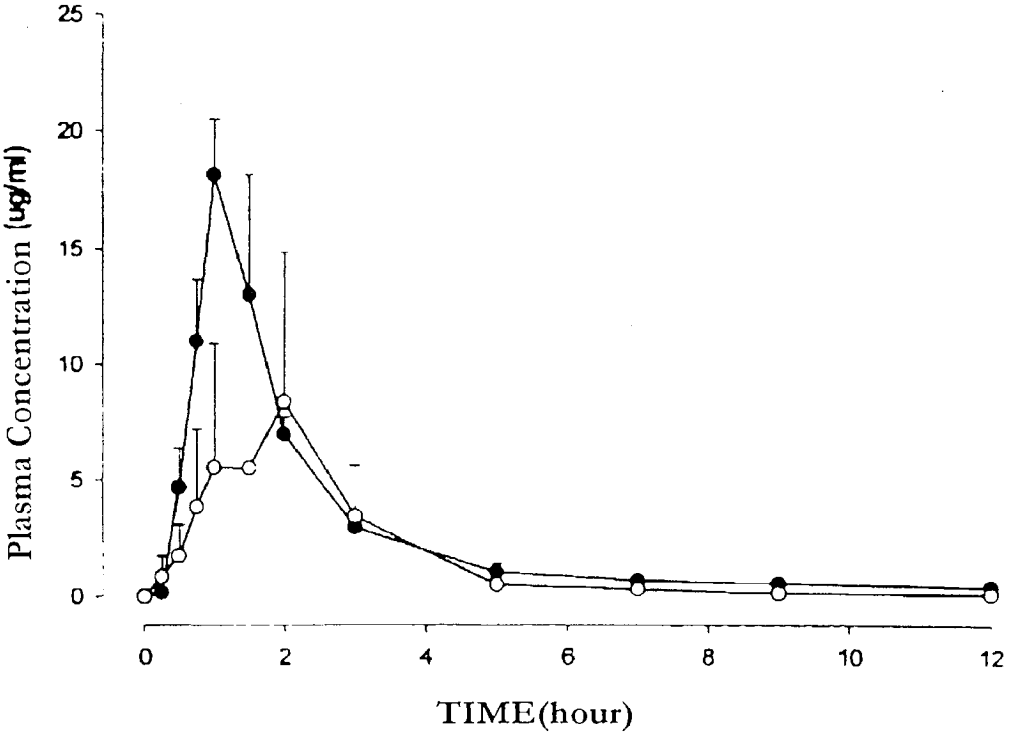
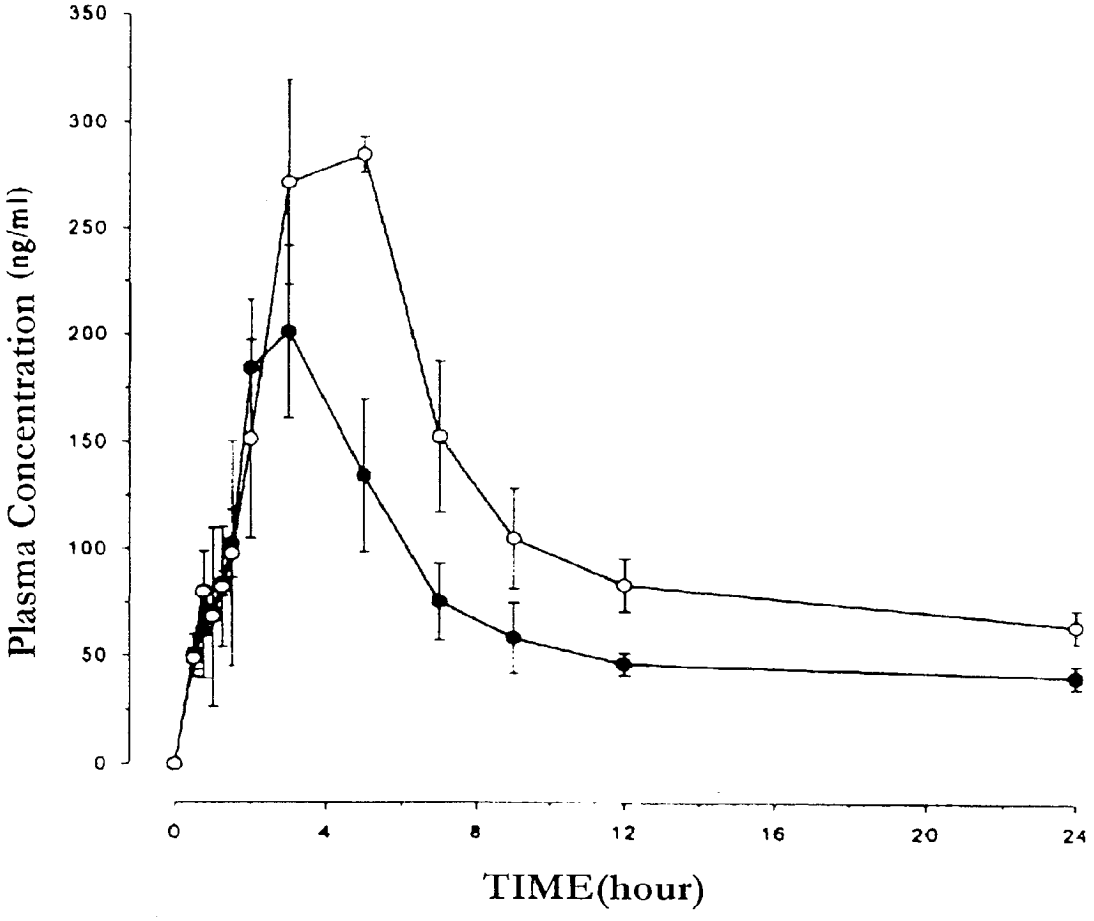


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00341

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: A 61 K 9/14, A 61 K 9/16, A 61 K 9/20, A 61 K 9/48, A 61 K 31/20, A 61 K 9/107, A 61 K 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 October 1999 (28.10.99)	03 December 1999 (03.12.99)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer Krenn Telephone No. 1/53424/435

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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(54) Title: CYCLOSPORIN FORMULATION

(57) Abstract: A pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, comprises a) a water-insoluble pharmaceutically active material; b) one or more propylene glycol esters of a fatty acid; c) surfactant; and either d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or e) a solid carrier. The composition is substantially free from ethanol.

CYCLOSPORIN FORMULATION

This invention relates to improved pharmaceutical compositions for the administration of water-insoluble pharmaceutically active substances especially, but not exclusively, cyclosporin.

In our European patent specification no. EP-A-0760237 there is described a pre-concentrate microemulsion composition comprising a water-insoluble pharmaceutically active material; a C_8 - C_{20} fatty acid mono-, di- or tri-glyceride from a vegetable oil or any mixture of two or more thereof; and a phospholipid and another surfactant. A stable oil-in-water microemulsion can be formed by mixing the preconcentrate composition with a hydrophilic phase. Unlike prior art microemulsion compositions, the microemulsion compositions of EP 0760237 are made by directly dissolving the active material in the oil phase and then dispersing the oil phase in the hydrophilic phase. This has certain advantages. For example, in the case of cyclosporin microemulsions, it eliminates or vastly reduces the tendency for solid microfine cyclosporin to be precipitated during use of the microemulsions, a problem encountered with many of the prior art microemulsions.

Whilst the microemulsions disclosed in EP-A-0760237 are generally very satisfactory in many ways, we have found that there is an upper limit to the bioavailability of the active material in the compositions of EP-A-0760237. We have now discovered that by judiciously alternating the components of the oil phase in the compositions of EP-A-0760237, the bioavailability of the active material can, surprisingly, be increased. The present compositions thus possess the advantages of the compositions of EP-A-0760237 together with, in addition, the advantage of increased bioavailability of the active material.

According to the present invention, there is provided a pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, which composition is substantially free from ethanol and comprises:

a) a water-insoluble pharmaceutically active material;

b) one or more propylene glycol esters of a fatty acid;

c) surfactant; and either

d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or

e) a solid carrier.

There is also provided a process for making a composition according to the invention, which process comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution either with component (d) or with component (e), and component (c) if not included earlier.

In the case of a microemulsion, the method of the invention thus comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c) but being free from hydrophilic phase, and then mixing the preconcentrate with the hydrophilic

phase, to form a stable oil-in water microemulsion, the composition being free from ethanol.

In the case of a solid composition, the method of the invention comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c), and then mixing the preconcentrate with the solid carrier, to form a solid, table composition of preconcentrate and carrier, the composition being free from ethanol.

In its broadest aspect, the present invention therefore encompasses two different formulations of the basic preconcentrate mixture. Both of these formulations possess the advantage of increased bioavailability of the active material.

Thus, in a first aspect, the invention provides a stable oil-in-water microemulsion composition wherein components (a) to (c) above have first been formed into a preconcentrate by wholly directly dissolving component (a) in component (b) optionally in the presence of component (c) (i.e. component (c) may be added later), and then mixing the preconcentrate with a hydrophilic phase. The microemulsion composition is generally liquid at room temperature and can, therefore, be advantageously provided in, for example, a soft gelatine capsule or as an oral solution such as an aqueous drink, for instance.

In a second aspect, the invention provides a stable, solid formulation comprising a blend of the basic preconcentrate mixture with a solid carrier. In this way, the preconcentrate mixture having increased bioavailability of the active material can, for example, be formulated into a free-flowing powder which, in turn can, for instance, be put into a hard gelatin capsule or compressed into a table. We generally prefer to formulate the composition of the invention in this way rather than as a microemulsion, since the solid formulation is simple to process and has excellent stability.

In the present invention, component (a) is a water insoluble pharmaceutically active material. The invention is particularly useful with the

cyclosporins, e.g. cyclosporin A. dihydrocyclosporin C, cyclosporin D and dihydrocyclosporin D. It is also useful with other water-insoluble substances such as, for example, water-insoluble peptides, or water-insoluble antimicrobial or antineoplastic substances. Examples include desmopresin, calcitonin, insulin, lenprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol, etoposide or mixtures thereof.

In the compositions of the invention, component (a) is in solution in component (b). Component (b) can be a propylene glycol ester of a fatty acid or a mixture of any two or more such esters. The fatty acids may optionally be derived from a vegetable oil and are preferably C_8 - C_{20} residues. Particular preferred compounds are propylene glycol monocaprylate (Caprgol 90) and propylene glycol monolaurate (Lauroglycol 90). We prefer to formulate the composition such that the weight ratio of component (a) to component (b) is from about 1:1 to about 1:10 but ratios outside this range can be used if desired.

These compounds, which increase the bioavailability of the active material can be used alone or in combination with one or more of the glycerides described in EP 0760237. For example, oleoyl macrogol-6 glycerides (Labrafil M 1944 CS), linoleoyl macrogol-6 glycerides (Labrafil M 2125 CS), and caprylocaproyl macrogol-8 glycerides (Labrasol) are particularly preferred compounds for use with the oils employed in the present invention.

Component (c) is a surfactant to provide the preconcentrate mixture and, where employed, the fully formed microemulsion with stability. Those skilled in the art will be aware of many surfactants which can be used, but we prefer to use polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene sorbitan monostearate. If desired, the surfactant can be mixed with a phospholipid, such as lecithin. We prefer to use a weight ratio of component (a) to surfactant of about 1:1 to about 1:50, but ratios outside this range can also be employed if desired. When a phospholipid is included in the

composition, we prefer to use a weight ratio of component (a) to phospholipid of about 1:05 to about 1:5.0, but, again, other ratios can be used.

In the case where the composition of the invention is provided as a microemulsion, component (d) is a hydrophilic phase. The preferred material is propylene glycol or diethylene glycol monoethyl ether (transcutol) but other substances can be used. Ethanol cannot be present. Water can of course also be present but it is not preferred. Despite the use of propylene glycol, component (a) remains wholly dissolved in the oil phase (component (b)).

Microemulsions are transparent due to the very small particle size of the dispersed phase, typically less than 200 nm. Such small droplets produce only weak scattering of visible light when compared with that from the coarse droplets (1 -10 nm) of normal emulsions. An essential difference between microemulsions and emulsions is that microemulsions form spontaneously and, unlike emulsions. required little mechanical work in their formulation. General reviews on microemulsions are provided by Attwood D. et al J. Colloid Interface Sci 46:249 and Kahlweit M. et al J. Colloid Interface Sci 118:436.

The microemulsions can be formed by diluting with aqueous liquid (e.g. water, fruit juice, milk etc.) to form an oil-in-water microemulsion, e.g. for oral administration. This aids in ready absorption as the surface area of the fat globules is largely increased. The role played by bile salts in the initial step of fragmentation of fat globules, essential for fat digestion, is circumvented.

The rate determining factor for the absorption of drug in the vehicle is not the enzymatic metabolism of triglycerides but rests primarily in the breakdown of the fat globules into micro particles since the enzymes (lipases) act mainly at the surface of the fat globules.

In the microemulsions of the invention, the amounts of the components, in percent by weight, are as follows:

Component	General	Usual	Preferred
Active pharmaceutical	1-12%	2.5-10%	7 -1 0%
Oil phase	20-80%	30-60%	25-40%
Surfactant	20-40%	25-60%	40-50%
Hydrophilic phase	10-60%	20-50%	25-30%

In the microemulsions, the weight percent of hydrophilic phase is generally up to about 75%, most usually from 15 to 50%, and preferably from 35 to 50%.

In the case where the composition of the present invention is provided as a blend of preconcentrate and solid carrier, component (e) is employed instead of component (d). Preferred solid carriers include colloidal silicon dioxide and polyvinyl pyrrolidone (cross Povidone) but other suitable inert solid substances can also be used, as will be clear to those skilled in the art. Typically, the solid carrier will be in the form of a dry powder. Generally, the preconcentrate mixture (comprising active material, oil and surfactant) is simply blended with the solid material such that the oily preconcentrate is absorbed by the material. Preferably, the blended mixture is provided in the form of a free-flowing powder. Such a powder can then be easily coated, for example, into a hard gelatin capsule or, alternatively, compressed into tablets, for instance. The technique of absorbing an oily phase (in this case an oily preconcentrate) on to a solid phase such as colloidal silicon dioxide followed by formulation into a final dosage form is a technique well known by those skilled in the art of formulation, so further details are considered unnecessary.

Both the microemulsion and solid compositions can consist only of the components described, or they can contain other substances. For example, in order to prevent oxidation/ rancidification of the natural oils, an antioxidant, e.g. □-to copherol can be used. Propyl gallate may be used as an alternative.

In order that the invention may be more fully understood, the following examples are given by way of illustration only.

Examples 1-3

Examples of compositions comprising a blend of preconcentrate and solid carrier are:

Example 1

Imusporin-25

Component	**mg/capsule**
Cyclosporin USP	25
Glyceryl Monolinoleate (Maisine 33-1)	17.25
Propylene glycol monocaprylate (Capryol 90)	17.25
Polyoxyl 35 Castor Oil NF (Cremophor EL)	50.00
Colloidal silicon dioxide	52.50
Crospovidone USP (PVP CL-M)	13.00
Net Fill Wt/cap (mg)	175.00

Example 2

Imusporin-50

Component	**mg/capsule**
Cyclosporin USP	50.00
Glyceryl Monolinoleate (Maisine 33-1)	34.50
Propylene glycol monocaprylate (Capryol 90)	34.50
Polyoxyl 35 Castor Oil NF (Cremophor EL)	100.00
Colloidal silicon dioxide	105.00
Crospovidone USP (PVP CL-M)	26.00
Net Fill Wt/cap (mg)	350.00

Example 3

Imusporin-100

Component	**mg/capsule**
Cyclosporin USP	100.00
Glyceryl Monolinoleate (Maisine 33-1)	69.00
Propylene glycol monocaprylate (Capryol 90)	69.00
Polyoxyl 35 Castor Oil NF (Cremophor EL)	200.00
Colloidal silicon dioxide	210.00
Crospovidone USP (PVP CL-M)	52.00
Net Fill Wt/cap (mg)	700.00

The blended preparations were made as follows:

1 Mix Maisine 35-1, Capryol 90 and Cremophor EL in a clean jacketed vessel.

2 Add Cyclosporin to the above vessel under stirring, continue stirring for about 70-75 mins. If required, heat the blend to not more than 50°C till the drug dissolves completely.

4 Cool the above blend to room temperature and strain through 150#.

5 Sift Aerosil and Crospovidone through 20# and 40# respectively. Mix in a suitable mixer.

6 Adsorb the above blend (step 4) over the mixture of Aerosil and Crospovidone.

7 Pass the powder blend of Cyclosporin through 20#.

8 Fill this blend in hard gelatin capsules or compressed with tablets.

The blends were then either fill into hard gelatin capsules or compressed into tablets.

Examples 4 - 8

Microemulsions of the invention were made of the compositions indicated, by dissolving the cyclosporin A in the oils and then forming the oil-in-water emulsions. The procedure was:

(a) dissolve the cyclosporin A in the mixture of oils with slight warming and under stirring to obtain a clear yellow liquid. Confirm the complete dissolution of the drug by microscopy.

(b) add the surfactant with stirring.

(c) add the hydrophilic phase with stirring

(d) add the alpho tocopherol and mix thoroughly.

Example 4

Preparation of microemulsion for administration in Soft Gelatin capsules:

Component	**mg/capsule**
Capryol 90	130
Castor oil	130
Polyoxyl-40 hydrogenated	400
Castor oil	-
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 5

Preparation of microemulsion for administration as oral solution:

Component	**mg/capsule**
Capryol 90	150
Maisine	125

Polysorbate-80 (Tween 80)	425
α-tocopherol	10
Transcutol	225
Cyclosporin A	100

Example 6

Preparation of microemulsion for administration as oral solution

Component	**mg/capsule**
Capryol 90	275
Polyoxyl-40 hydrogenated castor oil	425
α-tocopherol	10
Propylene glycol	225
Cyclosporin A	100

Example 7

Preparation of microemulsion for administration as oral solution:

Component	**%**
Capryol 90	130
Lauroglycol 90	130
Polysorbate 80 (Tween 80)	400
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 8

Preparation of microemulsion for administration as oral solution:

Component	mg/capsule
Capryol 90	14
Maisine	15
Polyoxyl-40 hydrogenated castor oil	45
α-tocopherol	1
Transcutol	25
Cyclosporin A	10

The oral solution which is filled into bottles can be administered using a syringe or more preferably with the aid of a metered dose pump with a dropper actuator.

The compositions described in Examples 4 to 8 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C). Ref 40°C-80% RH and 45°C, after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Neoral capsules containing 100mg cyclosporin A per capsule was also examined. It was observed from the above examination that the stability of solutions prepared according to the process of invention did not differ from the stability of the commercially available composition.

CLAIMS:

1. A pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, which composition is substantially free from ethanol and comprises:

a) a water-insoluble pharmaceutically active material;

b) one or more propylene glycol esters of a fatty acid;

c) surfactant; and either

d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or

e) a solid carrier.

2. A composition according to claim 1, which composition is a microemulsion comprising components (a), (b), (c) and (d).

3. A composition according to claim 1, which composition is a blend of said preconcentrate and said solid carrier comprising components (a), (b), (c) and (e).

4. A composition according to claim 1, 2 or 3, wherein component (a) is a cyclosporin, or another water-insoluble peptide, or a water-insoluble antimicrobial or antineoplastic substance or mixtures thereof.

5. A composition according to claim 4, wherein component (a) is cyclosporin A, dihydrocyclosporin C, cyclosporin D or dihydrocyclosporin D, or desmopresin, calcitonin, insulin, leuprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol or etoposide or mixtures thereof.

6. A composition according to any preceding claim, wherein component (b) is a propylene glycol ester of C_{12} to C_{18} fatty acids.

7. A composition according to any preceding claim, wherein said surfactant is polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate or mixtures thereof.

8. A composition according to any preceding claim, wherein component (c) further comprises a phospholipid.

9. A composition according to any preceding claim, wherein the weight ratio of component (a) to component (b) is from 1:1 to 1:10.

10. A composition according to claim 8 or 9, wherein the weight ratio of component (a) to said phospholipid is from 1:0.5 to 1:5.0.

11. A composition according to any preceding claim, wherein the weight ratio of component (a) to said surfactant is from 1:1 to 1:5.0.

12. A composition according to any of claims 1-9 and containing component (e), wherein component (e) is colloidal silicon dioxide, polyvinyl pyrrolidone or a mixture thereof.

13. A soft gelatin capsule or oral administration fluid which comprises a composition as claimed in any of claims 1 to 11

14. A tablet or hard gelatin capsule which comprises a composition as claimed in any of claims 1 to 12 when in said solid form.

15. A process for making a composition according to claim 1, which comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution either with component (d) or with component (e) and component (c) if not included earlier.

16. A process according to claim 150, wherein a preconcentrate composition is mixed with component (d).

17. A process according to claim 15, wherein a preconcentrate composition is mixed with component (e).

18. A method of making a pharmaceutical composition according to any of claims 1 to 11, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c) but being free from hydrophilic phase, and then mixing the preconcentrate with the hydrophilic phase, to form said stable oil-in-water microemulsion, the composition being free from ethanol.

19. A method of making a pharmaceutical composition according to any of claims 1-12, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c), and then mixing the preconcentrate with the solid carrier, to form a solid, stable composition if preconcentrated and carrier, the composition being free from ethanol.

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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3

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PCT/GB 00/04143

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 40051 A (ABBOTT LAB) 17 September 1998 (1998-09-17) examples 21,23,24 page 6, line 5 -page 6, line 8 page 6, line 34 -page 7, line 2	1,2,4-7, 9,11,15, 16,18
A	GB 2 222 770 A (SANDOZ LTD) 21 March 1990 (1990-03-21) examples 1.5.-1.10.	1,2, 4-11, 13-16,18

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Information on patent family members

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(54) Title: REMOVABLE GASTRIC BAND

(57) Abstract: A removable gastric band is provided which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present removable gastric band comprises an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises at least one aperture in the first zone and a button in the second zone, and where the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach. The removable gastric band can be easily paired with the use of a gastric electrostimulator and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. Such electrostimulation devices may either be incorporated into the removable gastric band or located at a distance from the removable gastric band.

REMOVABLE GASTRIC BAND

Related Application

This application claims priority from Italian Patent Application Number MI99A002641, filed December 7,1999.

Field of the Invention

The present invention relates to a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach.

Background of the Invention

Laparoscopic banding systems are available which provide for the use of an elongated main part that is placed around the stomach and closed over the stomach so as to reduce the diameter of the stomach to be able to treat the patient's obesity. Such currently available bands, however, present some drawbacks essentially due to the difficulty of application and/or removal of the gastric band. In fact, normally, the current bands' means of closing the elongated main part are almost always hard to manipulate; moreover, their connection entails the use of additional instruments and/or devices that further complicate the application and/or later removal of the gastric band for the surgeon.

Furthermore, to be able to remove the known bands, which must necessarily be done after a more or less long time interval, it is necessary to execute an additional surgical intervention and, consequently, to administer more anesthesia to the patient. The application and/or removal of the known bands also require the application of suture stitches, in addition to another intervention and more anesthesia. In particular, the bands used today are also hard to remove because they present little resistance to tissue adhesions and lack sufficient mechanical integrity to withstand tensile forces, both of which hinder their removal unless the patient is undergoing surgery.

It is desirable, therefore, to provide an improved gastric band which is both easier to implant within the patient and, when necessary, to remove from the patient.

Summary of the Invention

The present invention provides a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present invention provides a removable gastric band comprising an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach.

The present invention provides a removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The present invention also provides a method for treatment of obesity or for reducing weight in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The task proposed by the present invention is the realization of a removable gastric band that eliminates the above-noted drawbacks of the known gastric bands. Within the scope of this task, one important purpose of the invention is to realize a removable gastric band that can be removed without having to subject the patient to further intervention and, consequently, to additional general anesthesia.

Yet another purpose of the invention is to realize a removable gastric band that is easy to remove because it is highly resistant to adhesion to the tissue and has sufficient mechanical integrity to withstand tensile forces during removal. Yet another purpose of the invention is to realize a removable gastric band that can be applied without necessarily having to use suture stitches.

Yet another purpose of the invention is to realize a removable gastric band that can be applied and/or removed by the surgeon very simply and without having to use additional instruments or devices for that purpose. Another purpose of the invention is to realize a removable gastric band that is extremely easy to manipulate, so that it can be easily placed in and/or removed from the patient.

Brief Description of the Drawings

Figure 1 illustrates the gastric band according to the invention.

Figure 2 shows schematically the gastric band according to the invention being applied to the proximal tract of a patient's stomach;

Figure 3 shows the gastric band according to the invention applied to the proximal tract of the patient's stomach;

Figure 4 shows the gastric band according to the invention inflated so as to compress a portion of the patient's stomach;

Figure 5A shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band compressing the patient's stomach before the gastric band has been inflated;

Figure 5B shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band in relation to the patient's stomach after the gastric band has been inflated;

Figure 5C shows in cross-section sectional view a reinforcing member or element located within the elongated perimeter (i.e., the rib connecting the inner and outer surfaces, thereby forming an inflatable chamber or cavity) of the gastric band which reduces the tendency of the gastric band to twist around its longitudinal axis;

Figure 6A is a view of the gastric band attached to the patient's stomach with the inflation mechanism positioned to allow for inflation; and

Figure 6B is an expanded view of the inflation mechanism.

Detailed Description of the Invention

With reference to the figures described above, the removable gastric band according to the invention, indicated as a whole with reference number 1, comprises an elongated body 3 having a first or distal zone 30, a second or middle zone 32, a third or proximal zone 34, and a closure mechanism 2 for closing the elongated body 3 back upon itself so as to surround a portion, preferably the proximal tract, of the patient's stomach 4. The closure mechanism 2 preferably comprises a button 6 in the first zone 30 and a corresponding aperture 5 in the second zone 32 whereby the button 6 can fit

through the aperture 5 and fix or lock the elongated body 3 back onto to itself. Once locked into place, the gastric band 1 completely encircles and compresses a portion of the patient's stomach (see, e.g., Figures 3 and 4). Although only one aperture 5 is shown within the second zone 32, a plurality of such apertures can be provided if desired; using such a plurality of apertures allows the surgeon to more closely adjust the diameter of the encircling portion of the gastric band to the particular patient's situation and needs.

Appropriately, button 6 is suitably shaped and sized to allow it to be internally introduced into aperture 5, as well as to close, in an extremely simple but secure manner, the elongated main part 3 around stomach 4 and keep it in place. Although the button 6 and aperture 5 are preferably circular as shown in Figure 1, other shapes can be used so long as they provide the desired closing/locking action. Elongated body 3 presents at least an inner surface 7 and an outer surface 8 as more clearly shown in Figure 5A (deflated state) and Figure 5B (inflated state). Preferably, the elongated body 3 has an inflatable portion or internal cavity 9 formed by inner surface 7, outer surface 8, and ribs, welds, or closures 22 at the edges of the elongated body 3. Ribs 22 essentially form a closed space or internal cavity 9 in combination with the inner and outer surfaces 7 and 8 (see, e.g., Figure 5B). Such an inflatable member allows the elongated body 3 to be expanded when a physiological inflation medium (i.e., liquid or gas) 9 is introduced between inner surface 7 and outer surface 8. (Both the inflatable portion or internal cavity and the inflation medium, which effectively defines the size of the internal cavity, are referred to by common reference number 9 in the figures.) Preferably, button 6 is fluid-dynamically connected to inner surface 7 of elongated body 3 in such a way that, as the latter inflates, button 6 also inflates, as can be seen, for example, in Figure 3; this provides a more secure locking of the elongated body back upon itself. Preferably, the inner surface 7 is more easily expandable relative to outer surface 8 so that inflation of the elongated body 3 allows further compression, and thus more control of the compression, of the stomach. Generally, therefore, it is preferred that outer surface 8

undergoes little, if any, expansion when the physiological inflation liquid or gas 9 is introduced between inner surface 7 and outer surface 8. Compression of the stomach using the gastric band of the present invention allows for a reduction of the stomach volume as desired. The degree of compression can be modified as desired throughout the course of treatment by adding or removing inflation medium 9.

Furthermore, button 6 and aperture 5 are preferably sized relative to one another that once button 6 is passed through aperture 5 and inflated, the closure mechanism is securely activated but, once button 6 is deflated, the closure mechanism can easily be deactivated by simply pulling on one end of the gastric band (preferably by pulling on tube 20) to remove the gastric band from the abdomen. Furthermore, button 6 is preferably located outside of elongated main part 3 by a distance that can allow a substantial alignment of the first and second zones of elongated body 3, when the latter is closed around the stomach. Thus, when the elongated body 3 is inflated (and preferably button 6 is also inflated), there is no unsuitable and/or harmful superposition of two parts of the elongated body 3 that would provide an undesired enlargement at the zone where they are superposed. In other words, the inflatable portions of the gastric band do not overlap; such overlapping might result in undesirable and/or additional stomach compression in the area of overlap.

Preferably, button 6 is equipped with flap 10 that makes it easier to catch and insert the button 6 into aperture 5 using appropriate instruments. Flap 10 is appropriately made with no internal cavity and, therefore, is not inflatable. Flap 10 can be grasped quickly and simply by surgical endoscopic forceps 11 that is passed first through aperture 5 (see Figure 2). Once grasped, flap 10 and button 6 are pulled back through aperture 5 to lock the gastric band in place (see Figure 3).

As noted above, it is preferred that the button 6 expands at the same time as inner surface 7 of the elongated body 3. The expansion of button 6 should, however, be limited so that, once the gastric band 1 is locked firmly in place, the button 6 does not under go significant further expansion. For

example, the relative thicknesses of the walls of the button 6 and inner surface 7 can be controlled such that the inflation of the button will reach a definite value without expanding any further, independently of the inflation of inner surface 7 of elongated body 3. Thus, preferably the button 6 expands to a size sufficient to lock the closure mechanism 2 in place but not significantly larger.

The elongated body 3 is preferably designed so as to prevent or reduce the tendency of the elongated body 3 to rotate around its long axis as it is being placed in the proper position around the patient's stomach. For example, one or both of the ribs 22 at the edges of the elongated body 3 can contain stiffening elements 12 (see Figure 5C) which will reduce the tendency of the elongated body 3 to rotate or twist about its long axis without effecting the ability of the elongated body to fold back on itself and encircle the patient's stomach. Such stiffening elements 12 will reduce the tendency to twist as the gastric band is being positioned within the patient. Such stiffening or antirotation elements 12 will tend to stabilize the prosthesis and make the insertion easier. The ribs 22 at the edges of the elongated body 3 are preferably gently curved so as not to create problems either at the time of the implant or during removal by pulling of elongated body 3 from the outside; in other words, the ribs, as well as other portions of the gastric band, preferably present smooth and gently curved surfaces to allow the gastric band to slide easily around organs during implantation and removal.

The gastric band preferably has an inflation mechanism 15 comprising a reservoir 16 for receiving the inflation medium, preferably a physiological liquid or gas, for inflating both elongated body 3 and button 6. Preferably, the reservoir 16 has several concentric layers 17 to allow it to be pierced, for example with needle 18, without the inflation medium 9 being able to escape from the perforation. Preferably, reservoir 16 is constructed with multiple layers of material (preferably elastomeric or plastic materials) that, when pricked with needle 18, allows the hole to be made without skewing or leakage between the different layers 17. Such skewing or leakage would generally be mainly noticeable or chiefly accentuated during the expansion of

reservoir 16 when the inflation medium 9 would tend to leak. The external layer of reservoir 16, preferably constructed of biocompatible materials, is generally thicker than the other, internal layers and can even be rigid, since it preferably remains adjacent to the abdominal wall, more preferably within the subcutis, and presents such dimensions as to permit easy introduction through a surgical laparoscopic trocar. By maintaining the reservoir 16 near the abdominal wall, the compression of the stomach can more easily be modified as desired by addition or removal of the inflation medium 9. In some instances, it may be desired for the reservoir 16 to remain outside the abdominal wall.

The elongated body 3 can be inflated using the inflation medium introduced into the reservoir 16 using, for example, a syringe 18 as shown in Figures 6A and 6B. The elongated body is inflated until the desired degree of compression of the stomach occurs. The inflation of the gastric band is generally performed under the control of the endoscopist, who can observe, preferably using an endoscope from inside the stomach, the diameter of the gastric restriction induced by the inflation of the gastric band, particularly by inner surface 7. Preferably, essentially the entire length of the gastric band 3 encircling the stomach can be inflated using the inflation medium 9.

Reservoir 16 is preferably located in the third or proximal zone 34 of elongated body 3 and is connected to the second or middle zone 32 containing aperture 5 is present via tube 20. The length of tube 20 can be varied as needed for particular patients; preferably, tube 20 does not significantly expand when inflation medium 9 is added to the gastric band. In operation, the reservoir 16 is preferably not secured and remains in the subcutis of the abdominal wall. It may be located, using, for example, feel or ultrasound, for introduction of the inflation medium in order to inflate or deflate the gastric band. Using such a technique, the diameter of the gastric constriction provided by the gastric band can be modified or adjusted as desired. Preferably, reservoir 16 has a flap 21 which can be grasped using appropriate instruments to assist in the inflation or deflation operation.

Preferably, both the main portion of the elongated body 3 and the tube 20 have stiffening or antirotation elements 12 within the ribs 22 as shown in Figure 5C. For example, the stiffening elements 12 could be a thin steel, other metal, or other type wire that is fused into the plastic material of the rib 22. Such a stiffening element 12 reduces the tendency of the gastric band to rotate about its long axis before the closure mechanism is activated. Additionally, it makes the gastric band considerably stronger (i.e., acting as a reinforcing element); this added strength may be especially important when the gastric band is removed from the patient by pulling on the proximal end 34 from the outside. The stiffening element 12, when formed using a steel or other suitable metal wire, can also be observed using X-rays, thereby determining the exact position of the band inside the patient's abdomen. Preferably, such stiffening element 12 extends essentially the entire length of the elongated body 3 (i.e., through the first, second, and third zones, including tube 20).

When it is desired to remove the gastric band from the abdomen, it is generally preferred to remove at least a portion of the inflation medium 9 so that the closure mechanism 2 can more easily be disengaged. A significant portion of the inflation medium 9 can be removed using, for example, a syringe using essentially the same procedures as used for the initial inflation process. Alternatively, tube 20 can be cut using cutting device 11a to separate reservoir 16, as represented in Figure 3, to release inflation medium 9. Preferably, at least a portion of inflation medium 9 is removed prior to cutting tube 20 so as to minimize release of inflation medium 9 into the abdominal cavity. For this purpose, under local anesthesia, a small cutaneous incision is made in the abdominal wall to access reservoir 16, at which time tube 20 is cut and the reservoir 16 is removed from the abdominal cavity. After the closure mechanism 2 is disengaged, the gastric band 3 can be removed from the abdominal cavity by pulling on the tube 20 through the small cutaneous incision.

Preferably, the limit of expandability of inner surface 7 is linked to the limit of compressibility of the gastric walls and the two ends of the elongated

body must be blunted enough to allow sliding between the patient's tissues in the phase of removal from the abdomen. In the removal phase, the gastric band will behave as an abdominal drainage tube. Preferably, the materials of construction and the surface smoothness are such that they will impede the production of fibrotic scar adhesions, as normally occurs with drainage tubes or prostheses of silicone materials. Such a smooth surface helps to prevent tissue adhesion to the gastric band. Thus, once deflated and unbuttoned, the gastric band can be removed easily by pulling on one end through a small incision. Preferably, the gastric band will have sufficient strength to withstand the forces associated with removal by this technique.

The gastric band of the present invention can be easily paired with the use of a gastric electrostimulator 100 and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. The electrostimulator 100 may be incorporated into the design of the gastric band as shown in Figure 1 (i.e., attached to the inner surface 7) such that the electrostimulator 100 is in contact with the stomach when the gastric band is properly positioned. Alternatively, it may be separately implanted elsewhere within the abdominal cavity as shown in Figure 2 (e.g., attached to the antrum). If incorporated into the gastric band design, the electrostimulator 100 is implanted at the same time as, and held in place by, the gastric band, thereby eliminating separate attachment of the electrostimulator 100. In such a unitary design, however, the electrostimulator 100 must be removed at the same time as the gastric band. If such an electrostimulator 100 is separately placed at a distance from the gastric band, it may remain within the abdominal cavity after removal of the gastric band. The selection of the preferred location of such an electrostimulator 100 relative to the gastric band will depend largely on the particular patient's requirements and planed treatment regime. Both the electrostimulator 100 and the gastric band are preferably installed and/or removed at the same time, thereby reducing the extent of surgical intervention and anesthesia.

Conventional electrostimulation devices 100 may be used in the practice of this invention in combination with the gastric band 3. Such

devices include, for example, those described in U.S. Patent 5,423,872 (June 3, 1995) (an implantable gastric electrical stimulator at the antrum area of the stomach which generates sequential electrical pulses to stimulate the entire stomach, thereby artificially altering the natural gastric motility to prevent emptying or to slow down food transit through the stomach); U.S. Patent 5,690,691 (November 25, 1997) (a portable or implantable gastric pacemaker employing a number of electrodes along the greater curvature of the stomach for delivering phased electrical stimulation at different locations to accelerate or attenuate peristaltic movement in the gastrointestinal tract); U.S. Patent 5,836,994 (November 17, 1998) (an implantable gastric stimulator which incorporates direct sensing of the intrinsic gastric electrical activity by one or more sensors of predetermined frequency bandwidth for application or cessation of stimulation based on the amount of sensed activity); U.S. Patent 5,861,014 (January 19, 1999) (an implantable gastric stimulator for sensing abnormal electrical activity of the gastrointestinal tract so as to provide electrical stimulation for a preset time period or for the duration of the abnormal electrical activity to treat gastric rhythm abnormalities); U.S.Patent 6,041,258 (March 21, 2000) (electrostimulation device with improved handle for laparoscopic surgery); U.S. Patent Application Serial Number 09/640,201(filed August 16, 2000) (eletrostimulation device attachable to enteric or endo-abdominal tissue or viscera which is resistance to detachment); PCT Application Serial Number PCT/US00/09910 (filed April 14, 2000; Attorney Docket No. 3581/006 PCT) entitled "Gastric Stimulator Apparatus and Method for Installing" based on United States Provisional Application Serial Numbers 60/129,198 and 60/129,199 (both filed April 14, 1999); PCT Application Serial Number PCT/US00/10154 (filed April 14, 2000; Attorney Docket No. 3581/004 PCT) entitled "Gastric Stimulator Apparatus and Method for Use" based on United States Provisional Application Serial Numbers 60/129,209 (filed April 14, 1999) and 60/466,387 (filed December 17, 1999); and U.S. Provisional Patent Application Serial Number 60/235,660 (filed September 26, 2000) entitled "Method and Apparatus for Intentional Impairment of Gastric Motility and/or

Efficiency by Triggered Electrical Stimulation of the Gastric Tract with Respect to the Intrinsic Gastric Electrical Activity." All of these patents, patent applications, provisional patent applications, and/or publications are hereby incorporated by reference.

Moreover, the gastric band of the invention is of great clinical interest, especially in relation to problems inherent to prolonged permanence in the abdomen, that is, intragastric decubitus, perforation, strangulation, and the like. In practice it has been confirmed that the removable gastric band according to the invention is particularly advantageous because it can be removed without having to perform an additional surgical intervention and additional anesthesia on the patient, thanks especially to its qualities of resistance to pulling.

The invention thus conceived is susceptible to numerous modifications and variations, all falling within the scope of the inventive concept; furthermore, all of the details can be substituted with technically equivalent elements. In practice, other materials and dimensions can be used, depending on the demands and on the state of the technique.

Claims

That which is claimed is:

1. A removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

2. The removable gastric band of claim 1, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

3. The removable gastric band of claim 2, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be more securely closed around the section of the stomach when the cavity is inflated.

4. The removable gastric band of claim 3, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

5. The removable gastric band of claim 3, wherein the button has a flap for catching and easy introduction into the aperture.

6. The removable gastric band of claim 5, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

7. The removable gastric band of claim 6, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

8. The removable gastric band of claim 7, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

9. The removable gastric band of claim 8, wherein the reinforcing elements are radiopaque.

10. The removable gastric band of claim 7, wherein the reservoir has a flap for easy holding.

11. The removable gastric band of claim 8, wherein the reservoir has a flap for easy holding.

12. The removable gastric band of claim 2, wherein essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in placed around the stomach.

13. The removable gastric band of claim 7, wherein the essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in place around the stomach.

14. A method for treatment of obesity in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

15. The method of claim 14, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

16. The method of claim 15, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be

more securely closed around the section of the stomach when the cavity is inflated.

17. The removable gastric band of claim 16, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

18. The method of claim 16, wherein the button has a flap for catching and easy introduction into the aperture.

19. The method of claim 18, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

20. The method of claim 19, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

21. The method of claim 20, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

22. The method of claim 21, wherein the reinforcing elements are radiopaque.

23. The method of claim 20, wherein the reservoir has a flap for easy holding.

24. The method of claim 21, wherein the reservoir has a flap for easy holding.

25. The method of claim 15 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

26. The method of claim 25, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

27. The method of claim 25, wherein the electrostimulator is located separately from the gastric band.

28. The method of claim 20 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

29. The method of claim 28, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

30. The method of claim 28, wherein the electrostimulator is located separately from the gastric band.

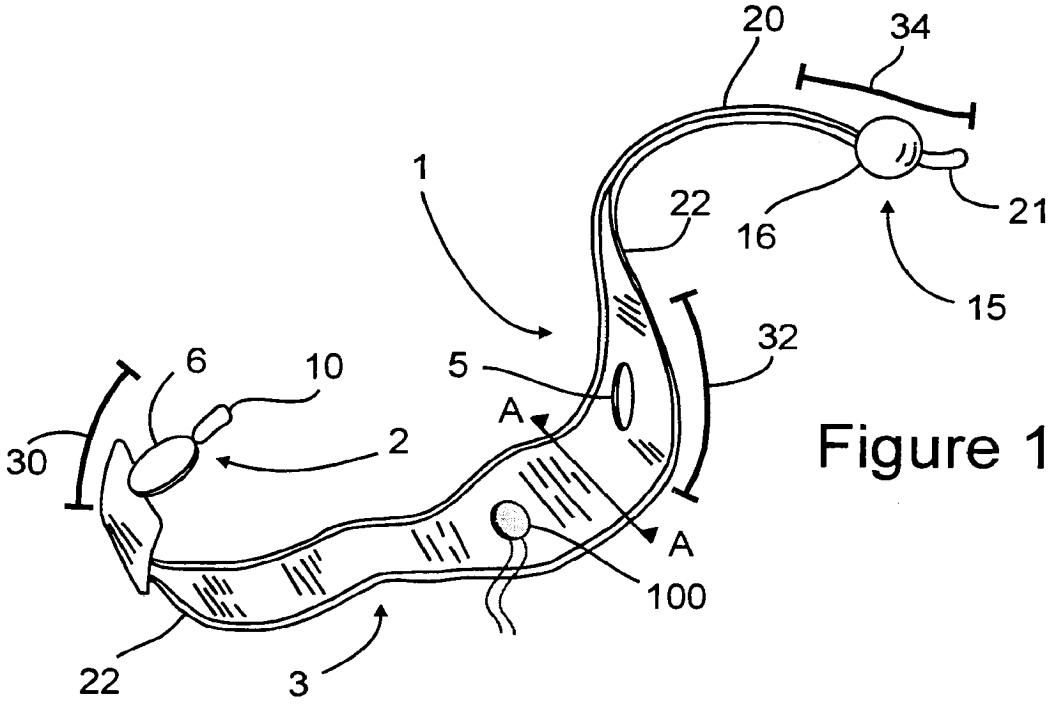


Figure 1

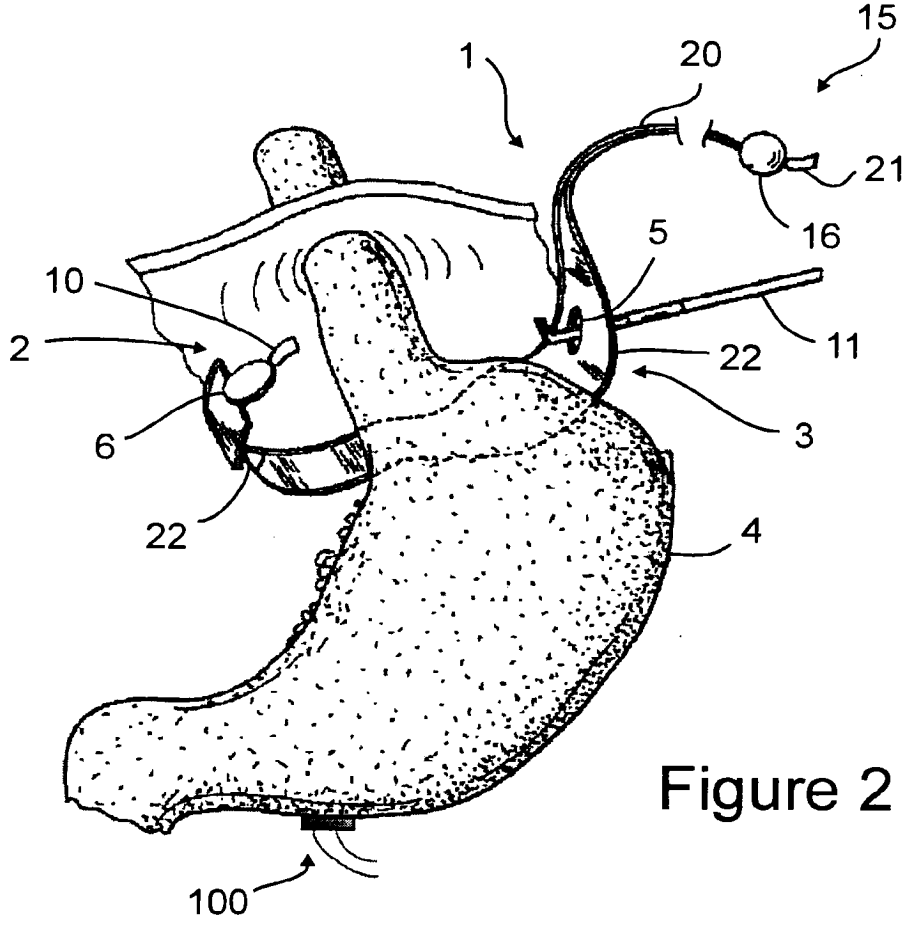
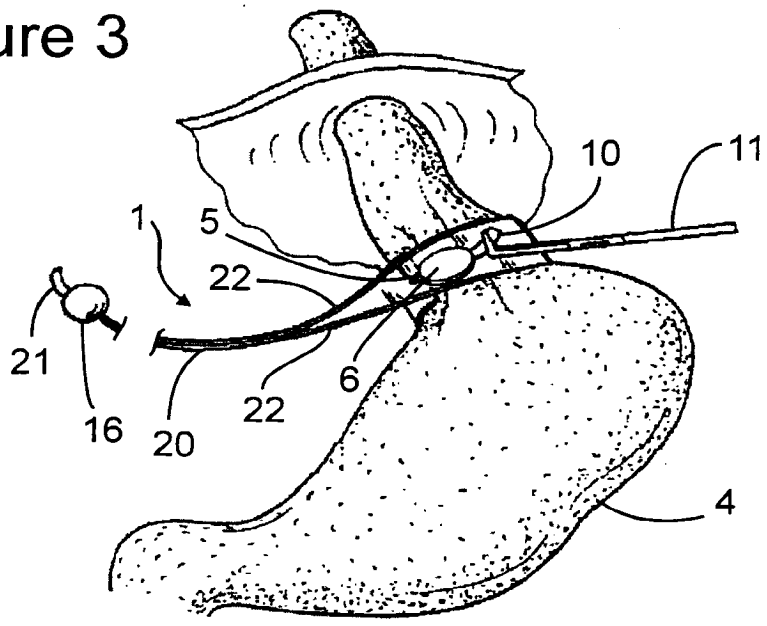


Figure 2

Figure 3



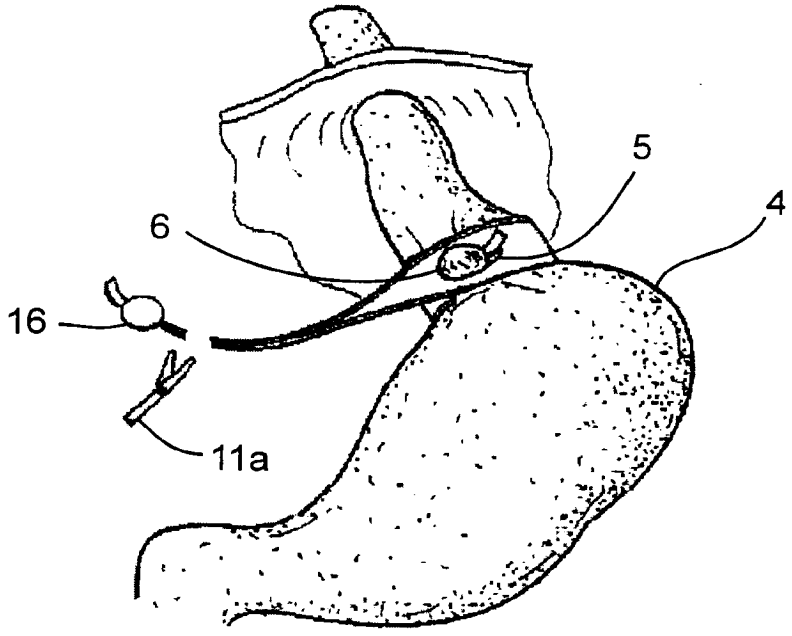


Figure 4

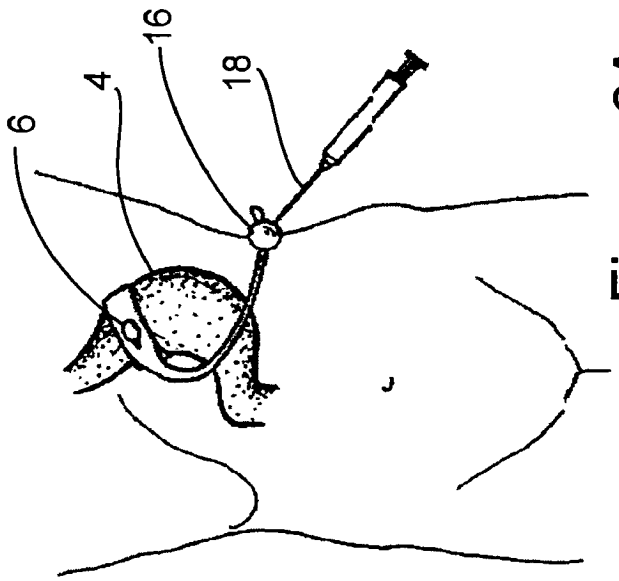


Figure 6A

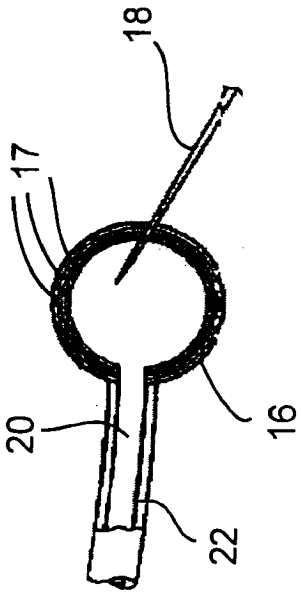


Figure 6B

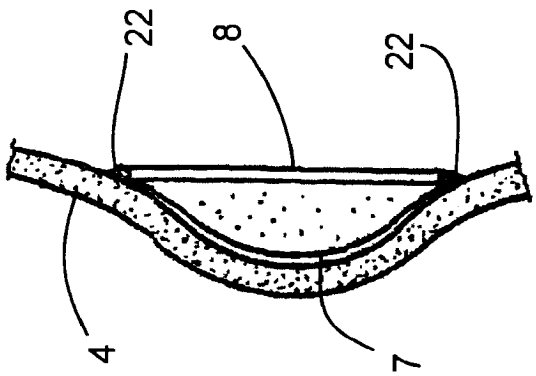


Figure 5B

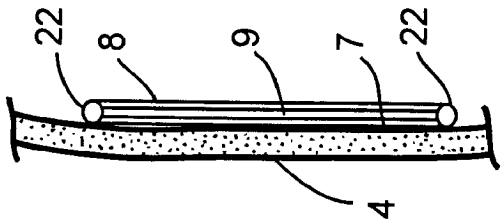


Figure 5A

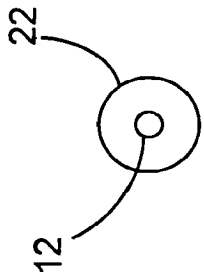


Figure 5C

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
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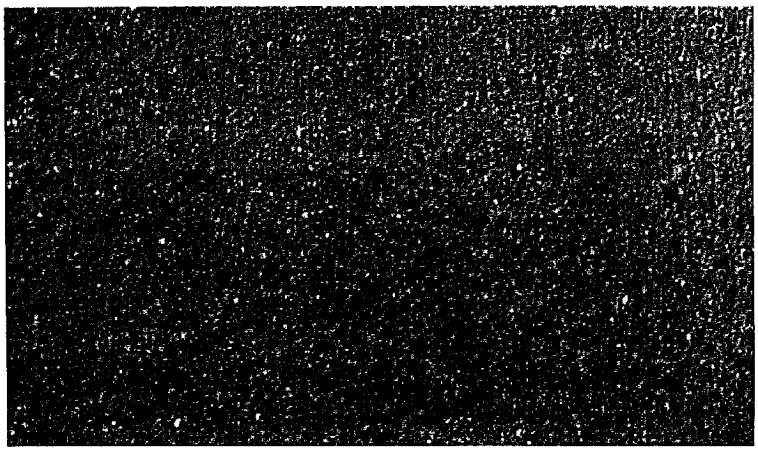
(84) Bestimmungsstaaten *(regional)*: ARIPO-Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Fortsetzung auf der nächsten Seite]

(54) **Title:** DISPERSIONS FOR FORMULATING SLIGHTLY OR POORLY SOLUBLE ACTIVE INGREDIENTS

(54) **Bezeichnung:** DISPERSIONEN ZUR FORMULIERUNG WENIG ODER SCHWER LÖSLICHER WIRKSTOFFE



1 Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

1 OPTICAL MICROSCOPE PHOTO OF THE EMULSION CONTAINING 5mg/mL AMPHOTERICIN B, FROM EXAMPLE 19

(57) **Abstract:** The invention relates to a dispersion, comprising an oily phase and an aqueous phase in the form of an O/W emulsion or a W/O emulsion, at least one active ingredient which is slightly or poorly soluble in the oily and the aqueous phases, in addition to optionally one or more emulsifiers and/or stabilisers. The dispersion is devoid of toxicologically questionable organic solvents and contains a dissolved quantity of said active ingredient that is higher than the additive quantity obtained by its maximum solubility in both the oily and the aqueous phase of the emulsion.

[Fortsetzung auf der nächsten Seite]

WO 02/09667 A2

Erklärung gemäß Regel 4.17:

— *Erfindererklärung (Regel 4.17 Ziffer iv) nur für US*

Veröffentlicht:

— *ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts*

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(57) Zusammenfassung: Die Erfindung betrifft eine Dispersion, die eine ölige Phase und eine wässrige Phase in Form einer O/W-Emulsion oder W/O-Emulsion, mindestens einen in der öligen und der wässrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfasst, wobei die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wässrigen Phase der Emulsion ergibt.

Dispersionen zur Formulierung wenig oder schwer löslicher Wirkstoffe

Die Erfindung betrifft Dispersionen, die eine ölige Phase, eine wäßrige Phase und in diesen beiden Phasen wenig löslichen, schwer löslichen bis zu unlöslichen Arzneimittelwirkstoff umfassen.

Wirkstoffe mit geringer Löslichkeit haben sehr oft das Problem einer unzureichenden Bioverfügbarkeit. Der generelle Lösungsansatz für dieses Problem ist die Erhöhung der Löslichkeit dieser Wirkstoffe. Beispiele hierfür sind die Lösungsvermittlung über Solubilisation, Bildung von Einschlußverbindungen (z. B. mit Cyclodextrinen) sowie die Verwendung von Lösungsmittelgemischen (K. H. Bauer, K.-H. Frömming, C. Führer, Pharmazeutische Technologie, Georg Thieme Verlag Stuttgart, 1991). Für viele Wirkstoffe führt dies jedoch nicht zu einer ausreichenden Erhöhung der Löslichkeit, insbesondere wenn Wirkstoffe gleichzeitig schwerlöslich in wäßrigen Medien und gleichzeitig schwerlöslich in organischen Medien sind. Hier scheiden z. B. Lösungsmittelgemische als Lösung für das Problem aus. Alternativ können gering wasserlösliche Wirkstoffe in Ölen gelöst werden, eine O/W-Emulsion hergestellt und diese dann oral oder parenteral (in der Regel i.v.) appliziert werden. Sehr viele Wirkstoffe, insbesondere Wirkstoffe mit gleichzeitig geringer Löslichkeit in wäßrigen und organischen Medien, sind jedoch nicht ausreichend in Ölen löslich. Nicht ausreichend bedeutet, daß aufgrund zu geringer Löslichkeit bei erforderlicher Dosis das zu applizierende Volumen der Emulsion zu groß wird.

In Wasser und in Ölen gering lösliche Wirkstoffe wie Amphotericin B können trotzdem in Emulsionen eingearbeitet werden (Seki et al. US 5 534 502). Um dies zu erreichen müssen jedoch zusätzliche organische Lösungsmittel eingesetzt werden. Diese Lösungsmittel müssen dann in Zwischenschritten der Emulsionsherstellung oder dem Produkt wieder entzogen werden (Davis, Washington, EP 0 296 845 A1) wobei jedoch ein gewisser Restlösungsmittelgehalt im Produkt verbleibt. Zusätzlich ist diese Herstellung sehr zeitaufwendig und kostenintensiv, so daß Produkte basierend auf dieser Technologie praktisch auf dem Markt nicht vertreten sind. Eine alternative Methode ist die Einlagerung von derartigen Substanzen wie Amphotericin B in die Phospholipid-Doppelmembran von Liposomen, Handelsprodukt ist beispielsweise Ambisome® (Janknegt et al., Liposomal and lipid formulations of amphotericin B, Clin. Pharmacokinet., 23, 279-291 [1992]). Nachteilig ist aber auch hier die sehr teure Herstellung, so daß es in der Regel nur in Notfällen eingesetzt wird, wenn eine andere Behandlung nicht zum Ziel führt bzw. nur bei Patienten eingesetzt wird, die finanziell in der Lage sind, die Behandlung zu bezahlen. Somit besteht eindeutig ein Bedarf an einer kostengünstigen Formulierung, die gleichzeitig möglichst einfach herzustellen ist, im Gegensatz zu Liposomen lagerstabil ist und eine Lyophilisation nicht erfordert sowie nicht von Restlösungsmitteln belastet ist.

Der vorliegenden Erfindung liegt daher die Aufgabe zugrunde, eine Dispersion zur Verfügung zu stellen, die einen wenig, schwer oder sogar bisher unlöslichen Wirkstoff in einer bisher nicht möglichen Menge gelöst enthält, wobei gleichzeitig die oben beschriebenen Nachteile der Verwendung zusätzlicher zur Formulierung bisher notwendiger organischer Lösungsmittel entfällt.

Gegenstand der vorliegenden Erfindung ist daher eine Dispersion auf der Basis einer O/W-Emulsion oder einer W/O-Emulsion beladen mit Wirkstoff, der in Wasser und gleichzeitig auch in Ölen wenig löslich oder schwer löslich bis hin zu unlöslich ist, wobei diese Dispersion frei von toxikologisch bedenklichen organischen

Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der Wasser- und der Ölphase der Emulsion ergibt.

Insbesondere ist die erfindungsgemäß gelöste Menge um den Faktor 2, bevorzugter 5, noch bevorzugter 10 oder noch größer als die additive Menge.

Die "additive Menge" wird durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen), wobei keine weiteren zusätzlichen organischen Lösungsmittel zum Einsatz kommen. Die erfindungsgemäße Dispersion enthält zusätzlich zu der additiven Menge ein überadditive Menge an gelöstem Wirkstoff.

Ein wichtiges erfindungsgemäßes Merkmal ist, daß bei gleicher Zusammensetzung hochenergetisch homogenisiert wird, im Vergleich zu niederenergetischem Dispergieren (Schütteln oder Blattrührer).

Die Herstellung der erfindungsgemäßen Dispersion erfolgt insbesondere unter Ausschluß von toxikologisch bedenklichen organischen Lösungsmitteln wie z.B. Methylenchlorid und Ethanol. Die Wirkstoffe werden unter Umgehung eines Zwischenschrittes direkt aus der festen Substanz in die Emulsion eingearbeitet.

Detaillierte Beschreibung der Erfindung

Generell ist es anerkannter Stand der Wissenschaft, daß die Moleküle eines schwerlöslichen oder gering löslichen Wirkstoffes aus dem festen Aggregatzustand (Pulver) über mindestens einen Zwischenschritt (z. B. molekulardisperse Verteilung in einem Lösungsmittel) in eine Emulsion als Trägersystem eingearbeitet werden müssen. Die Erfahrung zeigt, daß bei in Wasser und Öl gleichzeitig sehr gering löslichen Substanzen es nicht genügt,

eine Emulsion mit Kristallen des Wirkstoffes zu versetzen. So führt die teilweise praktizierte Zumischung von Amphotericin B-Lösung (Lösungsmittelgemisch) zu einer handelsüblichen O/W-Emulsion wie Intralipid oder Lipofundin zur Präzipitation des Wirkstoffes, es entstehen Amphotericin B-Kristalle, die sedimentieren und sich nicht mehr in der Emulsion auflösen.

Überraschender Weise wurde jedoch nun gefunden, daß die Herstellung eines Emulsionssystems mit gelöstem Wirkstoff auch direkt aus dem festen Aggregatzustand des Wirkstoffes möglich ist. Zur Herstellung der erfindungsgemäßen Dispersion wird der Wirkstoff in partikulärer Form der Wasserphase oder der Ölphase zugesetzt und anschließend alle Komponenten einem höher energetischen oder hochenergetischen Prozeß wie z. B. der Homogenisation, insbesondere der Hochdruckhomogenisation unterzogen. Der hochenergetische Prozeß der Hochdruckhomogenisation führt dazu, daß der Wirkstoff in die Emulsion molekulardispers eingearbeitet wird und keine Wirkstoffkristalle mehr im Polarisationsmikroskop detektierbar sind. Die erhaltenen Emulsionen sind überraschender Weise ähnlich stabil wie Systeme, die unter Einsatz von organischen Lösungsmitteln erzeugt worden sind.

Eine sehr einfache Art der Einarbeitung der Wirkstoffkristalle ist die Verreibung des Wirkstoffes mit einer handelsüblichen O/W-Emulsion (z. B. Lipofundin, Intralipid). Nach Anreiben befindet sich der Wirkstoff primär in der Wasserphase, es ist ein disperses System entstanden, das als innere Phase gleichzeitig Öltropfen und Wirkstoff-Kristalle enthält. Dieses disperse System wird dann homogenisiert oder hochdruckhomogenisiert (z. B. 1.500 bar und 5 – 20 Homogenisationszyklen). Es wird eine feindisperse Emulsion erhalten (Beispiel 1), in der am Ende des Homogenisationsprozesses keine Wirkstoff-Kristalle mehr nachweisbar sind. Die Kristalle haben sich daher nahezu vollständig oder vollständig aufgelöst, d.h. daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10

Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

Falls es gewünscht ist, kann der Wirkstoff jedoch auch in einer solchen Menge eingesetzt werden, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

Alternativ kann eine wäßrige Suspension des Wirkstoffes mit einer O/W-Emulsion gemischt werden. Es handelt sich wieder um ein disperses System mit einer dispergierten Phase aus Öltropfen und Wirkstoff-Kristallen. Dieses wird ebenfalls einem höher oder hochenergetischem Prozeß wie der Hochdruckhomogenisation unterzogen. Die Zumischung einer wäßrigen Suspension des Wirkstoffes eignet sich insbesondere dann, wenn die Wirkstoffkonzentration relativ gering ist. Zusätzlich kann die wäßrige Suspension des Wirkstoffes vor der Zumischung einem in den Lehrbüchern beschriebenem Mahlprozeß unterzogen werden, z. B. Naßmahlung mit einer Kolloidmühle, einer Kugelmühle oder einer Perlmühle oder durch Hochdruckhomogenisation vorzerkleinert werden.

Generell ist es günstig, den Wirkstoff in der Form sehr feiner Kristalle zu verwenden, d. h. in mikronisierter Form mit einer Teilchengröße im Bereich von ca. 0,1 µm - 25 µm (Kolloidmühle, Gasstrahlmühle).

Alternativ kann der Wirkstoff auch im Öl dispergiert werden. Das Öl mit den Wirkstoff-Kristallen wird dann in der Wasserphase dispergiert, wobei das dafür notwendige Tensid entweder der Wasserphase zugesetzt wird oder in der Ölphase gelöst wird bzw. jeweils dispergiert wird. Im Falle von Lecithin kann das Lecithin im Wasser dispergiert werden oder in der Ölphase unter leichtem Erwärmen gelöst werden.

Bei Einarbeitung der Wirkstoff-Kristalle in die Ölphase kann dies ohne Zusatz eines Tensids erfolgen. Das Tensid, z. B. Lecithin, wird anschließend zugesetzt. Alternativ können auch die Wirkstoff-Kristalle in eine Ölphase eingearbeitet werden, die bereits Tensid enthält.

Nach Einarbeitung der Wirkstoff-Kristalle in das Öl wird die Ölphase in Wasser dispergiert (z. B. mit einem hochtourigen Rührer) und die erhaltene Rohemulsion anschließend hochdruckhomogenisiert. Auch hier ist es günstig, die Wirkstoff-Kristalle möglichst klein einzusetzen. Zur weiteren Zerkleinerung der in die Ölphase eingearbeiteten Wirkstoff-Kristalle kann diese ölige Suspension vor dem Herstellen der Rohemulsion zunächst einer Mahlung unterzogen werden. Die Wirkstoff-Kristalle in der Ölphase werden durch diese Naßmahlung weiter zerkleinert, teilweise bis in den Nanometerbereich. Übliche Verfahren der Naßmahlung, die eingesetzt werden können, sind z. B. die Kolloidmühle und die Hochdruckhomogenisation der Ölphase. Generell ist die Kavitation einer wäßrigen Phase das anerkannte Prinzip der Zerkleinerung bei der Hochdruckhomogenisation, d. h. die Anwesenheit von Wasser ist zur Kavitation erforderlich. Öle mit einem zu Wasser extrem geringen Dampfdruck sind zur Kavitation nicht fähig. Trotzdem wurde überraschender Weise gefunden, daß eine zur Herstellung des neuen Trägersystems ausreichende Zerkleinerung auftritt.

Charakteristisch für die erfindungsgemäße Dispersion ist, daß der in der Emulsion eingearbeitete Wirkstoff in höherer Menge gelöst vorliegt als es sich additiv aus seiner maximalen Löslichkeit in der Wasser- und Ölphase der Emulsion ergibt und gleichzeitig zur Herstellung keine toxikologisch bedenklichen organischen Lösungsmittel eingesetzt wurden. Zu solchen toxikologisch bedenklichen organischen Lösungsmitteln gehören insbesondere Chloroform, Methylenchlorid, längerkettige Alkohole wie Hexanol und Octanol, aber auch ethanol in höheren Konzentrationen.

In der Regel handelt es sich bei den erfindungsgemäßen Wirkstoffen um Wirkstoffe, die wenig löslich (1 Teil löst sich in 30-100 Teilen Lösungsmittel) oder schwer löslich (1 Teil in 100-1000 Teilen Lösungsmittel), insbesondere aber sehr schwer löslich (1 Teil löst sich in 1.000 bis 10.000 Teilen Lösungsmittel) oder sogar unlöslich sind (> 10.000 Teile Lösungsmittel).

So beträgt die Löslichkeit von Amphotericin B in Wasser weniger als 0,001% (< 0,01 mg/ml) bei pH 6-7, das heißt dem pH-Wert der Emulsion. Die Löslichkeit von Amphotericin ist zwar höher bei pH 2 und pH 11 (0,1 mg/ml), jedoch sind diese Lösungen nicht intravenös applizierbar.

Die Löslichkeit von Amphotericin in Sojaöl (Long Chain Triglycerides – LCT) und in Miglyol 812 (Medium Chain Triglycerides – MCT), den Standardölen für die meisten auf dem Markt befindlichen Emulsionen zur parenteralen Infusion ist kleiner als 0,0001 mg/ml.

40g Emulsion aus Beispiel 1 bestehen zu 20% aus Öl (8g) und ca. 80% aus Wasser (32g). Somit lassen sich aufgrund der Löslichkeiten 8 x 0,0001 mg/ml plus 32 x 0,01 mg, d.h. insgesamt 0,3208 mg Amphotericin in 40g Emulsionsbestandteilen Öl und Wasser auflösen, d.h. 0,008 mg/ml. In der vorliegen erfindungsgemäßen Emulsion konnten 0,2 mg/ml Emulsion eingearbeitet werden (Beispiel 1) ohne daß mikroskopisch Kristalle von ungelöstem Arzneistoff detektierbar waren (Beispiel 12). Auch höhere Konzentration wie 1 mg/ml Emulsion konnten eingearbeitet werden (Beispiel 2), mit Laserdiffraktometrie waren keine der zur Herstellung eingesetzten Arzneistoffpartikel mehr detektierbar (Beispiel 11).

Bei einer gewünschten Dosis von z.B. 100 mg Amphotericin B ergibt sich bei den erfindungsgemäßen Dispersionen mit 1 bzw. 0,2 mg/ml Emulsion ein intravenös zu applizierendes Volumen von 100 bis 500 ml Emulsion. Somit werden mit der erfindungsgemäßen Emulsion

wenig lösliche und schwer lösliche Wirkstoffe erst in einem ausreichend kleinen Applikationsvolumen bei verträglichen pH-Werten applizierbar.

Gelöster Wirkstoff ist schnell verfügbar. Zur Erzeugung eines Depots kann mehr Wirkstoff in die Dispersion eingearbeitet werden als sich darin löst, d h. man erzeugt Kristalle, die als Depot wirken. Die Löslichkeit in Wasser und Ölphase betragen z.B. für Amphotericin B 0,008 mg/ml, die erfindungsgemäße Emulsion löst ohne detektierbare Kristalle z.B. 0,2 mg/ml (Beispiel 1). Arbeitet man 5 mg/ml Dispersion ein, so ist die Löslichkeit überschritten (übersättigtes System). Nach Hochdruckhomogenisation erhält man zusätzlich zum gelösten Wirkstoff noch hochfeine Arzneistoffkriställchen (Beispiel 15).

Die durch Mischung von Arzneistoff (Beispiel 15) oder einer Arzneistoffsuspension (analog Beispiel 6) mit einer Emulsion und anschließende Homogenisation hergestellten heterogenen, übersättigten Dispersionen sind dadurch gekennzeichnet, daß separat nebeneinander Öltropfen und hochfeine Kriställchen existieren, d.h. die Kristalle sind primär außerhalb der Öltropfen.

Die Bestimmung der Partikelgröße erfolgt mit Lichtmikroskopie unter Ermittlung der Anzahlverteilung. Alternativ erfolgt die Bestimmung mit Laserdiffraktometrie (Gerät: Coulter LS 230, Coulter Electronics, Krefeld, Germany), wobei die erhaltene Volumenverteilung in die Anzahlverteilung umgerechnet wird.

Sind in der Dispersion bei hoher Beladung mit Wirkstoff neben den Emulsionstropfen noch Arzneistoffkristalle vorhanden, so sind direkt nach der Herstellung mindestens 90%, bevorzugt 95% der Anzahl der Wirkstoffkristalle in der Anzahlverteilung kleiner als 5 µm. Bei Anwendung von hohen Drücken (z.B. 1000 bar) und einer ausreichenden Anzahl an Homogenisationszyklen erhält man hochdisperse Systeme. In Abhängigkeit von Druck und Zyklenzahl erhält man Dispersionen mit mindestens 90%, teilweise 95% und

insbesondere 99% der Anzahl der Kristalle in der Anzahlverteilung kleiner als 1 µm.

Oben wurde die in situ Erzeugung des Wirkstoff-Depots aus Kriställchen durch Herstellung der erfindungsgemäßen Dispersion mit einer Wirkstoffmenge oberhalb der Sättigungslöslichkeit des Systems beschrieben. Alternativ kann auch eine erfindungsgemäße Dispersion mit ausschließlich gelöstem Wirkstoff hergestellt werden, der man nachträglich Wirkstoffkristalle definierter Größe zumischt, z.B. mikronisierter Wirkstoff.

Zur Herstellung der erfindungsgemäßen Dispersion können handelsübliche O/W-Emulsionen eingesetzt werden (z.B. Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid und Salvilipid) oder es wird eine Emulsion aus Ölphase, Emulgator / Stabilisator und äußerer Phase (z.B. Wasser) hergestellt.

Beispiele für Bestandteile der Ölphase der Emulsionen sind: Sojaöl, Safloröl (Distelöl), langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT) wie z.B. Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride wie Stesolid, einzeln oder in Mischungen.

Zur Stabilisierung der Dispersionen können Emulgatoren und Stabilisatoren eingesetzt werden. Diese sind gegebenenfalls bereits in der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten Emulsion enthalten, Zusatz weiterer Emulgatoren und Stabilisatoren bei der Herstellung der Dispersion kann vorteilhaft sein.

Beispiele für Emulgatoren sind z.B. Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Tween 80, Natriumglykocholat und Natriumlaurylsulfat (SDS). Alternativ kann Stabilisierung durch Zusatz von Substanzen erfolgen die über andere Mechanismen als Emulgatoren stabilitätserhöhend wirken, z.B. über sterische

Stabilisierung oder Erhöhung der Zetapotentials. Solche Stabilisatoren sind z.B. Block-Copolymere wie z.B. Poloxamere (z.B. Poloxamer 188 und 407) und Poloxamine (z.B. Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide wie Hyaluronsäure und Chitosan und ihre Derivate, Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester wie Saccharosemonostearat und Antiflokkulatien wie Natriumcitrat. Emulgatoren und Stabilisatoren können einzeln oder in Mischungen verwendet werden. Typische Konzentrationen sind 0,1% bis 20%, insbesondere 0,5% bis 10%.

Als wäßrige äußere Phase der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten O/W-Emulsion können dienen: Wasser, Mischungen von Wasser mit anderen wassermischbaren organischen Flüssigkeiten, flüssige Polyethylenglykole (PEG, insbesondere PEG 400 und 600).

Die wäßrige äußere Phase kann auch Zusätze enthalten, z.B. Elektrolyte, Nichtelektrolyte (z.B. Glycerol, Glucose, Mannit, Xylit zur Isotonisierung), Gelbildner wie Cellulosederivate und Polysaccharide wie Xanthan und Alginate (z.B. zur Viskositätserhöhung).

Für die topische Applikation können der Dispersion Penetrationsverstärker (z.B. Azone, Laurinsäure) und für die Applikation zum Gastrointestinaltrakt Absorptionsverstärker (z.B. Gallensäuren, Lysophospholipide) zugesetzt werden.

Wirkstoffe zur Einarbeitung in die Emulsion sind neben Amphotericin B z.B. Ciclosporin, Buparvaquon und Atovaquon. Weitere Wirkstoffe sind Hormone (z.B. Estradiol), Antioestrogene und Kortikoide (z.B. Prednicarbat).

Die Applikation der Emulsion kann auf verschiedenen Wegen erfolgen, z.B. parenteral aber auch oral oder topisch. Bei

parenteraler Applikation sind alle gängigen Wege möglich, z.B. intravenös, intra- und subkutan, intramuskulär, intraartikulär, intraperitoneal etc.

Topische Emulsionen mit Ciclosporin können die Wirkstoffpenetration in die Haut verbessern aufgrund des hohen gelösten Anteils an Arzneistoff (erhöhter Konzentrationsgradient). Orale Applikation der Ciclosporin-Emulsion kann die Bioverfügbarkeit erhöhen da im Gegensatz zu mikronisiertem Ciclosporin ein erhöhter gelöster Anteil vorliegt.

Die Bioverfügbarkeit von oral appliziertem Amphotericin B ist aufgrund seiner geringen Löslichkeit nahezu Null. Orale Applikation der Amphotericin-Emulsion kann aufgrund des erhöhten gelösten Anteils ebenfalls die Bioverfügbarkeit erhöhen.

Die erfindungsgemäßen Emulsionen (z.B. mit Buparvaquon und Atovaquon) können nach intravenöser Injektion auch durch Anlagerung einer Targeting-Einheit (z.B. Apolipoprotein E in Kombination mit Apolipoprotein AI und AIV) für eine gewebsspezifische Arzneistoffapplikation eingesetzt werden (Targeting zum Gehirn). Erreger lokalisieren bei bestimmten Erkrankungen des monozytären phagocytierenden Systems (MPS) auch im Gehirn und sind bisher schwer einer Therapie zugänglich (z.B. Leishmaniosen, Toxoplasmose).

Die oben beschriebenen Systeme sind vom Typ O/W, d. h. Öltropfen sind dispergiert in einer Wasserphase. Es ist jedoch auch möglich, Dispersionen auf der Basis von W/O-Emulsionen zu produzieren. Ein grundsätzlicher Vorteil ist, daß die äußere Ölphase als eine Diffusionsbarriere fungiert und die Freigabe des Arzneistoffes verzögert. Derartige Dispersionen können nicht intravenös appliziert werden, aber sie können zum Beispiel intramuskulär oder subkutan als Depotformulierung injiziert werden. Applikation dieser W/O-Systeme am Auge erhöht die Verweilzeit aufgrund der erhöhten Viskosität und gleichzeitig

wird eine verlängerte Arzneistofffreisetzung erreicht. Bei topischer Applikation auf die Haut hat die Ölphase einen okklusiven Effekt, der zu einer erhöhten Arzneistoffpenetration führt. Daher besitzen diese W/O-Typ-Systeme einen Vorteil für spezielle Anwendungen. Bevorzugte Form der Erfindung ist jedoch die Dispersion auf der Basis des O/W-Typs.

Bei Öl-in-Wasser Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie 5 bis 99,5 Gew.-% wäßrige Phase, vorzugsweise 10 bis 95 Gew.-% wäßrige Phase, besonders bevorzugt 60 bis 95 Gew.-% wäßrige Phase und speziell 70-95% wäßrige Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Bei Wasser-in-Öl Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie aus 5 bis 30 Gew.-% wäßriger Phase, vorzugsweise 10 bis 25 Gew.-% wäßriger Phase, besonders bevorzugt 10 bis 20 Gew.-% wäßriger Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Die Bestandteile der Ölphase der Emulsionen sind – wie oben ausgeführt - insbesondere ausgewählt aus der Gruppe bestehend aus Sojaöl, Safloröl (Distelöl), langkettigen Triglyceriden (LCT), mittelkettigen Triglyceriden (MCT), wie z. B. Miglyole, Fischölen und Ölen mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierten Partialglyceriden, wie in Stesolid®, einzeln oder in Mischungen. Die mittelkettigen Triglyceride enthalten vorzugsweise wenigstens 90 % Triglyceride der Capryl-Säure (C8) und der Caprin-Säure (C10). Als Ölphase sind im Rahmen der Erfindung Gemische aus Sojaöl und MCT, vorzugsweise im Gewichtsverhältnis 5:1 bis 1:5, besonders bevorzugt zwischen 2:1 und 1:2 oder 1:1 geeignet.

Die Fettphase der erfundenen Dispersion kann aus Ölen bestehen, d.h. die Lipide sind bei einer Raumtemperatur von 20°C flüssig. Es besteht weiterhin die Möglichkeit, daß diese Öle mit Lipiden gemischt werden, die bei einer Raumtemperatur von 20°C fest sind.

Die Mischungsanteile von Öl zu festem Lipid können von 99 + 1 bis 1 + 99 (Gewichtsanteile) variieren. Bevorzugt sind Mischungen, die mindestens 10 Teile flüssiges Öl enthalten, speziell mindestens 30 Teile flüssiges Öl und insbesondere mindestens 50 Anteile flüssiges Öl.

In speziellen Fällen kann die Lipidphase der Dispersion zu 100% Lipide enthalten, die bei einer Raumtemperatur von 20°C fest sind. Schmelzen die Lipide nahe der Raumtemperatur, können Dispersionen erhalten werden, deren Lipidtröpfchen sich in einem Zustand einer "Unterkühlten Schmelze" befinden. Liegen sehr hochschmelzende Lipide vor, können – ungeachtet der durch die Thomson-Gleichung beschriebenen Schmelzpunktionsdepression – die Partikel der Dispersion aushärten. Die Thomson-Gleichung beschreibt, daß der Schmelzpunkt von Lipiden gegenüber ihrer "bulk"-Ware stark herabgesetzt wird, wenn diese in sehr feinen Partikeln auskristallisieren (z. B. Nanopartikel oder Partikel in einem Größenbereich von wenigen Mikrometern) (Hunter, R.J., Foundations of colloid science, Vol. 1, Oxford University Press, Oxford, 1986).

Beispiele für bei Raumtemperatur feste Lipide sind, Karnaubawachs, Hydroxyoctacosanylhydroxystearat, Chinesisches Wachs, Cetylpalmitat, Bienenwachs und ähnliche Wachse. Weitere Beispiele für feste Lipide beinhalten C_{20-40} Di- und Triglyceride, mit gesättigten und ungesättigten Fettsäuren, C_{20-40} Fettalkohole, C_{20-40} Fettamine und ihre Verbindungen, sowie Sterole.

Als Lipide zur Herstellung von Mischungen aus flüssigen und festen Lipiden sind geeignet: Natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit z. B. Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben. Besonders geeignet sind synthetische Monoglyceride, Diglyceride und Triglyceride als

individuelle Substanzen oder als Mischung (z. B. Hartfett), Imwitor 900, Triglyceride (z. B. Glyceroltrilaurat, Glyceroltrimyristat, Glyceroltripalmitat, Glyceroltristearat und Glyceroltribehenat) und Wachse wie z. B. Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB). Außerdem Kohlenwasserstoffe, wie z. B. Hartparaffin.

Die Tropfengröße der Öltropfen (O/W-Typ) oder Wassertropfen (W/O-Typ) in der Dispersion ist größer als 100 nm (bestimmt mit Photonenkorrelationsspektroskopie – PCS). Das empfohlene obere Größenlimit für die Tropfen ist 10 µm, anderenfalls kommt es zum Aufrahmen aufgrund der Flotation der Tropfen, was zu physikalischer Instabilität führt (Tropfenkoaleszenz). Um Flotation zu minimieren, sollte die Größe kleiner als 5 µm sein, vorzugsweise unterhalb von 1 µm (PCS-Durchmesser), was zu den sogenannten physikalisch "autostabilen" Dispersionen führt. Die optimale Stabilität wurde gefunden im Größenbereich ähnlich zu parenteralen Fettemulsionen mit PCS-Durchmessern von 200 nm bis 500 nm.

Der Gehalt an Stabilisatoren in parenteralen Zubereitungen sollte so niedrig wie möglich gehalten werden, um Toxizität und Störungen des Metabolismus zu minimieren. Von Lecithin-haltigen Emulsionen zur parenteralen Ernährung ist es bekannt, daß eine zu hohe Zuführung von Lecithin metabolische Störungen bewirken kann, typische Tagesvolumina appliziert sind hier z. B. 500 ml Emulsion und mehr. Dies führte zu der Entwicklung der Lecithin-reduzierten Emulsionen, d. h. man reduzierte den Lecithingehalt von 1,2% weiter auf nur 0,6% Lecithin. Einige Systeme zur Applikation von schwerlöslichen Arzneistoffen verwenden einen relativ hohen Emulgatorgehalt (z. B. Solubilisierung mit Tensiden, SEDDS – self-emulsifying drug delivery systems basierend auf der Solubilisation von Öl mit hohen Tensidkonzentrationen). Eine spezielle Eigenschaft der vorliegenden Erfindung ist, daß sie die Tensidbelastung minimiert. Eine typische Zusammensetzung des O/W-Types der erfindungsgemäßen Dispersion ist: 20 g Öl, 1,2 g Lecithin, 0,1 g Arzneistoff und 78,3 g

Wasser. Dies bedeutet, daß die 21,2 g produzierter Öltropfen aus 20 g Ölphase (= 94,3%) und 1,2 g Stabilisator (= 5,7%) bestehen.

Weitere Beispiele für Emulgatoren sind neben Lecithinen die Polyethoxysorbitanester (Tween®-Typen), wie beispielsweise Laurate (Tween 20/21), Palmitate (Tween 40), Stearate (Tween 60/61), Tristearate (Tween 65), Oleate (Tween 80/81), oder Trioleate (Tween 85), Natriumglycocholat und Natriumlaurylsulfat (SDS) sowie die Sorbitanfettsäureester (Span® -Typen). Besonders bevorzugt ist Tween 80.

Bevorzugt werden weiterhin Tenside, Emulgatoren und Stabilisatoren eingesetzt, die für die Anwendung am und im Menschen zugelassen sind (z.B. Hilfsstoffe mit dem GRAS-Status).

Speziell für die Dispersionen vom Typ W/O werden die typischen Wasser-in-Öl-Tenside zur Stabilisierung benutzt, manchmal in Mischungen, auch in Mischungen mit O/W-Emulgatoren. Beispiele hierfür sind die Fettalkohole, Ethylenglykolmonostearat, Glycerolmonostearat, Sorbitanfettsäureester (Span®-Serie, z. B. Span 20-, Span 40-, Span 60- und Span 80-Serie, speziell Span 85), Ether von Fettalkoholen mit Polyethylenglykol (PEG) (z. B. Brij®-Serie), Ester von Fettsäuren mit PEG (z. B. Myrj®-Serie).

Im allgemeinen werden wieder Tenside und Stabilisatoren mit einem anerkannten Status bevorzugt, z. B. GRAS-Substanzen (Generally Regarded As Safe – Food Additives – GRAS substances, Food Drug Cosmetic Law Reports, Chicago (1994), Food Additive Database der FDA, Internet: www.fda.gov, 1999).

Im Fall daß die erfindungsgemäßen Dispersionen – zusätzlich zu den Öltropfen – noch Partikel von ungelöstem Wirkstoff enthalten, sollte die Partikelgröße so klein wie möglich sein, zum Beispiel zwecks Erhalt der physikalischen Stabilität und zur Vermeidung von Sedimentation. Zusätzlich, im Fall der intravenösen Applikation, sollten die Partikel klein genug sein, um Kapillar-

blockade zu vermeiden. Die kleinsten Blutkapillaren sind ungefähr 5-6 µm im Durchmesser. Daher sollte der Partikeldurchmesser 90% unterhalb von 5 µm sein, vorzugsweise auch der Durchmesser 95% und insbesondere der Durchmesser 100% sollte unterhalb 5 µm sein (gemessen mit Laserdiffraktometrie nach Abtrennung der Partikel von der Dispersion durch Zentrifugation, Volumenverteilungsdaten). Es ist noch günstiger, wenn diese Durchmesser alle unterhalb von 3 µm sind, da dann eine Sicherheitsdistanz zur Größe der kleinsten Kapillaren vorhanden ist.

Am vorteilhaftesten ist eine Partikelgröße des ungelösten Arzneistoffes unterhalb von 1000 nm (mittlere Partikelgröße gemessen mit Photonenkorrelationsspektroskopie). Diese Größe ist weit weg von den 5-6 µm der kleinsten Kapillardurchmesser und schließt gleichzeitig jegliche Sedimentationseffekte aus (diese Partikelgröße sedimentiert nicht relativ unabhängig von der Dichte des Arzneistoffes). Im Fall, daß eine schnellere Auflösung der Arzneistoffkristalle nach Applikation der Dispersion notwendig ist, sollte der mittlere PCS-Durchmesser im Bereich 100 nm bis ungefähr 400 nm, bevorzugt unter 100 nm sein.

Generell ist es günstig, den Wirkstoff zur Herstellung der Dispersion in der Form sehr feiner Kristalle zu verwenden, d.h., in mikronisierter Form mit einer mittleren Teilchengröße im Bereich von ca. 0,1 µm – 25 µm (Kolloidmühle, Gasstrahlmühle). Bevorzugt sind mittlere Teilchengrößen von 0,1µm – 5 µm, besonders bevorzugt von kleiner als 1 µm.

Der pH-Wert der erfindungsgemäßen Dispersionen liegt typischerweise zwischen 4 und 8, vorzugsweise zwischen 5 und 7,5, besonders bevorzugt zwischen 6 und 7,5 und wird in der Praxis bestimmt durch die Applikationsform.

Die Dispersion gemäß der Erfindung kann ferner eine wirksame Menge eines Antioxidanz, wie beispielsweise Vitamin E, insbesondere das Isomer alpha-Tocopherol enthalten. Alternativ

können auch beta- oder gamma-Tocopherol, oder Ascorbylpalmitat verwendet werden. Der Zusatz kann zwischen 10 mg und 2000 mg, vorzugsweise zwischen 25 mg und 1000 mg, bezogen auf 100 g Triglyceride betragen.

Eine typische Dispersion gemäß der Erfindung kann somit, bezogen auf die anwendungsfertige Gesamtzusammensetzung z.B. umfassen: 0,05 bis 1,0 Gew.-%, vorzugsweise 0,05 bis 0,5 Gew.-% des Wirkstoffes, 0,05 bis 2 Gew.-% eines Emulgators oder Emulgatorgemisches, beispielsweise Tween 80 und/oder Ei-Lecithin, dispergiert in einer O/W-Emulsion, die, bezogen auf die Emulsion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 20 Gew.-% Triglyceride enthält. Bei den Triglyceriden handelt es sich vorzugsweise um Sojabohnenöl, mittelkettige Triglyceride (wenigstens 90 % C8/C10) sowie Gemische aus Sojabohnenöl und mittelkettigen Triglyceriden (wenigstens 90 % C8/C10) im Gewichtsverhältnis 1:2 bis 2:1, vorzugsweise 1:1. Daneben können noch, bezogen auf die Gesamtzusammensetzung, 0,5 bis 5 Gew.-%, vorzugsweise 1 bis 3 Gew.-% übliche Isotonisierungsmittel, wie Glycerol, und 0,005 bis 0,05 Gew.-% Antioxidantien, wie beispielsweise alpha-Tocopherol enthalten sein. Ein besonders bevorzugter Wirkstoff ist insbesondere Amphotericin B. Zusätzlich können auch Konservierungsmittel zugesetzt werden. Die trifft insbesondere bei Abpackung der Dispersion in Gefäße zur Mehrfachentnahme zu.

Die Dispersion enthält den Wirkstoff gelöst in einer Menge, die größer ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit jeweils in der Wasser- und der Ölphase der Emulsion ergibt, wobei die "additive Menge" unter Normalbedingungen (20°C, Normaldruck) durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen) wird.

In der Dispersion sind typische Wirkstoffkonzentrationen 0,01 Gew.-% bis 30 Gew.-%, vorzugsweise 0,1 Gew.-% bis 10 Gew.-%,

besonders bevorzugt 1 Gew.-% bis 5 Gew.-%, bezogen auf die Gesamtmenge der Dispersion.

Arzneistoffe von besonderem Interesse – neben Amphotericin B – sind Vancomycin und Vecuronium. Des weiteren können schwerlösliche Arzneistoffe aus den Gruppen der Prostaglandine, z. B. Prostaglandin E_2, Prostaglandin $F_{2\alpha}$ und Prostaglandin E_1, Proteinase-Hemmstoffe, wie z. B. Indinavir, Nelfinavir, Ritonavir, Saquinavir, Zytostatika, z. B. Paclitaxel, Doxorubicin, Daunorubicin, Epirubicin, Idarubicin, Zorubicin, Mitoxantron, Amsacrin, Vinblastin, Vincristin, Vindesin, Dactiomycin, Bleomycin, Metallocene, z B. Titanmetallocendichlorid, und Lipid-Arzneistoff-Konjugate, wie z. B. Diminazenstearat und Diminazenoleat, und generell schwerlösliche Antiinfektiva wie Griseofulvin, Ketoconazol, Fluconazol, Itraconazol, Clindamycin, insbesondere antiparasitische Arzneistoffe, z. B. Chloroquin, Mefloquin, Primaquin, Pentamidin, Metronidazol, Nimorazol, Tinidazol, Atovaquon, Buparvaquon, Nifurtimox und antiinflammatorische Arzneistoffe, wie z. B.Ciclosporin, Methotrexat, Azathioprin, verwendet werden.

Dispersionen, die antiinflammatorische Arzneistoffe enthalten, können topisch, oral und parenteral angewendet werden. Im Falle einer topischen Anwendung auf der Haut, kann der Arzneistoff in das tiefere Gewebe penetrieren, wo entzündliche Prozesse stattfinden. Mit einer topischen Anwendung auf Schleimhäuten, wie z. B. am Auge, können Erkrankungen wie das "Trockene Auge"-Syndrom behandelt werden, dem ein entzündlicher Prozeß zugrunde liegt. Eine topische Anwendung auf den Schleimhäuten der Vagina ist ebenso vorteilhaft, z. B. ganz besonders für Antiinfektiva. Die Dispersion spreitet gut auf der Schleimhautoberfläche und gewährleistet so eine gleichmäßige Verteilung des Arzneistoffs. Insbesondere wenn diese Dispersion Öltröpfchen und zusätzlich sehr feine Arzneistoffkristalle enthält, da diese feinen Kristalle auf der vaginalen Schleimhaut haften und sich dort langsam auflösen und damit für eine verlängerte Arzneistoff-

wirkung sorgen (Depotwirkung). Für eine Anwendung am Auge ist es vorteilhaft, wenn man Dispersionen verwendet, die positiv geladen sind. Die Wechselwirkungen der positiv geladenen Partikel mit den negativ geladenen Zellmembranen verlängern die Verweilzeit des Arzneistoffs am Wirkort.

Die orale Anwendung der erfundenen Dispersion ist geeignet, die Bioverfügbarkeit von schwerlöslichen Arzneistoffen, die oral nicht ausreichend verfügbar sind, zu erhöhen. Beispiele hierfür sind Paclitaxel und Amphotericin B. Anstelle von wäßrigen Dispersionen können auch, durch Sprühtrocknung oder Gefriertrocknung überführte, trockene Formen verwendet werden.

Die parenterale, insbesondere die intravenöse Anwendung von arzneistoffhaltigen Dispersionen kann Nebenwirkungen reduzieren, z. B. bei Doxorubicin, Daunorubicin und Amphotericin B. Intravenös angewendete Dispersionen können durch Modifizierung der Oberfläche mit Apolipoproteinen gezielt zu gewünschten Zielorganen, wie Gehirn oder Knochenmark gelenkt werden. Dies ist bei Arzneistoffen, die keinen oder nur geringen Zugang zum Gehirn haben, von besonderem Interesse. Typische Beispiele hierfür sind zytotoxische Substanzen wie Doxrubicin. Eine gezielte Aufnahme zytotoxischer Dispersionen in das Gehirn ermöglicht die Behandlung von Hirntumoren, die bisher nur operativ oder lokal, z. B. mit implantierten therapeutischen Systemen und mit arzneistoffhaltigen Implantaten behandelt werden können. Dispersionen, die Antiinfektiva mit geringer Blut-Hirn-Schranken-Permeabilität enthalten, können nun genutzt werden, um diese Antiinfektiva zur Behandlung von persistierenden Parasiten durch die Blut-Hirn-Schranke zu transportieren.

Die Organverteilung von intravenös applizierten Arzneistoffträgern wird von deren physiko - chemischen Eigenschaften, wie z. B. Partikelgröße, Partikelladung und Oberflächenhydrophobie bestimmt. Negativ geladene Partikel werden zum Beispiel wesentlich schneller von den Makrophagen der Leber aufgenommen als

ungeladene Partikel (Wilkens, D, J. and Myers, P. A., Studies on the relationship between the electrophoretic properties of colloids and their blood clearance and organ distribution in the rat. Brit. J. Exp. Path. 47, 568-576, 1966). Um die In-vivo-Organverteilung zu modifizieren, kann die Ladung der erfindungsgemäßen Dispersion geändert werden, speziell positiv geladene Dispersionen sind vorteilhaft. Die positiv geladene Dispersion kann im Bereich der Einstichstelle an den negativ geladenen Zelloberflächen haften bleiben. Nach intravenöser Applikation der negativ geladenen Dispersion interagieren die Partikel mit negativ geladenen Proteinen, speziell mit Albumin, das mengenmäßig bedeutendste Protein im Blut. Aufgrund seiner Funktion als Dysopsonin kann es durch Adsorption an der Tropfenoberfläche und Bildung einer Albumin-Adsorptionsschicht die Verweilzeit der erfundenen Dispersion im Blut verlängern (z. B. verminderte Aufnahme durch Makrophagen der Leber).

Positiv geladene Dispersionen gemäß der Erfindung, können unter Verwendung positiv geladener Emulgatoren, Mischungen von positiv geladenen und ungeladenen Stabilisatoren (z. B. Poloxamere) und/oder negativ geladenen Emulgatoren (z. B. Lecithin) hergestellt werden. Positiv geladene Dispersionen, gemäß der Erfindung, haben ein positives Zetapotential. Das Zetapotential der Dispersionspartikel wird mit elektrophoretischer Messung in destilliertem Wasser (durch Zugabe von Natriumchlorid auf eine Leitfähigkeit von 50 µS/cm eingestellt) oder im Originaldispersionsmedium (äußere Phase der Dispersion) gemessen. Beispiele für positiv geladene Emulgatoren und Stabilisatoren sind Stearylamin, Cetypyridiniumchlorid (CPC), für positiv geladene Lipide N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniumchlorid (DOTMA), Didodecyldimethylammoniumbromid (DDAB), 2,3-Dioleyloxy-N-[2(spermidincarboxamid)ethyl]-N,N-dimethyl-1-propylammoniumtrifluoroacetat (DOSPA), 3β-[N-(N',N'-Dimethylaminoethan)carbamoyl]-cholesterol (DC-Chol).

Die Herstellung positiv geladener Dispersionen kann unter Verwendung positiv geladener Emulgatoren oder Emulgatormischungen im Produktionsprozeß durchgeführt werden (De novo-Herstellung). Der positiv geladene Emulgator kann alternativ auch zu einer negativ geladenen Dispersion zugefügt werden. Der Emulgator muß in ausreichender Menge zugefügt werden, damit eine Ladungsumkehr von negativ nach positiv eintritt.

Nähere Beschreibung des Produktionsprozesses: Die Mischung aus Lipid, Arzneistoff, Wasser und Emulgator oder andere Stabilisatoren muß einem hochenergetischem Dispergierprozeß unterzogen werden. Sollen Mischungen von Ölen und festen Fetten im Homogenisationsansatz verwendet werden, ist es vorteilhaft, das feste Fett bei erhöhter Temperatur im Öl zu lösen. Die bevorzugte Methode die erfindungsgemäße Dispersion herzustellen, ist die Hochdruckhomogenisation, z. B. mit Kolben-Spalt-Homogenisatoren oder Jet Stream-Homogenisatoren. Befindet sich Wasser in der äußeren Phase der Dispersion, wird die Homogenisation zwischen 0°C und 100°C durchgeführt. Die beste Dispergierung und schnellste Auflösung des schwerlöslichen Arzneistoffs wird erreicht, wenn die Homogenisation deutlich über Raumtemperatur durchgeführt wird, z. B. zwischen 35°C und 100°C. Die optimale Homogenisationstemperatur bei gleichzeitiger Berücksichtigung der chemischen Stabilität des Arzneistoffs wurde zwischen 45°C und 65°C ermittelt. Liegt ein extrem temperaturempfindlicher Arzneistoff vor, sollte die Homogenisation in der Nähe des Gefrierpunktes von Wasser durchgeführt werden (z. B. ungefähr 4°C).

Werden für die äußere Phase der Dispersion andere Flüssigkeiten als Wasser verwendet, die einen höheren Siedepunkt als Wasser besitzen, kann auch bei höheren Temperaturen oder unter 0°C (z. B. PEG 600) homogenisiert werden.

Im Fall von Mischungen aus Lipiden, Mischen von Öl und festem Lipid als "bulk"-Waren kann zu einer festen "bulk"-Mischung

führen – obwohl die daraus in der Dispersion produzierten Partikel flüssig sind (Thomson-Effekt). In diesem Falle sollte die Homogenisation bei einer Temperatur durchgeführt werden, die über dem Schmelzpunkt der "bulk"-Mischung liegt. Daßelbe gilt bei alleiniger Verwendung von festen Lipiden zur Herstellung der Dispersion gemäß der Erfindung. Der angelegte Homogenisationsdruck kann zwischen 10 und 11.000 bar liegen. Werden die Dispersionen mit 11.000 bar produziert, ist die resultierende Dispersion steril, da unter diesem hohen Druck Bakterien und Viren zerrissen werden. Ist eine Sterilisation durch Homogenisation nicht erwünscht, liegt der bevorzugte Produktionsdruck zwischen 200 bar und annähernd 4000 bar. Die in der Industrie in Produktionslinien verwendeten Hochdruckhomogenisatoren arbeiten gewöhnlich in einem Bereich von 200 bar bis 700 bar, daher wäre es nicht notwendig neue Maschinen anzuschaffen, wenn bei diesen Drücken gearbeitet wird. Die Produktion bei niedrigeren Drücken erfordert jedoch eine höhere Anzahl an Durchläufen (Zyklen). Muß eine höhere Anzahl an Durchläufen vermieden werden (z. B. begründet durch Aspekte der chemischen Stabilität des Arzneistoffes), sollte ein höherer Druck angewendet werden, der von 700 bar bis 4000 bar reicht. Für den Bereich 700-1500 bar können Homogenisatoren von APV Gaulin (Lübeck, Deutschland) verwendet werden, für den Bereich 700-2000 bar sind Maschinen der Firma Niro Soavi (Lübeck, Germany) geeignet, des weiteren ermöglichen spezielle Homogenisatoren der Firma Stansted (Stansted, UK) bei Drücken bis zu 4000 bar zu arbeiten.

Um die Dispersion herzustellen kann jede Homogenisatorausstattung verwendet werden, die eine genügend hohe Leistungsdichte erreicht, d. h. typischerweise über 10^4 W/m^3. Bei einigen Homogenisatoren kann die Leistungsdichte (dissipierte Energie pro Volumeneinheit der Dispergierzone) nicht errechnet werden, da die genaue Größe der Dispergierzone nicht bekannt ist (z. B. Microfluidizer). In diesem Fall muß die Eignung der Maschine für die Herstellung der erfundenen Dispersion auf empirischem Wege ermittelt werden. Beispiele für Homogenisatoren vom Kolben-Spalt-

Typ sind die Maschinen von den Firmen APV Gaulin, Niro Soavi, Stansted und French Press, ein Beispiel für Jet Stream-Homogenisatoren ist der Microfluidizer (Microfluidics, Inc., USA).

Die Erfindung wird durch die nachfolgenden Beispiele näher erläutert, ohne sie jedoch zu beschränken.

Beispiele

Beispiel 1

8 mg Amphotericin B wurden mit 40 g Lipofundin N 20 % angerieben (0,2 mg Amphotericin B/ml Emulsion) und die erhaltene Dispersion mit einem Ultra-Turrax-Rührer 5 Minuten bei 8000 Umdrehungen pro Minute gerührt. Anschließend wurde die Dispersion mit einem Micron LAB 40 bei 1.500 bar mit 20 Zyklen hochdruckhomogenisiert. Die Partikelgröße wurde mit einem Laserdiffraktometer bestimmt (Coulter LS 230, Coulter Electronics, USA). Der Durchmesser 50 % (D50%) der Volumenverteilung betrug 0,164 µm, D90% 0,340 µm, D95% 0,387 µm, D99% 0,466 µm und D100% 0,700 µm.

Beispiel 2

Es wurde ein Emulsionssystem mit Amphotericin B wie in Beispiel 1 hergestellt, die eingearbeitete Menge an Amphotericin B betrug jedoch 40 mg (d. h. 1 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50% 0,160 µm, D90% 0,362 µm, D95% 0,406 µm, D99% 0,485 µm und D100% 0,746 µm.

Beispiel 3

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die eingearbeitete Amphotericin B-Menge betrug jedoch 80 mg (d. h. 2 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50%

0,194 µm, D90% 0,381 µm, D95% 0,423 µm, D99% 0,494 µm und D100% 0,721 µm.

Beispiel 4

40 mg Amphotericin B-Pulver wurden mit 40 g Öl (Mischung 50 : 50 aus LCT und MCT) angerieben und die erhaltene Suspension wie in Beispiel 1 mit einem Ultra-Turrax für 5 Minuten gerührt. Anschließend wurde die Suspension mit einem Hochdruckhomogenisator Micron LAB 40 hochdruckhomogenisiert mit 2 Zyklen bei 150 bar, 2 Zyklen bei 500 bar und anschließend 20 Zyklen bei 1.500 bar. 8 g der erhaltenen öligen Suspension wurden dann in 32 g Wasser dispergiert, das 1,2 % Lecithin enthielt. Dispergierung erfolgte mit einem Ultra-Turrax für 5 Minuten bei 8000 Umdrehungen/Minute. Die erhaltene Dispersion wurde dann mit dem Micron LAB 40 hochdruckhomogenisiert bei 500 bar mit 10 Zyklen. Es wurden folgende Durchmesser gemessen: D50% 0,869 µm, D90% 2,151 µm, D95% 2,697 µm, D99% 3,361 µm.

Beispiel 5

Es wurde eine Emulsion analog Beispiel 4 hergestellt, allerdings erfolgte die Herstellung der Emulsion mit Hochdruckhomogenisation nicht bei Raumtemperatur, sondern in einem temperaturkontrolliertem LAB 40 bei 50°C. Es wurden folgende Durchmesser gemessen: D50% 0,647 µm, D90% 1,537 µm, D95% 1,768 µm, D99% 2,152 µm und D100% 3,310 µm.

Beispiel 6

Es wurde eine Amphotericin B-Emulsion durch Hochdruckhomogenisation analog Beispiel 1 hergestellt (0,2 mg Amphotericin B/ml Emulsion), die Hochdruckhomogenisation der Emulsion erfolgte bei Raumtemperatur. Der Arzneistoff wurde in 1,2%iger wäßriger Tween 80-Lösung angerieben, die Suspension vorhomogenisiert und 80 mg dieser Suspension mit 40g Lipofundin N 20% gemischt. Es wurden

folgende Durchmesser gemessen: D50% 0,142 µm, D90% 0,282 µm, D95% 0,331 µm, D99% 0,459 µm und D100% 0,843 µm.

Beispiel 7

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 1 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,245 µm, D90% 0,390 µm, D95% 0,426 µm, D99% 0,489 µm, D100% 0,700 µm.

Beispiel 8

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 2 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,237 µm, D90% 0,389 µm, D95% 0,426 µm, D99% 0,491 µm, D100% 0,701 µm.

Beispiel 9

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Hochdruckhomogenisation der Emulsion erfolgte bei 60°C. Es wurden folgende Durchmesser gemessen: D50% 0,197 µm, D90% 0,388 µm, D95% 0,436 µm, D99% 0,532 µm und D100% 0,953 µm.

Beispiel 10

Es wurde eine Emulsion analog Beispiel 7 hergestellt, der Homogenisationsdruck betrug jedoch 500 bar anstatt 1500 bar. Es wurden folgende Durchmesser gemessen: D50% 0,263 µm, D90% 0,401 µm, D95% 0,435 µm, D99% 0,493 µm und D100% 0,657 µm.

Beispiel 11

Die Partikelgrößenverteilung des Amphotericin B-Pulvers wurde mit Laserdiffraktometrie und Lichtmikroskopie analysiert. Abbildung 1 (oben) zeigt die Teilchengrößenverteilungskurve des Pulvers

nach Dispergierung in Wasser ermittelt mit Laserdiffraktometrie sowie die Partikelgrößenverteilung nach Einarbeitung in das erfindungsgemäße Emulsionssystem aus Beispiel 2 (Abbildung 1, unten). Im Emulsionssystem sind keine Amphotericin B-Kristalle mehr detektierbar, Amphotericin B wurde in das Emulsionssystem inkorporiert.

Beispiel 12

Die Amphotericin B-Emulsion wurde im Vergleich zu in Wasser dispergierten Amphotericin B-Kristallen mit Lichtmikroskopie untersucht. Abbildung 2 zeigt die lichtmikroskopische Aufnahme des Amphotericin B-Pulvers im polarisierten Licht, aufgrund der Anisotropie der Kristalle erscheinen sie hell. Abbildung 3 zeigt die lichtmikroskopische Aufnahme im polarisierten Licht nach Einarbeitung von Amphotericin B in das Emulsionssystem (Beispiel 1), anisotrope Strukturen sind nicht mehr detektierbar, das gesamte Bild ist nahezu schwarz. Für die Lichtmikroskopie wurde das Emulsionssystem unverdünnt auf den Objektträger aufgetragen.

Beispiel 13

Buparvaquon wurde analog zu Amphotericin B wie in Beispiel 6 in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,399 µm, D90% 0,527 µm, D95% 0,564 µm, D99% 0,635 µm und D100% 0,843 µm.

Beispiel 14

Atovaquon wurde analog zu Beispiel 1 anstelle von Amphotericin B in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,297 µm, D90% 0,437µm, D95% 0,475 µm, D99% 0,540 µm und D100% 0,744 µm.

Beispiel 15

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die Menge an eingearbeitetem Amphotericin betrug jedoch 5 mg/ml Emulsion. Die Löslichkeit in der Dispersion für Amphotericin war überschritten, neben Öltropfen lagen Arzneistoffkristalle vor (heterogene Dispersion).

Beispiel 16

Es wurde eine Amphotericin B-Emulsion durch Zumischung von 40 mg Amphotericin B zu 40 ml Lipofundin N 20 % hergestellt (d. h. Amphotericin B 1 mg/ml Emulsion). Die Mischung wurde mit 10 Zyklen bei 1500 bar und 45°C homogenisiert. Diese Emulsion wurde durch Autoklavieren bei 121°C für 15 Minuten (gemäß Deutschen Arzneibuches) sterilisiert. Der PCS-Durchmesser vor Autoklavierung betrug 203 nm, der Polydispersitätsindex 0,102, nach Autoklavierung lag der Durchmesser bei 208 nm, der Polydispersitätsindex bei 0,137.

Beispiel 17

100 mg Amphotericin B-Pulver wurden in 900 mg sterilen Wasser dispergiert, vorhomogenisiert und unter Verwendung von Pistill und Mörser in 20 g MCT-Öl mit 1,2% Lecithin eingearbeitet. Das Öl wurde in 80 g Wasser dispergiert und diese Mischung in einem Microfluidizer Typ Microfluidix M110y homogenisiert (d. h. Amphotericin B 1 mg/ml Emulsion). Die Homogenisation wurde bei 1000 bar für 10 Minuten durchgeführt. Der PCS-Durchmesser vor Autoklavierung betrug 192 nm, der Polydispersitätsindex 0,113, nach Autoklavierung lag der Durchmesser bei 196 nm, der Polydispersitätsindex bei 0,109.

Beispiel 18

Die unverdünnte Amphotericin B-Emulsion aus Beispiel 17 wurde auf größere Partikel und Amphotericin B-Kristalle mittels Lichtmikroskop untersucht. Abbildung 4 zeigt nur wenige größere Tröpfchen, Amphotericin B-Kristalle konnten nicht detektiert werden.

Beispiel 19

Es wurden Emulsionen, wie in Beispiel 16 beschrieben, hergestellt, wobei jedoch 15 Homogenisationszyklen durchgeführt wurden. Es wurden zwei Dispersionen hergestellt, die 1 mg/ml und 5 mg/ml Amphotericin B enthielten. Die Emulsionen wurden mit Lichtmikroskopie untersucht. Die lichtmikroskopische Aufnahme der Dispersion mit 1 mg/ml zeigt ein Emulsionssystem ohne detektierbare Amphotericin B-Partikel (Abb. 5), in der Dispersion mit 5 mg/ml Amphotericin B sind neben den Emulsionströpfchen kleine Amphotericin B-Kristalle detektierbar (Abb. 6)

Beispiel 20

Es wurde eine Amphotericin B-Emulsion, wie in Beispiel 16, hergestellt. Die Emulsion wurde 20 Zyklen bei einer Produktionstemperatur von 65°C homogenisiert. Der mittlere PCS-Durchmesser betrug 255 nm, der Polydispersitätsindex 0,098. Die Partikelgröße wurde mittels Laserdiffraktometrie mit einem Coulter LS 230 (Coulter Electronics, USA) durchgeführt. Der Durchmesser 50% war 0,247 µm, der Durchmesser 90% 0,410 µm, der Durchmesser 99% 0,566 µm und der Durchmesser 100% 0,938 µm. Die Amphotericin B-Konzentration lag bei 1 mg/ml, Sterilisation wurde mittels Autoklavieren bei 121°C für 15 Minuten durchgeführt. Die Arzneistoffkonzentration wurde mit HPLC analysiert, wobei in zwei Proben 93,8% und 91,0% wiedergefunden wurden.

Beispiel 21

100 mg Cyclosporin wurden mit 40 g Lipofundin N 20% angerieben. Die Homogenisation wurde mit 20 Zyklen bei 1500 bar und 25°C durchgeführt. Der mittlere PCS-Durchmesser betrug 234 nm, der Polydispersitätsindex 0,099. Der Laserdiffraktometerdurchmesser D50% lag bei 0,218 µm, der D90% bei 0,381 µm und der D100% bei 0,721 µm. Mit Lichtmikroskopie konnten keine Cyclosporin-Partikel detektiert werden (polarisiertes Licht, Dunkelfeld). Das Zetapotential der Emulsion wurde in destillierten Wasser mit einer eingestellten Leitfähigkeit von 50 µS/cm (durch Zugabe von Natriumchlorid) gemessen. Die Feldstärke lag bei 20 V/cm, die Umrechnung der elektrophoretischen Mobilität in das Zetapotential erfolgte mit der Helmholtz-Smoluchowski Gleichung. Das Zetapotential betrug -51 mV.

Beispiel 22

Es wurde eine Cyclosporin-Emulsion wie in Beispiel 21 beschrieben hergestellt. Während der Produktion wurden jedoch 0,5% Cetylpyridiniumchlorid (CPC) zugefügt. Die Emulsion war positiv geladen, das Zetapotential betrug +32 mV.

Beispiel 23

Es wurde eine Cyclosporin-Emulsion, wie in Beispiel 21 beschrieben, hergestellt. Während der Produktion wurden jedoch 1,0% Stearylamin zugefügt. Der PCS-Durchmesser betrug 247 nm, der Polydispersitätsindex 0.088. Der Laserdiffraktometerdurchmesser 50% lag bei 0,229 µm, der Durchmesser 90% bei 0,389 µm und der Durchmesser 100% bei 0,721 µm. das Zetapotential betrug +24 mV.

Beispiel 24

Eine Cyclosporin-Emulsion wurde de novo hergestellt. Die Zusammensetzung bestand aus 0,1% Cyclosporin, 0,5% Poloxamer 188, 0,5%

Eilecithin Lipoid E80, 0,15% Stearylamin, 10% Miglyol 812 und 2,25% Glycerol als Isotonisierungszusatz und Wasser ad 100%. Das Lecithin wurde in der Öl-Phase dispergiert, eine Prä-Emulsion wurde unter Zusatz der anderen Bestandteile durch Hochgeschwindigkeitsrühren hergestellt, das Cyclosporin-Pulver wurde im letzten Schritt zugefügt. Diese Mischung wurde bei 45°C mit 20 Zyklen und 1500 bar homogenisiert. Der PCS-Durchmesser betrug 226 nm, der Polydispersitätsindex 0,111. Der Laserdiffraktometerdurchmesser 50% lag bei 0,200 µm, der Durchmesser 90% bei 0,406 µm und der Durchmesser 100% bei 1,154 µm. Die Emulsion war positiv geladen, das Zetapotential betrug +31 mV.

Beispiel 25

Eine O/W-Dispersion wurde produziert mit der Zusammensetzung von 10 g Wasserphase, die 25 mg Amphotericin enthielt, 0,5 g Span 85, 0,25 Tween 80 und Miglyol 812 ad 50 g. 1,0 ml Amphotericin Suspension (2,5% Amphotericin/ml), stabilisiert mit 2,4% Lecithin Lipoid E 80 wurden gemischt mit destilliertem Wasser auf ein Gesamtgewicht von 10 g. Tween 80 wurde zur Wasserphase hinzugefügt, Span 85 zur Ölphase. Das Wasser wurde im Öl durch hochtouriges Rühren dispergiert. Die erhaltene Prä-Emulsion wurde bei 90°C homogenisiert unter Anwendung von 1500 bar und 20 Homogenisationszyklen. Größenanalytik wurde durchgeführt mit Laserdiffraktometrie (Mastersizer E, Malvern Instruments, United Kingdom). Der Durchmesser 50% war 2,25 µm, der Durchmesser 90% 4,21 µm.

Erklärungen zu Abbildungen:

Abb. 1: Partikelgrößenverteilung des Amphotericin-Pulvers vor Einarbeitung in die Dispersion (oben) und Partikelgrößenanalyse der erfindungsgemäßen Dispersion nach Einarbeitung des Amphotericin-Pulvers (unten, Beispiel

2), die Arzneistoffpartikel sind nicht mehr detektierbar (Laserdiffraktometrie)

Abb. 2: Lichtmikroskopische Aufnahme des Amphotericin-Pulvers vor Einarbeitung in die O/W-Emulsion (Beispiel 1) (Polarisations-Aufnahme im Dunkelfeld, anisotrope Kristalle erscheinen weiß, Balken wie in Abb. 3 (10 µm)).

Abb. 3: Lichtmikroskopische Aufnahme der O/W-Emulsion nach Einarbeitung des Amphotericin-Pulvers aus Abb. 2 (Beispiel 1) (Polarisations-Aufnahme, im Dunkelfeld nur schemenhafte Reflexe der isotropen Emulsionstropfen, Balken 10 µm).

Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/ml Amphotericin B aus Beispiel 19.

Abb. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/ml Amphotericin B aus Beispiel 19.

Patentansprüche

1. Dispersion, die eine ölige Phase und eine wäßrige Phase in Form einer O/W-Emulsion oder einer Wasser-in-Öl (W/O) Emulsion, mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfaßt, dadurch gekennzeichnet, daß die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wäßrigen Phase der Emulsion ergibt.

2. Dispersion nach Anspruch 1, dadurch gekennzeichnet, daß der Arzneistoff zusätzlich zum gelösten Zustand noch in hochdisperser fester kristalliner Form vorliegt, wodurch sich eine Dispersion mir einer heterogenen dispersen Phase aus Öltropfen und aus Arzneistoffkristallen ergibt.

3. Dispersion nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß mindestens 90%, bevorzugter 95% der vorhandenen Kristalle kleiner als 5 µm sind und insbesondere 100% kleiner als 5µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie), wobei besonders bevorzugt 90% kleiner als 3 µm, bevorzugter 95% kleiner als 3 µm und insbesondere 100% kleiner als 3µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

4. Dispersion nach Anspruch 3, dadurch gekennzeichnet, daß mindestens 90%, bevorzugt 95% und insbesondere 99% der Kristalle kleiner als 1 µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

5. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Öl-in-Wasser-Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 99,5 Gew.-%, vorzugsweise 10 bis 95 Gew.-% insbesondere 60 bis 95 Gew.-% und speziell 70-95% wäßrige Phase enthält.

6. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Wasser-in-Öl (W/O) Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 25 Gew.-% insbesondere 10 bis 20 Gew.-% wäßrige Phase enthält.

7. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Emulgator und/oder Stabilisator enthält.

8. Dispersion nach Anspruch 7, dadurch gekennzeichnet, daß sie, bezogen auf die Gesamtmenge Dispersion, weniger als 15%, bevorzugt weniger als 10% und insbesondere weniger als 2%, bevorzugt 0,6% bis 1,2% Emulgator und/oder Stabilisator enthält.

9. Dispersion nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß sie als Emulgatoren Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Sorbitanestern (insbesondere Span 85), Polyethylenglykolsorbitanester (insbesondere Tween 80), Natriumglycocholat, Natriumlaurylsulfat (SDS) oder Gemischen derselben und/oder als Stabilisatoren Block-Copolymere, insbesondere Poloxamere (bevorzugt Poloxamer 188 und 407) oder Poloxamine (bevorzugt Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide (bevorzugt Hyaluronsäure oder Chitosan und ihre Derivate), Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (bevorzugt Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester (bevorzugt Saccharosemonostearat) oder Natrium-

citrat einzeln oder in irgendeiner Mischung derselben enthält.

10. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie eine O/W-Emulsion umfaßt und die zur Herstellung der Dispersion verwendete ölige Phase (Lipidphase) nur bei Raumtemperatur feste Lipide oder nur bei Raumtemperatur flüssige Lipide umfaßt oder eine Mischung aus einem oder mehreren bei Raumtemperatur flüssigen Lipiden mit einem oder mehreren bei Raumtemperatur festen Lipiden umfaßt.

11. Dispersion nach Anspruch 10, dadurch gekennzeichnet, daß die Mischung aus flüssigem Lipid und festem Lipid von 99 + 1 bis zu 1 + 99 variiert (Gewichtsteile), insbesondere in der Mischung der Anteil von flüssigem Lipid mindestens 10 Teile beträgt, bevorzugt mindestens 30 Teile und insbesondere mindestens 50 Teile.

12. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Partikel aus folgenden einzelnen Lipiden oder deren Mischungen hergestellt werden: natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben insbesondere synthetische Monoglyceride, Diglyceride und Triglyceride als individuelle Substanzen oder als Mischung, vorzugsweise Hartfett, oder Imwitor 900, Triglyceride, insbesondere Glyceroltrilaurat, Glycerolmyristat, Glycerolpalmitat, Glycerolstearat und Glycerolbehenat, und Wachse, insbesondere Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB), sowie Kohlenwasserstoffe, insbesondere Hartparaffin.

13. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als Ölphase Sojaöl, Safloröl, langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT), insbesondere Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride (bevorzugt wie in Stesolid) einzeln oder in Mischungen enthält.

14. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als wäßrige Phase Wasser, Mischungen von Wasser mit wassermischbaren organische Flüssigkeiten, insbesondere flüssigen Polyethylenglykolen (PEG) (bevorzugt PEG 400 und 600) enthält.

15. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die wäßrige Phase Zusätze enthält, insbesondere Elektrolyte, Nichtelektrolyte (bevorzugt Glycerol, Glucose, Mannit, Xylit zur Isotonisierung) und/oder Gelbildner (bevorzugt Cellulosederivate zur Viskositätserhöhung).

16. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die eingesetzte Emulsion eine O/W-Emulsion ist und Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid oder Salvilipid ist.

17. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Arzneiwirkstoffen zur Behandlung des menschlichen und tierischen Körpers.

18. Dispersion nach Anspruch 17, dadurch gekennzeichnet, daß sie einen oder mehrere Arzneistoffe aus den Gruppen der Anaesthetika, Antibiotika, Antimykotika, Antiinfektiva, Kortikoide, Hormone, Antioestrogene, Antispetika, gefäßaktive Substanzen, Glaukomittel, Beta-Blocker, Cholinergi-

ka, Sympathomimetika, Carboanhydrase-Hemmer, Mydriatika, Virustatika, Mittel zur Tumortherapie, Antiallergika, Vitamine, antiinflammatorische Wirkstoffe sowie Immunsuppressiva enthalten, insbesondere Cyclosporin, oder irgendeine Kombination daraus enthält.

19. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladen ist.

20. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladene Stabilisatoren enthält, insbesondere Natriumlaurylsulfat (SDS), Stearylamin, und/oder positiv geladene Phospholipide und/oder positiv geladene Lipide.

21. Dispersion nach Anspruch 20, dadurch gekennzeichnet, daß sie die eingesetzte Emulsion eine O/W-Emulsion ist und intravenös appliziert werden kann, wobei neben positiven Stabilisatoren auch Mischungen mit Lecithin und/oder nichtionischen Stabilisatoren eingesetzt werden können, insbesondere Poloxamer Polymere.

22. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Cyclosporin als Wirkstoff enthält.

23. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß als Wirkstoff ein Antimykotikum (bevorzugt Amphotericin B), ein Antiinfektivum (bevorzugt Buparvaquon oder Atovaquon), ein Immunsuppressivum (bevorzugt Cyclosporin A oder eines seiner natürlichen und synthetischen Derivate), ein Mittel zur Tumortherapie (bevorzugt Paclitaxel oder Taxotere) enthält.

24. Verfahren zur Herstellung einer Zusammensetzung gemäß einem der Ansprüche 1 bis 23, dadurch gekennzeichnet, eine wäßrige Phase und eine ölige Phase, die nicht oder nur

teilweise miteinander mischbar sind, sowie gegebenenfalls ein oder mehrere Emulgator(en) und/oder Stabilisator(en) und eine feste Phase, die mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff umfaßt, miteinander gemischt werden und die erhaltene Mischung aus flüssigen und festen Phasen einem hochenergetischen Homogenisationsprozeß mit einem Homogenisator unterzogen werden, wobei keine toxikologisch bedenklichen organischen Lösungsmittel verwendet werden.

25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß der Wirkstoff ohne vorherige Auflösung als Feststoff in die flüssigen Phasen der Dispersion eingearbeitet wurde.

26. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff mit einer O/W-Emulsion oder einer W/O-Emulsion angerieben oder gemischt wird und diese Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

27. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff in einer Emulgatorlösung dispergiert wird, diese Dispersion homogenisiert wird, anschließend mit einer O/W-Emulsion oder einer W/O-Emulsion gemischt wird und die so erhaltene Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

28. Verfahren nach einem der Ansprüche 24 bis 27, dadurch gekennzeichnet, daß als Homogenisator ein Rotor-Stator-Homogenisator (vorzugsweise eine Kolloidmühle) oder ein Hochdruckhomogenisator (vorzugsweise ein Kolben-Spalt-Homogenisator (APV Gaulin, French Press, Niro, Stansted) oder ein Rohrhomogenisator (jet stream) (Microfluidizer oder Nanojet)) eingesetzt wird.

29. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß sich der Wirkstoff am Ende des Homogenisationsprozesses vollständig oder nahezu vollständig aufgelöst hat, so daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10 Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

30. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

31. Verfahren nach einem der Ansprüche 24 bis 30, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 5 µm, bevorzugt einen Durchmesser 95% kleiner als 5 µm und insbesondere einen Durchmesser 100% kleiner als 5µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

32. Verfahren nach Anspruch 31, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 3 µm, bevorzugt einen Durchmesser 95% kleiner als 3 µm und insbesondere einen Durchmesser 100% kleiner als 3µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

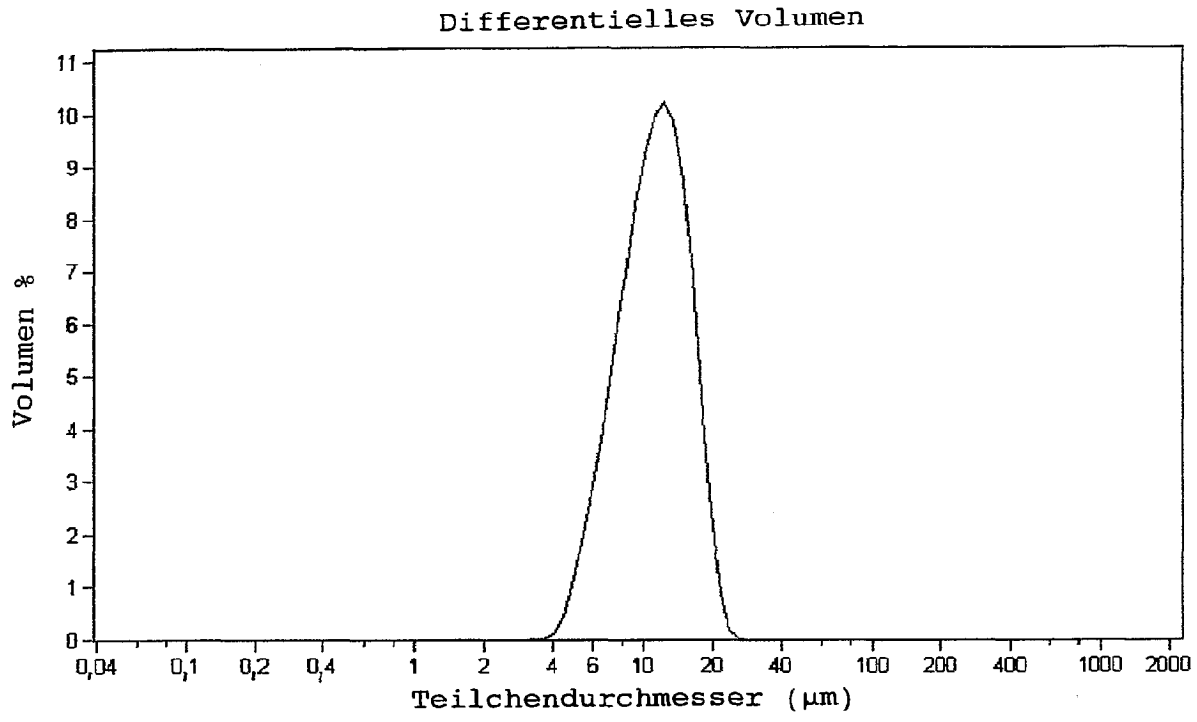
33. Verfahren nach Anspruch 32, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen mit Photonenkorrelationsspektroskopie (PCS) bestimmten Durchmesser kleiner als 1000 nm aufweisen.

34. Verwendung der Dispersion gemäß einem der Ansprüche 1 bis 23 oder hergestellt gemäß einem der Ansprüche 24 bis 33 zur Herstellung eines Arzneimittels.

35. Verwendung nach Anspruch 34, zur Herstellung eines Arzneimittels zur Behandlung von Mykosen, vorzugsweise systemischen Mykosen, Entzündungen, Allergien, Tumorerkrankungen, kardiovaskulären Erkrankungen, viralen und anderen Infektionen und zur Durchführung von Anästhesien.

36. Verwendung nach Anspruch 34 oder 35, dadurch gekennzeichnet, daß das Arzneimittel topisch, oral, peroral sowie parenteral, insbesondere intravenös, intra- und subkutan, intramuskulär, intraartikulär oder intraperitoneal wird, vorzugsweise am Auge angewendet wird und vorzugsweise Cyclosporin enthält.

37. Verwendung nach einem der Ansprüche 34 bis 36, dadurch gekennzeichnet, daß das Arzneimittel eine verlängerte Verweilzeit im Blut zeigt, verglichen mit negativ geladenen Dispersionen.



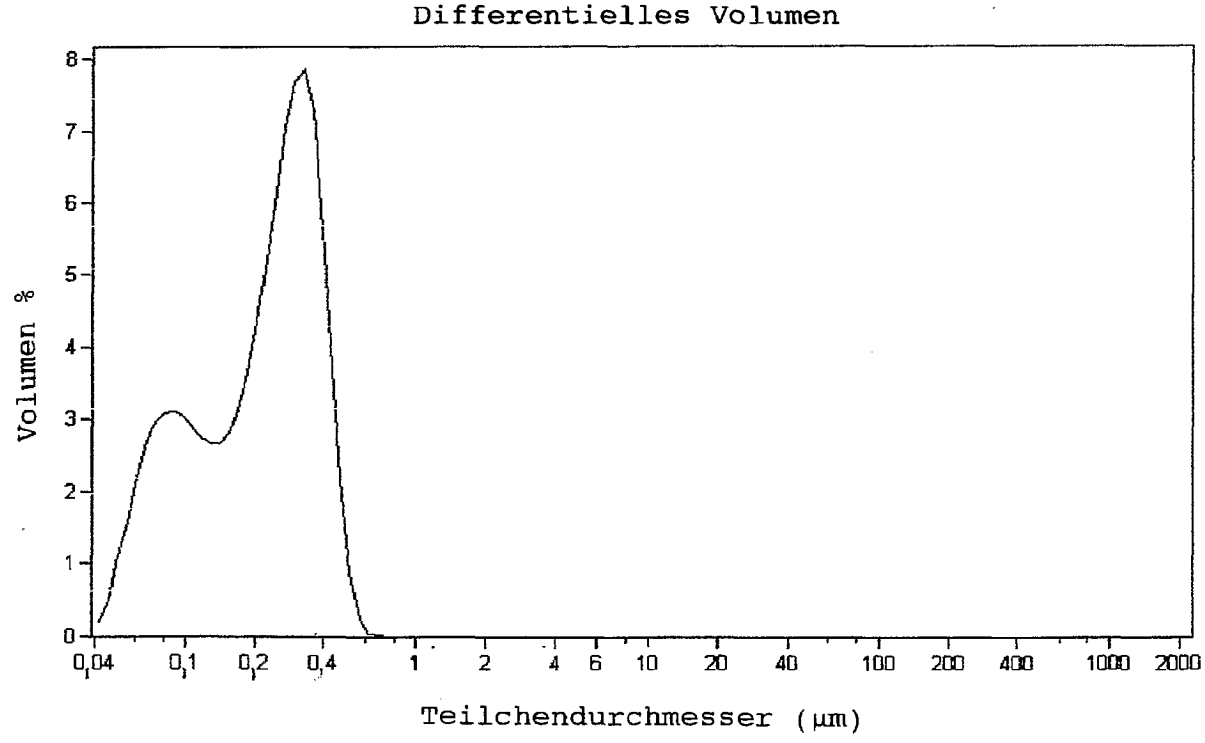


Abbildung 1

Abbildung 2

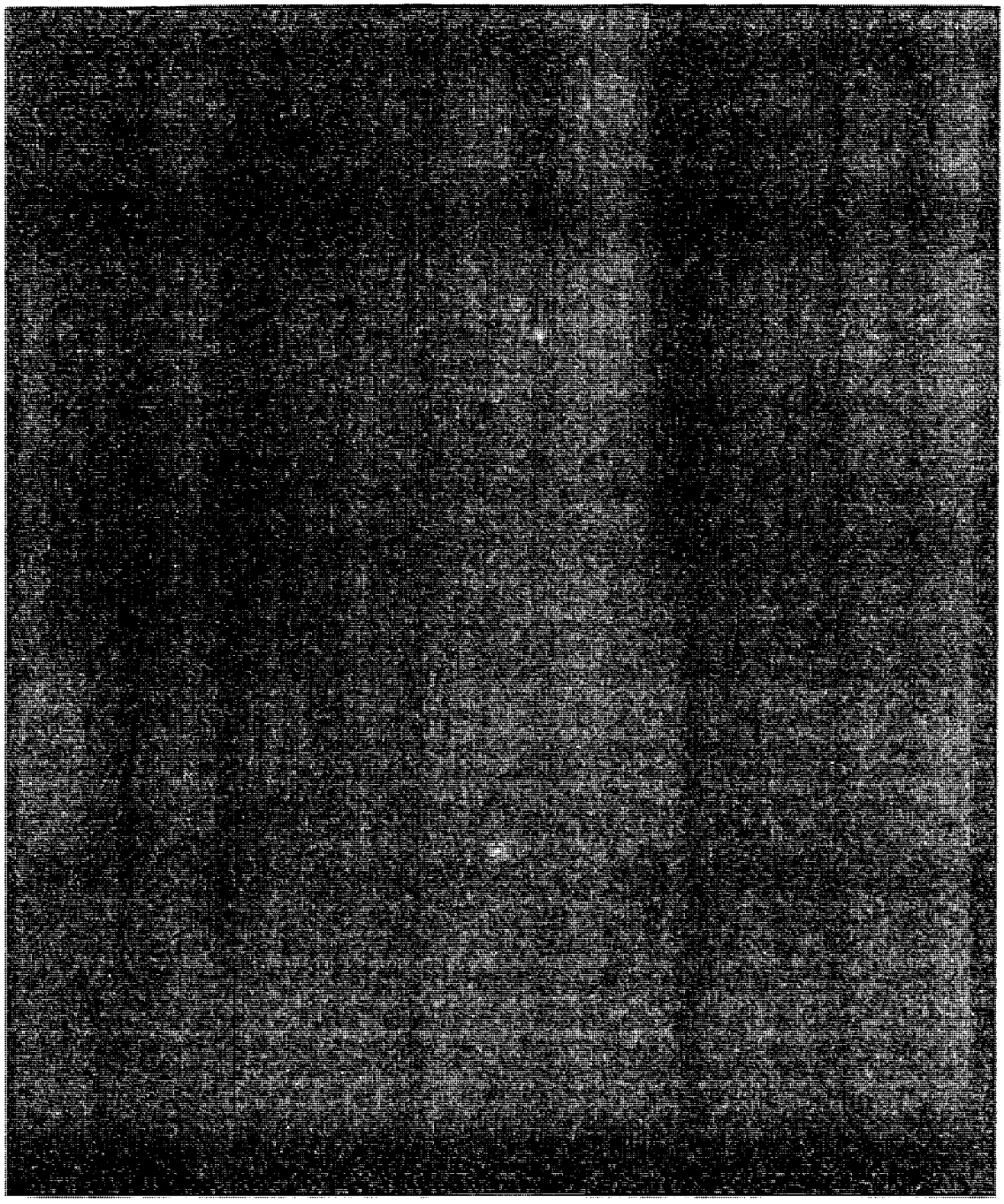


Abbildung 3

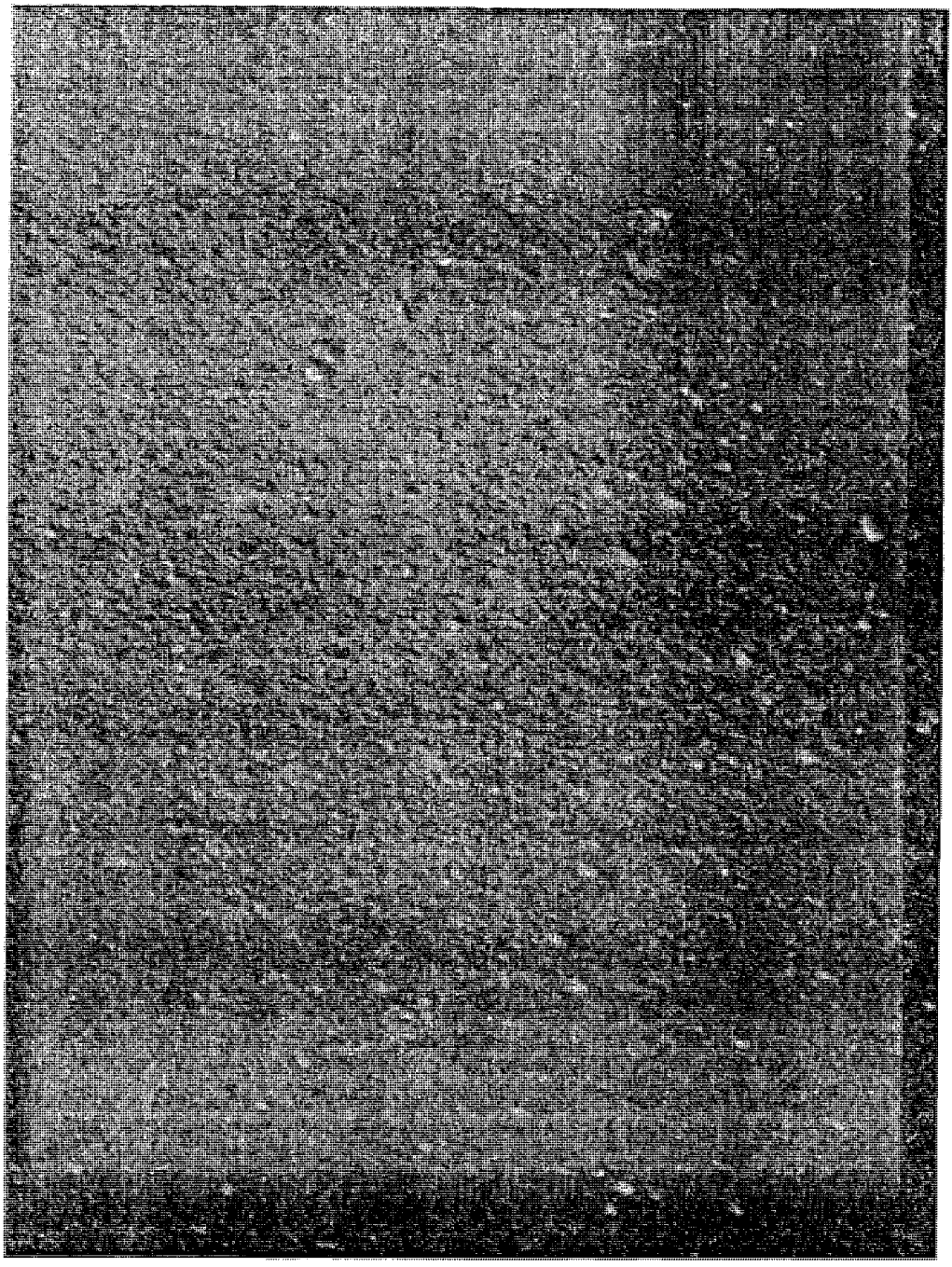


Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/mL Amphotericin B aus Beispiel 19.

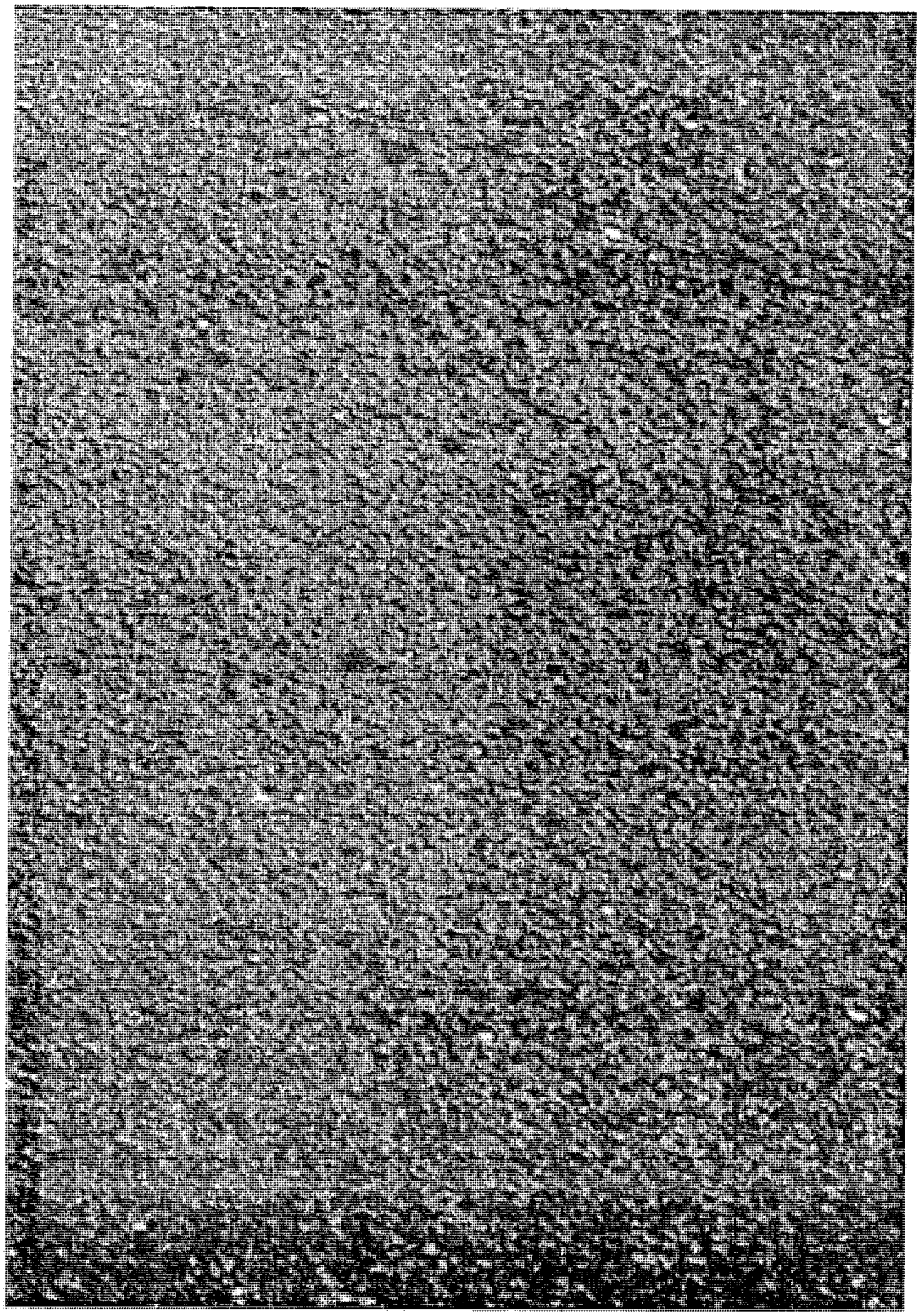


Fig. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

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(54) Title: COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

(57) Abstract: The present invention discloses a topical composition for prevention and alleviation of wrinkling which comprises one or two or more selected from the group consisting of Phenytoin, Valproic acid, Cyclosporin A, Nifedipine, Diltiazem, Verapamil HC1 and Amolldipine as an active ingredient having an effect of boosting collagen synthesis.

COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

Technical Field

The present invention relates to a topical composition for prevention and alleviation of skin wrinkles which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, in conjunction with conventional components of a formulation for transdermal absorption such as cream, ointment, lotion, skin tonic, gel, pack, patch or patch-type administering apparatus.

Background Art

Skin aging is developed by both endogenous causes, for example, aging, and environmental causes. The effects of aging are shown as wrinkles in the skin, which include neck wrinkles, worry lines, frown lines, crow's feet, the folds from the side of the nose to the corners of the mouth, and fine lines around the eyes, below the lips, and over the face. Skin wrinkles caused by aging, though there are individual differences, commonly occur in individuals in their early twenties and increase with age. With aging, the amount of dermal collagen of skin is decreased and alterations in elastic fibers occur, whereby the skin relaxes and fine wrinkles appear. Meanwhile, collagen is a major matrix protein produced by fibroblasts of the skin, being present in the extracellular matrix. It is a primary protein comprising 30 % by weight of proteins in the human body, and has a firm structure of a triple helix. It is known that collagen functions to provide structural stability to the skin, durability of connective tissues and cohesion of tissues while supporting cell coherence, cell proliferation, and induction of differentiation of unspecialized cells. Also, it is known that collagen is broken down by exposure to UV, an

environmental cause of skin aging, and the damage by UV is proportional to the accumulated time of exposure thereto. UV denatures collagenous fibers, causing wrinkles and decreasing elasticity of the skin. Other environmental causes known to promote skin aging include wind, heat and smoking.

As mentioned above, collagen is closely related with skin aging. The amount of collagen in the dermis is decreased with aging and by UV radiation. Collagen decreases by 65 % from age 20 to age 80. Such a decrease of collagen makes the skin thin and further, is closely associated with the formation of skin wrinkles.

Studies have been widely performed to find a method for the prevention and alleviation of skin wrinkles, elucidating important roles of collagen. The studies also elucidated that when collagen synthesis is activated in skin, dermal matrix components are increased, which has effects including alleviation of wrinkles, and increased elasticity and strength of skin. Therefore, using collagen having a moisture retention effect, some collagen-incorporated cosmetics have been developed. Such cosmetics, however, are poor in holding moisture, since the cosmetics are applied to the surface of skin and high molecular weight collagen is poor in transdermal absorption. As a result, their use fails to provide an intrinsic improvement in skin appearance. In the prior art, retinoic acid, TGF-β , protein derived from an animal placenta (JP8-231370), betulinic acid (JP8-208424) and *Chlorella* extract (JP9-40523, JP10-36283) are disclosed as substances for promoting collagen synthesis. As for retinoic acid, it is unstable and has a problem in its safety due to causing irritation and redness upon application the skin, limiting the available dosage thereof. As for other above substances including *Chlorella* extract, their effects of increasing collagen synthesis are weak, so they hardly improve skin appearance. Recently, several new procedures for treating wrinkles by promoting collagen synthesis have been introduced. Examples include ultrasonic treatment, skin scaling, laser peeling, botulinum toxin injection and Restilene injection. These procedures, however, have disadvantages in terms of cost effectiveness and duration of their effects. Thus, it is desirable to search for and develop a

highly effective agent for promoting collagen synthesis.

Disclosure of the Invention

Therefore, the present inventors have conducted studies to develop a compound having an effect of promoting collagen synthesis, and found that phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine which are already known as anticonvulsants, immunosuppressants or calcium channel inhibitors have very strong effects of promoting collagen synthesis in human fibroblast cell lines. Further, it was found that as applied to the skins of rats and mice, the compounds exhibited strong inhibition and alleviation effects of wrinkles, proving the effects of inhibiting and preventing signs of skin aging such as skin wrinkles. Accordingly, the present invention is directed to a composition comprising phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl or amoldipine as an active ingredient having an effect of promoting collagen synthesis.

Phenytoin and valproic acid have been widely used as anticonvulsants for treatment of epilepsy, and their effects on collagen synthesis are documented (USP5686489; Minerva Stomatol., 47(9): 387-398, Sep. 1998). Cyclosporine A has been widely used as an immunosuppressant for suppressing rejection of tissues after transplantation, and its effects on collagen synthesis are reported (J Periodontol., 72(7): 921-931, Jul. 2001). Nifedipine, diltiazem, verapamil HCl and amoldipine have already been used as calcium channel inhibitors, and their effects on collagen synthesis are also reported (J Periodontol., 72(8), Aug. 2001; Proc Natl Acad Sci USA, 93(11): 5478-5482, May 1996; J Urol., 156(6): 2067-2072, Dec. 1996). However, the above drugs are not disclosed for use as topical agents applied to the skin for preventing and alleviating skin wrinkles, as in the present invention.

Hereinafter, a topical composition for preventing and alleviating skin wrinkles will be described in detail, in conjunction with experimental

examples and examples.

Experimental example 1: Effect of active ingredients of the invention on promoting collagen production in fibroblasts

To investigate the effects of active ingredients of the invention on promoting collagen production in fibroblasts in cellular level, respective active ingredients were added to cultures of fibroblasts derived from a human. The synthesized collagen was measured using a modification of a method proposed by Martens (Gut, 33: 1664-1670, 1992) to evaluate the effects of the active ingredients. The experimental protocol in detail is as follows.

Human-derived fibroblasts were transferred to a 24 well plate and cultured in a medium containing 10 % fetal bovine serum (FBS) for 24 hours, followed by washing twice with phosphate buffered saline. The cells were then incubated in a medium containing 1 % FBS in the presence of phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl or amoldipine at final concentrations of 10^{-8} to 10^{-5} M. After 1 hr incubation, cultures were added with 10 μ Ci of ^{3}H-proline per well, followed by a final incubation for 24 hours. After the incubation was terminated, cells from each group were harvested and two fractions of each culture were prepared. One fraction from each culture was treated with collagenase. To all fractions was added trichloroacetic acid to precipitate proteins. The amount of radioactivity incorporated into collagenase-sensitive protein was measured and compared with that of the other fraction which was not treated with collagenase. The difference in radioactivity was attributed to the promoting effect of the compound. Samples without an active ingredient served as a control group, the amount of collagen synthesized being 100 %. The results are shown in Table 1.

Table 1: Effect of promoting collagen production in fibroblasts (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	215.28*	298.35	360.65	381.54
Valproic acid	100.00	201.13	283.24	332.11	370.21
Cyclosporine A	100.00	212.11	293.21	352.31	372.27
Nifedipine	100.00	204.31	292.21	330.30	358.16
Diltiazem	100.00	199.15	276.25	321.23	362.12
Verapamil HCl	100.00	183.25	280.23	331.09	355.12
Amoldipine	100.00	182.42	280.07	330.42	355.26

* Rate of collagen production = (collagen production of experimental group/ collagen production of control group) x 100

As shown in Table 1, the active ingredients in experimental groups have effects of promoting collagen production with increasing concentration of the compounds, ranging from the minimum of 182.42 % to the maximum of 381.54 % in a dose-dependent manner, compared to the control group which contains no active ingredient of the invention. This demonstrates that the active ingredients of the invention have excellent effects on promoting collagen synthesis.

Experimental example 2: Promotion of collagen production in rat skin

The effects of application of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on promoting collagen production in animal skin were investigated. The synthesized collagen was measured using a modification of a method proposed by Mard L DaCosta et al. (Surgery, 123: 287-293, 1998).

In brief, 5-week male SD rats were grouped with 5 rats per group. The rats were each incised 1 cm in the center of their abdomens and PVA sponges (Unipoint ind.) were inserted therein. After suturing, as for

experimental groups, respective active ingredients to be examined were applied to the PVA sponge-embeded regions in a volume of 200 ㎕ every day for 10 days. Upon autopsy, the PVA sponge was removed to quantify hydroxyproline. The PVA sponge was added with 4 ㎖ of 6 N HCl, hydrolized at 130℃ for 3 hours and was subjected to complete drying. 50 ㎕ of methanol was added and the solution was incubated at 110℃ until HCl was removed. 1.2 ㎖ of 50 % isopropanol was added to dissolve the remaining precipitate. 200 ㎕ of chloramine-T (sodium p-toluensulfochloramide trihydrate) solution was added while stirring, and let stand for 10 min. After adding 1.2 ㎖ of Ehrlich reagent and mixing, the solution was incubated at 50℃ for 90 min. The resulting solution was cooled to room temperature and absorbance at 558 ㎚ was measured. Hydroxyproline standard solutions were prepared by dissolving 1 ㎎ hydroxyproline in 1 ㎖ HCl and diluting it to concentrations of 0, 0.2, 0.4, 0.8, 1 ㎎ each relative to 25 ㎕ of 6 N HCl. The standard solutions were hydrolyzed at 130℃ for 3 hours. The quantified value of hydroxyproline, relative to hydroxyproline value (100 %) of the control group which was applied with solvent only, are shown in Table 2.

Table 2: Effect of promoting collagen production in animal skin (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	132.58*	143.51	167.41	182.47
Valproic acid	100.00	128.05	139.24	157.72	178.13
Cyclosporine A	100.00	131.02	143.07	164.82	179.26
Nifedipine	100.00	129.92	142.41	161.43	185.88
Diltiazem	100.00	122.44	136.76	157.45	175.23
Verapamil HCl	100.00	135.63	147.39	167.06	183.32
Amoldipine	100.00	132.50	149.65	163.84	181.12

* Rate of collagen production = (hydroxyproline value of experimental group/hydroxyproline value of control group) x 100

As shown in Table 2, the active ingredients increased collagen production in rat skin and the rates of increase ranged from the minimum of 122.44 % to the maximum of 185.88 %, compared to the control group to which no active ingredient of the invention was applied. This demonstrates that the active ingredients of the invention strongly promote dermal collagen synthesis.

Experimental example 3: Effect on inhibiting the generation of wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on inhibiting the generation of wrinkles in hairless mice were investigated.

6-week hairless mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective compounds at a concentration of 10^{-8} to 10^{-3} M. The control groups were applied with solvent only, without any active ingredient. The experimental protocol in detail is as follows. Hairless mice were radiated using simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 12 weeks, thereby generating wrinkles. Respective active ingredients or the solvent only were applied twice every day (specifically, on radiation days, the application was performed at 30 min before and after the radiation), at a volume of 100 $\mu\ell$ each for 10 weeks from the first radiation day. Degrees of inhibition of generated wrinkles were determined. The determination was performed by visual observation with naked eyes and photography. The degrees of inhibition of wrinkles in the compound-treatment groups (experimental groups) were compared with the control group (Score 0) and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 3a to 3c.

Table 3a: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-8} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	2	8
Exp. 2	Valproic acid	0	1	1	8
Exp. 3	Cyclosporine A	0	1	2	7
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	1	8
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	10	0	0	0

Table 3b: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-5} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	1	3	6
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	10	0	0	0

Table 3c: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-3} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	0	2	8
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	0	1	9
Exp. 7	Amoldipine	0	0	2	8
Control 1	-	10	0	0	0

As shown in Tables 3a to 3c, the active ingredients inhibited the generation of wrinkles by a high degree in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on inhibiting the generation of wrinkles.

Experimental example 4: Effect of alleviating wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on alleviating photo-induced wrinkles in 6-week hairless mice were investigated.

Mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective active ingredients at a concentration of 10^{-8} to 10^{-3} M. The control groups were the mice applied with solvent only without any active ingredient. The experimental protocol is as follows. Hairless mice were radiated using a simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 10 weeks, thereby generating wrinkles. Then, respective active ingredients or the solvent only were applied at a volume of 100 $\mu\ell$ each, twice a day for 6 weeks. Degrees of wrinkle

reduction were determined. The determination was performed by visually observing the compound-applied region with naked eyes, and the region was photographed. The degrees of alleviation of wrinkles in the compound-treatment groups (experimental groups) were compared with those of the control group and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 4a to 4c.

Table 4a: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-8} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	2	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	3	5
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	2	6
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	9	1	0	0

Table 4b: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-5} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	3	6
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	1	7
Exp. 7	Amoldipine	0	2	2	6
Control 1	-	9	1	0	0

Table 4c: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-3} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	0	2	8
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	8	2	0	0

As shown in Tables 4a to 4c, the active ingredients exhibited a high level of alleviation effects on the photo-induced wrinkles in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on alleviating wrinkles.

The results from the experiments employing the active ingredients of the invention for evaluating effects of promoting collagen synthesis in fibroblasts derived from human, rats and mice demonstrate that phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine with concentrations of 10^{-8} to 10^{-3} M each have excellent effects of promoting collagen synthesis.

A topical composition comprising an active ingredient of the invention may include any formulations applicable to skin, for example, cream, ointment, lotion, skin tonic, gel, pack, aerosol types thereof, patch and patch-type apparatus with micro needles. The compositions were especially prepared in formulations of cream, ointment and pack and applied to human skin for evaluating reduction of wrinkles. It was found that they significantly reduce wrinkle density.

Hereinafter, the present invention will be described in detail, in conjunction with examples and comparative examples. It is noted that these

examples are provided only for illustrative purposes, and the present invention is not to be construed as being limited to those examples.

Preparation of variable formulations comprising an active ingredient of the invention

Agents topically applicable to the skin were prepared with compositions given in Tables 5 to 7, employing each active ingredient and other supplementary components according to the invention. In the invention, ointment, cream, pack, essence, skin softner, nutrient emulsion, patch and patch-type apparatus with micro needles, each topically applicable to the skin, were prepared. It is noted that though only formulations employing phenytoin and cyclosporine A as active ingredients were prepared herein, the examples are not intended to limit the formulations and active ingredients.

Table 5: Formulation of ointment

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Diethyl Sebacate	8	8	8	8	8
Spermaceti	5	5	5	5	5
Polyoxyethylene*	6	6	6	6	6
Sodium benzoate	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.1	-	-	-
Cyclosporine A	-	-	0.00001	0.1	-
Total weight with Vaseline added	100	100	100	100	100

* Polyoxyethylene oleic ether phosphate

Table 6: Formulation of cream

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Stearic acid	15.0	15.0	15.0	15.0	15.0
Setanol	1.0	1.0	1.0	1.0	1.0
Potassium hydroxide	0.7	0.7	0.7	0.7	0.7
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	3.0	3.0	3.0	3.0	3.0
Preservative	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 7: Formulation of pack

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	4.0	4.0	4.0	4.0	4.0
Polyvinyl alcohol	15.0	15.0	15.0	15.0	15.0
Ethanol	8.0	8.0	8.0	8.0	8.0
Polyoxyethylene oleic ethyl	1.0	1.0	1.0	1.0	1.0
Paraoxy methyl benzoate	0.2	0.2	0.2	0.2	0.2
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.1	0.5	-	-	-
Cyclosporine A	-	-	0.1	0.5	-
Total weight with purified water added	100	100	100	100	100

Table 8: Formulation of essence

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Cyclometicon	15.0	15.0	15.0	15.0	15.0
Caprilic/capric triglyceride	3.0	3.0	3.0	3.0	3.0
Mineral oil	3.0	3.0	3.0	3.0	3.0
beeswax	1.0	1.0	1.0	1.0	1.0
Cetyl dimethicone copolyol	3.0	3.0	3.0	3.0	3.0
Glycerin	5.0	5.0	5.0	5.0	5.0
Magnesium sulfate	3.0	3.0	3.0	3.0	3.0
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Phenytoin	0.01	0.05	-	-	-
Cyclosporine A	-	-	0.01	0.05	-
Total weight with purified water added	100	100	100	100	100

Table 9: Formulation of skin softner

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	2.0	2.0	2.0	2.0	2.0
Hyaluronic acid	1.0	1.0	1.0	1.0	1.0
Polyoxyethylene oleic ether	0.1	0.1	0.1	0.1	0.1
Polyoxyethylene hydrogenated castor oil	0.1	0.1	0.1	0.1	0.1
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 10: Formulation of nutrient emulsion

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Setanol	1.0	1.0	1.0	1.0	1.0
Beeswax	0.5	0.5	0.5	0.5	0.5
Vaseline	2.0	2.0	2.0	2.0	2.0
Squalene	6.0	6.0	6.0	6.0	6.0
Ethanol	3.0	3.0	3.0	3.0	3.0
1,3-butyleneglycol	4.0	4.0	4.0	4.0	4.0
Polysolbait 60	1.0	1.0	1.0	1.0	1.0
Solbitan sesqi oleate	0.3	0.3	0.3	0.3	0.3
Carboxy-vinylpolymer	0.3	0.3	0.3	0.3	0.3
Triethanol amine	0.3	0.3	0.3	0.3	0.3
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 11: Formulation of patch

(unit: weight %)

Component	Compound	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Polymer	carboxymethylcellulose polyacrylic acid	1 2	1 2	1 2	1 2	1 2
Cross link agent	acetaldehyde	0.1	0.1	0.1	0.1	0.1
Humectant	glycerin	30	30	30	30	30
Inorganic filling agent	caolin	0.1	0.1	0.1	0.1	0.1
Preservative	paraoxy methyl benzoate paraoxy propyl benzoate	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05
Buffer	monosodium phosphate	0.1	0.1	0.1	0.1	0.1
	sodium tripoly phosphate	0.05	0.05	0.05	0.05	0.05
Active ingredient	phenytoin cyclosporine A	0.01 -	0.05 -	- 0.01	- 0.05	- -
Total weight with purified water added		100	100	100	100	100
Support		cotton	cotton	cotton	cotton	cotton
Protective film		silicon	silicon	silicon	silicon	silicon

With regard to a patch-type apparatus with micro needles, a main body of the patch apparatus, a reservoir which contains a solvent for a drug, is comprised of a polymer support for securing an entire patch type apparatus as well as preventing a drug such as phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine from being permeable thereto. The solvent for a drug may be water, polyethyleneglycol, transcutol or ethanol and is contained inside the reservoir. As for the polymer support, polyethylene, polypropylene, non-woven fabric or cotton fabric are available. The drug mentioned above is dispersed in powdered form in a lower part of the reservoir. The patch-type apparatus is an instrument for administering a drug transdermally, characterized by further comprising a support for micro needles and a number of micro needles. As for a support for micro needles, it is made of a polymer gel such as celluloses, polypropylene, fluorocarbon or polycarbonate and it has a swelling property

as the solvent is released after adhesion to the skin. As for micro needles, they are distributed and fixed perpendicular to the support for micro needles, and they come into contact with the skin. More particularly, 10 to 50 micro needles are attached per unit area (㎠) of the support for needles and each has a channel through which a drug can pass, the channel being 1 to 1000 ㎛ in diameter and the needles being fixed outward 0.01 to 1 ㎜ in length. The apparatus has an adhesive layer at its lower part which has a role in adhering the apparatus to the skin, the adhesive layer being made of a material such as polyacrylate or polybutene. It should be noted that the adhesive layer has no adverse effects on skin and is not dissolved by a solvent. Further, no decrease in adhesive property by a solvent is permitted. Finally, there is a protective film attached to the adhesive layer film, which is easily removable upon using the apparatus, to prevent a drug from being leaked, and protecting an adhesive. Hereinafter, the present invention will be described in detail in conjunction with examples, not to be construed as being limited to those examples.

Preparation of patch-type apparatus with micro needles comprising an active ingredient of the invention

Comparative example 1

1 g of 3 % gelatin solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A comparative matrix was thus obtained.

Example 1

0.001 % phenytoin was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A phenytoin-dispersed matrix was thus obtained.

Example 2

0.001 % cyclosporine A was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A cyclosporine A-dispersed matrix was thus obtained.

Evaluation of prevention and treatment effects on skin aging by a composition comprising an active ingredient of the invention

To evaluate the effect of the formulations prepared in above comparative example and examples including the examples as set forth in Tables 5 to 11 on alleviating skin wrinkles, female subjects aged 35 - 60 were employed. 760 females were placed into 38 groups, 20 subjects per group. Respective examples and comparative examples were applied to the face twice per day for 3 months (in case of the packs of Table 7, they were removed 30 min after application). The degrees of alleviating wrinkles were determined by a survey and an image analysis of wrinkles after 3 months. As for the survey, the degrees of alleviation of wrinkles and increase of elasticity were determined as one of 4 stages, that is, none, slight, moderate and high, as compared with the conditions before applying respective compositions, and the corresponding subjects were counted. The data are shown in Tables 12. For the evaluation by an image analysis of wrinkles, one replica of the region right below the eye of each subject was taken using Xantopren (Bayer) before beginning the experiment. Another replica was taken in the same region immediately after finishing the experiment. The replicas were subjected to an image analysis. Wrinkle density was measured by a two dimensional analysis. The measurements were represented as decrease rates, relative to wrinkle densities before the experiment. The results are shown in Table 13.

Table 12: Alleviation of wrinkles in human females

Degree of alleviation	Example	None	Slight	Moderate	High
Ointment	Ex. 1	1*	3	4	12
	Ex. 2	0	2	4	14
	Ex. 3	0	4	7	9
	Ex. 4	0	3	6	11
	Comp. Ex. 1	17	3	0	0
Cream	Ex. 1	0	1	8	11
	Ex. 2	0	1	6	13
	Ex. 3	0	2	7	11
	Ex. 4	0	3	7	10
	Comp. Ex. 1	13	7	0	0
Pack	Ex. 1	0	0	9	11
	Ex. 2	0	2	5	13
	Ex. 3	0	3	4	14
	Ex. 4	0	1	5	14
	Comp. Ex. 1	15	5	0	0
Essence	Ex. 1	0	2	5	13
	Ex. 2	0	0	5	15
	Ex. 3	0	3	6	11
	Ex. 4	0	1	7	12
	Comp. Ex. 1	12	7	1	0
Skin softner	Ex. 1	0	2	8	10
	Ex. 2	0	1	7	12
	Ex. 3	0	3	7	10
	Ex. 4	0	4	5	11
	Comp. Ex. 1	16	4	0	0
Nutrient emulsion	Ex. 1	0	1	5	14
	Ex. 2	0	0	5	15
	Ex. 3	0	1	7	12
	Ex. 4	0	2	7	11
	Comp. Ex. 1	13	7	0	0
Patch	Ex. 1	0	1	4	15
	Ex. 2	0	0	4	16
	Ex. 3	0	1	7	12
	Ex. 4	0	1	9	10
	Comp. Ex. 1	15	5	0	0
Micro-needle patch	Ex. 1	0	1	4	15
	Ex. 2	0	1	9	10
	Comp. Ex. 1	15	5	0	0

*: the number of subjects counted

Table 13: Effect of decreasing wrinkle density in human females

Example	Ointment	Cream	Pack	Essence	Skin softner	Nutrient emulsion	Patch
Ex. 1	45 %	43 %	40 %	39 %	44 %	45 %	46 %
Ex. 2	44 %	41 %	38 %	37 %	42 %	43 %	48 %
Ex. 3	50 %	40 %	41 %	40 %	48 %	46 %	45 %
Ex. 4	48 %	50 %	44 %	39 %	45 %	42 %	44 %
Comp. Ex. 1	98 %	98 %	94 %	97 %	99 %	98 %	96 %

As shown in Table 12, the examples according to the invention provide excellent effects of alleviating wrinkles and increasing skin elasticity. Specifically, more than 80 % showed high levels of improving effects. As shown in Table 13, when the examples comprising an active ingredient of the invention were applied to the subjects, wrinkle densities were considerably decreased to about 37 to 50 %, compared to that before the experiment. Also, when a patch-type administering apparatus with micro needles was applied, examples 1 and 2 exhibited significant decreases in wrinkle densities, 70 % and 60 % respectively, indicating that the examples are superior to the comparative example (98 %) (data not shown).

The above experimental results demonstrate that when the active ingredients of the invention are topically applied to the skin in the form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type apparatus with micro needle, skin wrinkles generated by intrinsic or extrinsic causes are effectively alleviated.

Industrial Applicability

As apparent from the above description, the present invention provides a topical composition which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, exhibiting the effects of inhibiting, alleviating and preventing skin aging, such as skin wrinkles.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

Claims:

1. A topical composition comprising one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis for prevention and alleviation of skin wrinkles.

2. The composition as set forth in claim 1, wherein the active ingredient is contained at an amount of 0.00001 to 30.00 % by weight, relative to the total weight of the composition.

3. The composition as set forth in claim 1 or claim 2, wherein the composition is formulated in a form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type administering apparatus with micro needles.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 : A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

CAPLUS(STN), SCISEARCH(STN), PASCAL(STN), BIOTECHNO(STN), INVESTEXT(STN), JICST-EPLUS(STN), KOSMET(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WOLF J. S. JR; SOBLE J. J.; RATFLIFF T. L.; CLAYMAN R. V."Ureteral cell cultures II : Collagen production and response to pharmacologic agents", Journal of urology, USA, 1996, Vol.156, No.6, p.2067-72	1-3
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A	US 5686489 A (Tristrata Technology, Inc.) 11 NOV 1997 cited in the application	1-3

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

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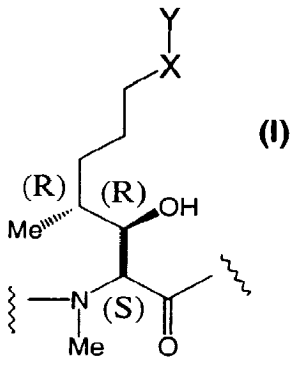
(54) Title: CYCLOSPORIN ANALOGS FOR THE TREATMENT OF LUNG DISEASES



(57) Abstract: The present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof. In formula (I), the formula for residue A is formula (II), where X is absent, -C1-C6 alkyl-, or -C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH_2-OC(O)R2; -C(S)-O-R1; and -C(S)- S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, 15 heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is -(D)Ala-, -(D)Ser- or -[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or -[O-(2-acyloxyethyl)(D)Ser]-. In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or pharmaceutically acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

WO 03/030834 A2

Cyclosporin Analogs for the Treatment of Lung Diseases

Technical Field

The present invention relates to novel cyclosporin analogs and methods for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

Background of the Invention

Respiratory diseases, such as asthma and other diseases characterized by airflow obstruction, are a global problem. Millions of people worldwide, both children and adults, suffer from these medical conditions. These diseases reduce quality of life by impairing the ability of sufferers to perform everyday tasks, and in some cases, cause death. One of the major respiratory diseases is asthma.

Asthma is a disease of unknown etiology in which the bronchi are inflamed and as a consequence obstructed. This narrowing results from a combination of bronchial smooth muscle contraction, mucosal oedema, inflammatory cell infiltrate and partial or total occlusion of the lumen with mucus, cells and cell debris. Bronchial obstruction is either partially or totally reversible, and this important feature distinguishes asthma from chronic bronchitis.

Asthma is an extremely common disease with a worldwide prevalence of 5% to 8%. In the developed world it is the most common chronic illness and, for reasons that are unclear, the disease is on the increase. It is now accepted that asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction. This type of airflow obstruction is often reversible either spontaneously or with treatment and causes associated increase in airway responsiveness to a variety of stimuli.

The illness has a wide clinical spectrum ranging from mild episodic bronchospasm (easily controlled by the occasional use of a bronchodilator) to a very severe intractable asthma sometimes resistant to treatment with high doses of

oral corticosteroids. Steroid resistance occurs in less than 5% of people with asthma. This translates to thousands of people. These patients with severe chronic disease may be dependent on corticosteroids and their disease is often so severe that full reversibility can be difficult or impossible to demonstrate.

Chronic obstructive airways disease, chronic obstruction lung disease and 'smoker's chest' have all been used to describe what is now known as COPD. COPD is characterized by progressive irreversible airway obstruction. It can lead to death from respiratory or cardio-respiratory failure. COPD consists of two subsets: chronic bronchitis and emphysema. In practice, it is very difficult to define the contribution of each of these two conditions to the obstruction of the airway and this has led to the displacement of these labels by the non-specific term COPD. The pathology of COPD is not fully elucidated, but features include hypertrophy of mucus-secreting glands, inflammation (including infiltration with lymphocytes) and goblet cell hyperplasia.

The treatment of COPD consists of bronchodilators, intermittent courses of antibiotics and, in some patients, inhaled and/or oral corticosteroids. The latter is claimed to reduce the decline in lung function in COPD.

Cystic fibrosis is an inherited condition. Excess viscid mucus is produced. This leads to recurrent chest infections and progressive bronchiectasis. Approximately 50% of cystic fibrosis sufferers have bronchial hyperresponsiveness and there is an increased incidence of atopy. There is widespread airway narrowing and wheeze. Most cystic fibrosis sufferers take bronchodilators, some take inhaled corticosteroids. And at least one study had reported benefit with oral corticosteroids.

Current drugs for treating asthma are corticosteroids (such as beclomethasone, triamcinolone), beta adrenergics (such as epinephrine, albuterol, bitolterol), NSAIDS, leukotriene antagonists, Xanthines (methyl xanthines such as theophylline, oxtriphylline) and anticholinergics (such as atropine, ipratropium bromide).

Corticosteroids are the mainstay of treatment of chronic asthma and they revolutionized the treatment of this disease when they were first introduced in the 1950's. Oral corticosteroids have today been largely replaced by inhaled corticosteroids, although severe asthmatics still require medication by mouth.

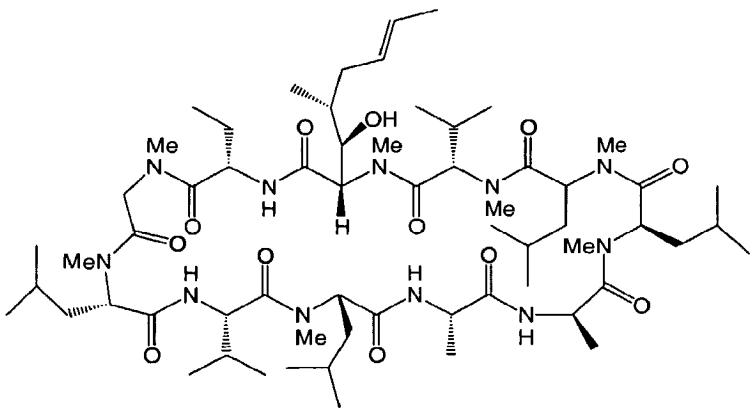
Inhaled cortisteroids are relatively safe and extremely effective in most patients, and improved the quality of life for millions of asthmatic sufferers. For those with severe asthma, however, oral therapy with corticosteroids is required. When taken for more than a few days oral corticosteroids have a number of serious side effects. These include growth retardation in children, severe osteoporosis (especially in old age), decreased responsiveness of the pituitary adrenal axis to stress, fluid retention, diabetes and precipitation of psychosis.

Furthermore, an appreciable number of patients have apparent corticosteroid resistance or unreponsiveness. Patients considered successfully treated with inhaled or oral steroids often have to be content with 60% of their predicted lung function. Further increasing the dose of oral corticosteroids runs the risk of concomitant side effects.

Although corticosteroids are effective for asthma, they are not ideal drugs. Over the years doctors have occasionally used immunosuppressive agents as adjuncts to corticosteroids in patients with extremely severe disease. Examples of immunosuppressive drugs are azathioprine, methotrexate, mycophenolic acid and prodrug, leflunomide, Cyclosporin A, ascomycin, FK-506 and rapamycin.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological activity, in particular immunosuppressive, anti-inflammatory or anti-parasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite

cyclosporin, *Cyclosporin A* represented as follows:



Since the original discovery of cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprising cyclosporins is thus now substantial and includes, for example, the naturally occurring Cyclosporins A through Z, for example, $[Thr]^2$, $[Val]^2$, $[Nva]^2$ and $[Nva]^{2-}$, $[Nva]^5$-Cyclosporin (also known as Cyclosporins C, D, G and M respectively), $[(D)MeVal]^{11}$-Cyclosporin (also known as Cyclosporin H), [cf., Traber et al.;1, Helv. Chim. Acta, 60, 1247-1255 (1977); Traber et al.; 2, Helv. Chim. Acta, 65, 1655-1667 (1982); Kobel et al.; Europ. J. Applied Microbiology and Biotechnology, 14, 273-240 1982); and Von Wartburg et al.; Progress in Allergy, 38, 28-45, 1986)]; as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the MeBmt- residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, for example, $[3\text{-O-acetyl-MeBmt}]^1$–Cyclosporin (also known as Dihydro-cyclosporin D), $[(D)Ser]^8$-Cyclosporin, $[MeIle]^{11}$-Cyclosporin, $[MeAla]^6$-Cyclosporin, $[(D)\ Pro]^3$-Cyclosporin etc., employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc., 15 Suppl. 1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products, 50, 123 (1986).

There is increasing evidence that chronic inflammation in asthma is mediated via a network of cytokines emanating from inflammatory and structural cells in the airways. The prominent eosinophilic inflammation that characterizes asthma appears to be orchestrated by cytokines derived from type 2 T-helper (Th2)-like lymphocytes, suggesting that immunosuppressants might be beneficial in the control of asthma (*see* for example, "Pharmacokinetics, pharmacodynamics, and safety of inhaled cyclosporin A after single and repeated administration in healthy male and female subjects and asthmatic patients," Rohatagi, S. et al., Aventis Pharmaceutical, Collegeville, PA, USA. J. Clin. Pharmacol. (2000), 40(11),

1211-1226). Cyclosporin A (hereinafter "CsA") is active against CD4+ lymphocytes and might, therefore, be useful for asthma. A trial of low-dose oral CsA in patients with steroid-resistant asthma indicated that it can improve control of symptoms in patients with severe asthma on oral steroids.

The mechanism of CsA action in asthma is of interest. CsA binds to the ubiquitous protein cyclophilin, in the cytosol, and the complex in turn binds to calcineurin, which is a calcium and calmodulin dependent serine threonine phosphatase. Calcineurin is necessary for the cytoplasmic portion of the transcription factor NF-AT, a nuclear factor of activated T-cells, to translocate to the nucleus and bind to its nuclear portion to become an active transcription factor. NF-AT forms a complex with AP-1 and regulates the transcription of the IL-2 gene, together with other genes, for example, IL-5. CsA prevents the cytoplasmic portion of NF-AT from translocating, resulting in reduced transcription of IL-2. CsA has a specific inhibitory effect in CD4+ cells through this transcription mechanism, but may also have inhibitory effects on other cells, including mast cells and eosinophils, through mechanisms that have not yet been defined.

Recently, three controlled trials of CsA in asthma have been reported. [Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **1992**; 339: 324-328; Niwanowska E, Dworski R, Domala B, Pinis G. Cyclosporin for steroid-dependent asthma. *Allergy*, **1991**; 46: 312-315; Lock SH, Kay AB, Barnes NC. Double-blinded, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* **1996**; 153: 509-14; Nizankowska E, Soja J, Pinis G, Bochenek G, Sladek K, Domagala B, et al. Treatment of steroid-dependent bronchial asthma with cyclosporin. *Eur Respir J* **1995**; 8: 1091-1099.]

CsA 5 mg/kg/day allowed a significant reduction in the use of corticosteroids by 60%. Side effects with systemic CsA were increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasis and paraesthesia. The systemic toxicity of CsA limits its use for the treatment of asthma, COPD and other related lung diseases. Therefore, it is desirable to synthesize analogs of CsA which retain CsA's potential utility as a primary or adjunct therapy for respiratory diseases, while reducing or eliminating CsA's systemic toxicity.

Summary of the Invention

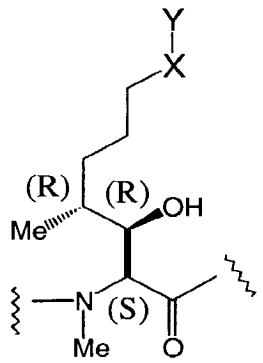
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 3 8 11

(I)

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH$_2$-OC(O)R2; -C(S)-O-R1; and -C(S)-S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or a pharmaceutically

acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject.

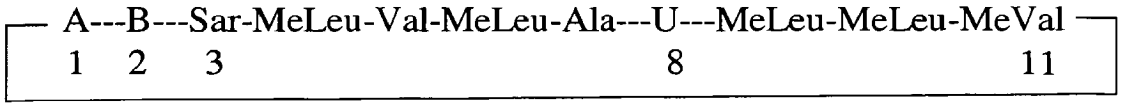
In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. In a preferred embodiment, the present invention relates to the processes for the production of cyclosporin analogs of formula I, with the structure of residue A as illustrated above.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

Detailed Description of the Invention

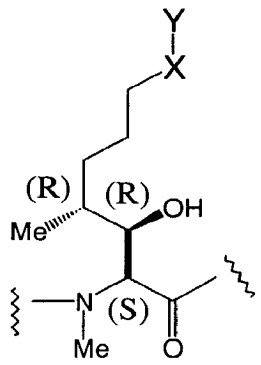
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production. The patents and publications identified in this specification indicate the knowledge in this field and are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure will prevail.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:



I

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; and -C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In formula I, abbreviation of amino acid residues, for example, -Ala-, -MeLeu-, -αAbu-, etc., are in accordance with conventional practice and are to be understood as having the L-configuration unless otherwise indicated (for example, -(D)Ala- represents a residue having the D-configuration). Abbreviation of residues preceded by "Me-" represents a α-N-methylated amino acid residue, for example, "Me-Leu" is a α-N-methylated-Leucine residue. Individual residues of a molecule of the cyclosporin analog of the present invention are numbered, as in the art, clockwise and starting with the residue -MeBmt-, corresponding to residue 1. The same numerical sequence is employed throughout the present specification and claims.

In a most preferred embodiment, a cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where residue B is -αAbu- and residue U is -(D)Ala-. In another preferred embodiment, the cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where X is absent in residue A, residue B is -αAbu- and residue U is - (D)Ala-.

Representative compounds of the invention include, but are not limited to, the following compounds as illustrated below:

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOH; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Cl$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_2CH_2O\ CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$C(=O)SCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is -$CH_2CH_2CH_2$- and Y = -$COOCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOFmoc; residue B = –αAbu-, and residue U = –(D)Ala-.

Cyclosporin analogs of the invention are accordingly useful for the treatment of diseases or conditions responsive to or requiring topical anti-inflammatory, immunosuppressive or related therapy, for example, topical administration for the treatment of such diseases or conditions of the eye, nasal passages, buccal cavity, skin, colon or, especially, airways or lung. In particular, cyclosporin analogs of the invention permit topical anti-inflammatory, immunosuppressive or related therapy with the concomitant avoidance or reduction of undesirable systemic side effects, for example general systemic immunosuppression.

Cyclosporin analogs of the invention useful for the treatment of diseases and conditions of the airways or lung, in particular, inflammatory or obstructive airway diseases. They are especially useful for the treatment of diseases or conditions of the airways or lungs associated with or characterized by inflammatory cell infiltration or other inflammatory events accompanied by inflammatory cell accumulation, for e.g., eosinophil and/or neutrophil. Most preferably, they are useful for the treatment of asthma.

Cyclosporin analogs of the invention are useful in the treatment of asthma of whatever type of genesis including both intrinsic and, especially, extrinsic asthma. They are useful for the treatment of atopic and non-atopic asthma, including allergic asthma, bronchitic asthma, exercise induced asthma, occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. Treatment of asthma is also to be understood as embracing treatment of "wheezy-infant syndrome," that is treatment of subjects, for example, of less than 4 to 5 years of age, exhibiting wheezing symptoms, in particular at night, and diagnosed or diagnosable as "wheezy infants," an established patient category of major medical concern and now more correctly indentified as incipient or early-phase asthmatics. Cyclosporin analogs of the invention are in particular useful for the treatment of asthma in subjects whose asthmatic status is either steroid dependent or steroid resistant.

Cyclosporin analogs of the invention are also useful for the treatment of bronchitis or for the treatment of chronic or acute airways obstruction associated therewith. Cyclosporin analogs of the invention may be used for the treatment of bronchitis of whatever type or genesis, including, for example, acute bronchitis,

arachidic bronchitis, catarrhal bronchitis, chronic bronchitis, croupous bronchitis, phthinoid bronchitis and so forth.

Cyclosporin analogs of the invention are in addition useful for the treatment of pneumoconiosis (an inflammatory, commonly occupational, disease of the lungs, frequently accompanied by airways obstruction, whether chronic or acute, and occasioned by repeated inhalation of dusts) of whatever type or genesis, including, for example, aluminosis, anthracosis, asbestosis, berylliosis, chalicosis, ptilosis, siderosis, silicosis, tabacosis and, in particular, byssinosis.

Cyclosporin analogs of the invention may also be used for the treatment of eosinophil-related disorders of the airways (e.g. involving morbid eosinophilic infiltration of pulmonary tissues) including hypereosinophilia as it effects the airways and/or lungs as well as, for example, eosinophil-related disorders of the airways consequential or concomitant to Loffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoan) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma and eosinophil-related disorders affecting the airways occasioned by drug-reaction.

The word "treatment" as used herein in relation to the treatment of diseases of the airways and lungs, in particular asthma, is to be understood as embracing both symptomatic and prophylactic modes, that is for immediate treatment, for e.g., of acute inflammation (symptomatic treatment) as well as for advance treatment to prevent, ameliorate or restrict long term symptomatology (prophylactic treatment). The term "treatment" as used in the present specification and claims in relation to such diseases is to be interpreted accordingly as including both symptomatic and prophylactic treatment, for e.g., in the case of asthma, symptomatic treatment to ameliorate acute inflammatory events and prophylactic treatment to restrict on-going inflammatory status and to ameliorate future bronchial exacerbation associated therewith.

Cyclosporin analogs of the invention may also be used to treat any disease or condition of the airways or lungs requiring immunosuppressive therapy, for e.g., the treatment of autoimmune diseases, or as they affect, the lungs (for example, for the treatment of sarcoidosis, alveolitis or chronic hypersensitivity pneumonitis) or for the maintainance of allogenic lung transplant, for e.g., following lung or heart lung transplantation.

As previously indicated, for the above purposes, cyclosporin analogs of the invention will be administered topically within the airways, for e.g., by the pulmonary route or by inhalation. As also previously noted, while having potent efficacy when administered topically, cyclosporin analogs of the invention exhibit reduced systemic toxicity. Cyclosporin analogs of the invention thus provide a means for the treatment of diseases and conditions of the airways or lung, for example, as hereinabove set forth, with the avoidance of unwanted systemic side effect, e.g. consequent to inadvertent swallowing of drug substance during inhalation therapy. It is estimated that during the course of manoeuvres required to effect administration by inhalation, up to 90% or more of total drug substance administered will normally be swallowed rather than inhaled.

By the provision of cyclosporin analogs which are topically active, e.g. effective when inhaled, but systemically inactive the present invention makes cyclosporin therapy available to subjects for whom such therapy might otherwise be excluded, e.g. due to the risk of systemic, in particular immunosuppressive, side effect.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' opthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as COPD asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly

following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs; by inhibiting P-glycoprotein, they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Accordingly, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a cyclosporin analog of the invention in combination with a pharmaceutically acceptable carrier or excipient. In particular, compositions pertaining to the present invention are useful for treating a subject for a reversible obstructive airway disease.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients, as described throughout the present specification. Such treatment of the disease may be done by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compound may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Dosages of the cyclosporin analogs of the present invention employed in practicing the method of the present invention will of course vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (for e.g., in terms of body weight, age and so forth) as well as the effect desired. In general, for treating diseases or conditions of the airways or lungs, for e.g., inflammatory or obstructive airway disease such as asthma, cyclosporins of the invention can be suitably administered topically to the airways or lungs, for e.g., but not limited to, inhalation, at dosages from about 20 to about 400 mg/day, preferably from about 50 to about 300 mg/day, most preferably from about 200 to about 300 mg/day. Dosages will appropriately be administered from a metered delivery system in a series of from 1 to 5 puffs at each administration, with administration performed once to four times daily. Dosages at each administration will thus conveniently be from about 5 to 100 mg/day, more preferably from about 12.5 to about 100 mg/day, e.g. administered with a metered delivery device capable of delivering, for e.g., 1 to 25 mg cyclosporin per actuation. For purposes of oral administration, more preferable doses may be in the range from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

Definitions

The terms "C_1-C_3-alkyl" or "C_1-C_6-alkyl" as used herein refer to saturated, straight- or branched-chain hydrocarbon radicals containing between one and

three or one and six carbon atoms, respectively. Examples of C_1-C_3 alkyl radicals include methyl, ethyl, propyl and isopropyl, and examples of C_1-C_6-alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl and n-hexyl.

The term "C_1-C_6-alkoxy" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom. Examples of C_1-C_6-alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *tert*-butoxy, neopentoxy and n-hexoxy.

The term "C_1-C_6-alkylthio" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through a sulfur atom. Examples of C_1-C_6-alkylthio include, but are not limited to, thiomethoxy, thioethoxy, thiopropoxy, thio-isopropoxy, *n*-thiobutoxy, *tert*-thiobutoxy, neothiopentoxy and n-thio-hexoxy.

The term "aryl" as used herein refers to a carbocyclic ring system having one or more aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including multi-cyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from lower alkyl, substituted loweralkyl, haloalkyl, alkoxy, thioalkoxy, lower alkylenedioxy, lower alkylidenedioxy, amino, alkylamino, dialkylamino, acyamino, cyano, hydroxy, acyl, halo and/or trifluoromethyl, mercapto, nitro, carboxylaldehyde, carboxy, alkoxycarbonyl, carbamoyl, sulfamoyl, lower alkoxycarbonylamino, lower alkanoyl, ureido, amidino and carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "C_3-C_6-cycloalkyl-" as used herein refers to carbocyclic groups of 3 to 6 carbons, respectively; for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "heterocyclics", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined

to the rest of the molecule via any of the ring atoms, such as, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

The term "subject" as used herein refers to a mammal or animal. Preferably the mammal is a human. A subject refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs and the like.

The term "pro-drug" as used herein refers to pharmacologically acceptable derivatives, for example, but not limited to, esters and amides, such that the resulting biotransformation product of the derivative is the active drug. Pro-drugs are known in the art and are described generally in, e.g., Goodman and Gilman's "Biotransformation of Drugs," in the Pharmacological Basis of Therapeutics, 8th Ed., McGraw Hill, Int. Ed. 1992, page 13-15, which is hereby incorporated by reference in its entirety.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate,

oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a compound of the present invention formulated together with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminun hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgement of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert

diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required.

Pharmaceutically acceptable diluents or carriers may be diluents or carriers acceptable for topical application at the intended site of therapy, e.g. diluents or carriers acceptable for topical administration pulmonary, dermally, nasally, ocularly or rectaly.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide,

calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

Forms in topically administrable form, e.g. enabling or facilitating topical administration, include, e.g. dry powder preparations of the active ingredient (i.e. cyclosporin analog of the invention) in substantially pure form, for example as employed in the art for delivery from a dry powder inhalation device. Means or devices enabling or facilitating topical administration include, in particular, inhalation devices as well as containers and the like from which the active ingredient may be delivered in a form capable of topical application. Preferred embodiments as defined under C will be such as permit topical administration within the airways or lungs, e.g. by inhalation.

It is clear that safety may be maximized by delivering the drugs by the inhaled route either in nebuliser form or as dry powder. Clearly the great advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or of chronic sinusititis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

Preparation of forms suitable for administration by inhalation may be carried out by methods known in the art. It should be noted that several antibiotics have recently developed for topical inhaled usage, particularly in cystic fibrosis, where they have been shown to be effective against pseudomonas infections. Various inhalants are described. For example, in DE 1491707, GB 1,392,945, GB 1,457,351, GB 1,457,352, NL 147939, DE 1491715, GB 1,598,053, EP 5585, EP 41783, EP 45419, EP 360463 and FR 2628638. DE 1491715, in particular, is said to be suitable for inhalation therapy intended for bronchial or lung diseases.

For this purpose cyclosporin analogs of the invention may be employed in any suitable finely dispersed or finely dispersible form, capable of administration

into the airways or lungs, for example in finely divided dry particulate form or in dispersion or solution in any appropriate (i.e. pulmonarily administerable) solid or liquid carrier medium. For administration in dry particulate form, cyclosporin analogs of the invention may, for example, be employed as such, i.e. in micronised form without any additive materials, in dilution with other appropriate finely divided inert solid carrier or diluent (e.g. glucose, lactose, mannitol, sorbitol, ribose, mannose or xylose), in coated particulate form or in any other appropriate form as know in the art for the pulmonary administration of finely divided solids.

Pulmonary administration may be effected using any appropriate system as known in the art for delivering drug substance in dry or liquid form by inhalation, e.g. an atomizer, nebulizer, dry-powder inhaler or like device. Preferably a metered delivery device, i.e. capable of delivering a pre-determined amount of cyclosporin analog at each actuation, will be employed. Such devices are known in the art.

For nasal administration, cyclosporin analogs of the invention will suitably be administered in liquid form from a nasal applicator. Suitable topical forms for the treatment of diseases or conditions of the skin will include, for example, creams, gles, ointments, pastes, cataplasms, plasters, transdermal patches and the like. Formulations for dermal application will appropriately contain a skin penetration enhancer, e.g. as know in the art, for example azone. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, again as known in the art. For rectal administration, i.e. for topical therapy of the colon, cyclosporin analogs of the invention may be administered in suppository or enema form, in particular in solution, e.g. in vegetable oil or like oily system for use as a retention enema.

According to the present invention, cyclosporin analogs may be used for the manufacture of a topical preparation for the treatment, with or without the concurrent use of other drugs. For the above purposes, cyclosporin analogs of the invention may be employed in any dosage form appropriate for topical administration to the desired site. For example, for the treatment of diseases of the airways or lungs, cyclosporin analogs of the invention may be administered via the pulmonary route, by inhalation from an appropriate dispenser device.

Dosage for the topical preparation will in general be one tenth to one hundredth, of the dose required for oral preparation.

Abbreviations

Sar:	Sarcosine
MeLeu:	N-Methyl-Leucine
Val:	Valine
Ala:	Alanine
MeVal:	N-Methyl Valine
Et:	Ethyl
Ph:	Phenyl
Fmoc:	9-Fluorenylmethoxycarbonyl-
MeBmt:	N-Methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine
α-Abu:	α-Aminobutyric acid

Synthetic Methods

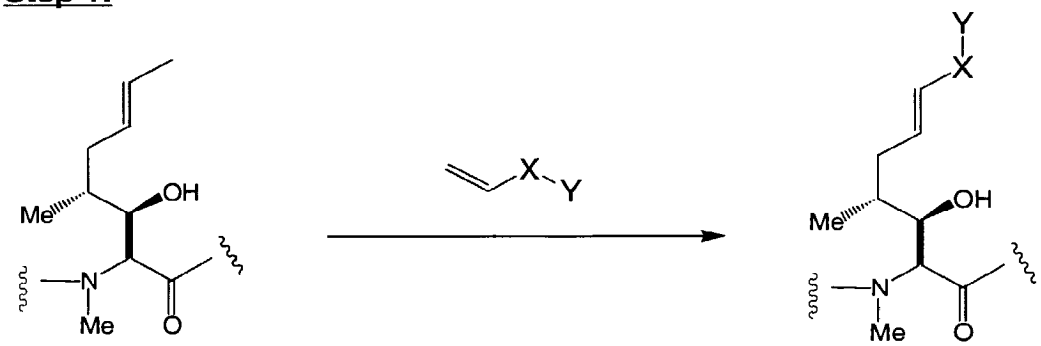
The compounds and processes of the present invention will be better understood, but are not limited to, the following synthetic scheme which illustrates the methods by which the compounds of the present invention (illustrated by formula I) may be prepared. The groups X and Y, and the amino acid residues B and U in formula I are as defined earlier in the specification. The starting material for Scheme I, illustrated by formula I where A'= -MeBmt-, may be, for example, but not limited to, a fermentation product or a synthetic product made by solution phase chemistry. Preferably, the starting material is commercially available. The starting material as a fermentation product may be made from highly productive strains, for example, but not limited to, *Sesquicillopsis rosariensis* G. ARNOLD F605; *Tolypocladium inflatum* wb6-5; Fusant, *Tolypocladium inflatum* KD461 etc. (in U.S. Patent Nos. 5,256,547; 5,856,141 etc.). Alternately, the starting material may be made by solution phase chemistry either by sequentially assembling amino acids or by linking suitable small peptide fragments, where the units are linked by, for example, but not limited to, amide, ester or hydroxylamine linkages (described in, *Müller, Methoden der organischen*, Chemie Vol. XV/2, pp 1 to 364, Thieme Verlag, Stuttgart, 1974; Stewart, Young, Solid Phase Peptide Synthesis, pp 31 to 34, 71 to 82, Pierce Chemical Company, Rockford, 1984; Bodanszky, Klausner, Ondetti, Peptide Synthesis, pp 85 to 128, John Wiley & Sons, New York, 1976 and other standard books on solution phase peptide chemistry). For amide linkages particular preference is given to the azide method, the symmetric and mixed anhydride method, *in situ* generated or preformed active esters and methods using

coupling reagents (e.g., dicyclohexylcarbodiimide, N,N-dimethyl-4-aminopyridine, N-hydroxy-benzotriazole, PyBrop® etc.). Classical solution phase chemistry using standard Z- and Boc- methodology may be used.

Residue A, which is -MeBmt- in the starting material is further modified, as illustrated in the following reaction scheme.

Scheme:

Step 1:

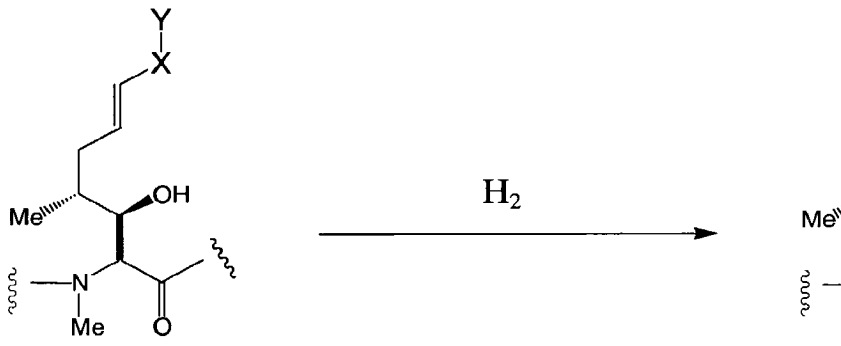


A' = -MeBmt- A", wherein X, Y are as defined (i)

The process for the preparation of the compounds of formula I comprises reacting a compound of formula I, where A' = -MeBmt- (for example, Cyclosporin A) with an olefin having a terminal double bond with catalysts such as Grubb's ruthenium alkylidene, Grubbs dihydroimidazole ruthenium, Shrock-Hoveyda molybdenum catalysts or benzylidene catalysts [*see* (a) US Patent 6,111,121; (b) Reviews: Synlett, **1999**, *2*, 267; (c) Reviews: Ivin, K J; Mol, J.C. *Olefin Metathesis and Metathesis Polymerization*, 2nd ed., Academic Press, New York, **1997**; (d) *J. Org. Chem.*, **1999**, *64*, 4798-4816; (e) *Angew. Chem.*, Int. Ed. English, **1997**, *36*, 2036-2056; (f) *Tetrahedron* **1998**, *54*, 4413-4450.] or Nolan's ruthenium catalyst [see (a) International Patent Application No. WO 00/15339; (b) *Org. Lett.*, **2000**, *2*, 1517-1519; (c) *J. Org. Chem.*, **2000**, *65*, 2204-2207] or Molybdenum catalysts [see (a) *J. Am. Chem. Soc.*, **1990**, *112*, 3875 (b), *J. Am. Chem. Soc.*, **1996**, *118*, 10926-10927] in the presence of a lithium salt such as lithium bromide, lithium chloride, lithium trifluoroacetate, lithium triflate of a lewis acid such as titanium isopropoxide in an organic solvent. The organic solvent used may be solvents such as, for example, dichloromethane, chloroform, toluene, benzene, tetrahydrofuran, dimethylformamide and the like or mixtures thereof. The reaction may be carried

out from room temperature to about 100 °C for 1-7 days to provide a compound of formula I, where residue A' is converted to residue A" having formula (i).

Step 2:



A", wherein X, Y are as defined (i)

A, wherein X, Y are as defined

The compounds of formula I in an organic solvent, where residue A" has formula (i), are then subjected to standard hydrogenation conditions using a catalyst such as, but are not limited to, a catalytic amount of palladium on carbon in a hydrogen atmosphere to provide the saturated compounds of formula I, where in particular, residue A" having formula (i) is converted to residue A, as described throughout the specification.

The organic solvents used can be solvents such as methanol, ethanol, ethyl acetate or mixtures thereof. Other catalysts useful to assist hydrogenation may be, for example, but not limited to, platinum metal or its oxide [*see* standard books on catalytic hydrogenation, e.g., Rylander, P.N., *Hydrogenation Methods*, Academic Press: NY, 1985; *Catalytic Hydrogenation in Organic Synthesis*, Academic Press: NY, 1985; Červený, L., *Catalytic Hydrogenation*, Elsevier: NY, 1986 etc.]. The reaction may be carried out at room temperature or elevated temperature, for example, but not limited to, 50 °C or 100 °C.

Examples

The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intentended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications,

including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods fo the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Cyclosporin methyl ester (0.030 mg, 0.024 mmol) and palladium on carbon (0.0012 mg, 0.0012 mmol) were added to a flask and the flask was evacuated and backfilled with hydrogen gas three times. Anhydrous methanol (3 ml) was added and the reaction was stirred for 18 h at ambient temperature under an atmosphere of hydrogen. After filtration and concentration in vacuo, the product was isolated as a white solid (0.021 mg, 70 % yield). Electrospray mass spectrum (ESMS) M+H: 1248.91

Example 2: Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 2 was prepared from cyclosporin ethyl ester and palladium on carbon according to the procedures described in Example 1. ESMS M+H: 1262.3

Example 3: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 3 was prepared from cyclosporin propyl ester and palladium on carbon according to the procedures described in Example 1.

Example 4: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 4 was prepared from cyclosporin benzyl ester and palladium on carbon according to the procedures described in Example 1.

Example 5: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 5 was prepared from cyclosporin fluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 6: Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 6 was prepared from cyclosporin difluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 7: Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 7 was prepared from cyclosporin trifluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1.

Example 8: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 8 was prepared from cyclosporin trifluoroethyl ester ester and palladium on carbon according to the procedures described in Example 1.

The cyclosporin analogs of the present invention have potent immunosuppressive and anti-inflammatory activity. In particular, they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. In vivo this activity is apparent following topical administration, e.g., pulmonary route.

The immunosuppressive and anti-inflammatory properties of cyclosporin analogs of the invention may be demonstrated in standard test models *in vitro* and *in vivo* for example as follows.

Example 9: Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with Cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide

representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green (Biomol AK-111).

Compounds in DMSO (2.4µl) were added to a 96-well microplate and mixed with 50µl assay buffer (50mM Tris-HCl, pH 7.5; 100mM sodium chloride; 6mM magnesium chloride; 0.5mM dithiothreitol, 0.025% NP-40, 500µM calcium chloride, 0.27µM Calmodulin) containing 10µM Cyclophilin and 3nM Calcineurin. After warming to 37 °C for 60 mins, the enzymatic reaction was initiated by addition of phosphopeptide (7.5µl) to give a final concentration of 94µM. Phosphate release after 60 min at 37 °C was determined by addition of Biomol Green (100 µl) and measurement of the absorbance at 620nm after 15 mins at room temperature.

IC_{50} values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 0.1 to 0.0015 µM.

Example 10. NFAT reporter gene assay

NFAT activation follows precisely the activation of calcineurin by increased free calcium levels in the cytoplasm. Researchers from diverse fields are interested in the NFAT family of transcription factors, which are potential targets for newer and safer immunosuppressive drugs. In addition, the activation of NFAT proteins involves various cellular signal transduction pathways, including calcium mobilization and MAP kinase pathways linked to T-cell receptors and Ras1. To assist researchers probing the activity of NFAT proteins, Stratagene has developed a PathDetect cis-reporter plasmid, the pNFAT-Luc reporter plasmid (Stratagene, Inc. catalog # 219094), containing the NFAT binding site from the human IL-2 gene.2,7-9. The NFAT cis-reporting system includes the transfection-ready pNFAT-Luc reporter plasmid and the pCIS-CK negative control plasmid.

Construction of the pNFAT-Luc Plasmid:

The backbone of the 5749-base-pair pNFAT-Luc plasmid is the pFR-Luc reporter plasmid of the aforementioned PathDetect trans-reporting system. To this backbone, the GAL4 binding element was replaced with four direct repeats of the NFAT binding sequence (–286 to –257) from the IL-2 gene promoter, the most studied and widely used NFAT binding sequence. For all reporter plasmids of the PathDetect cis-reporting systems, activation of the luciferase gene indicated

interaction of uncharacterized gene products, extracellular stimuli, growth factors, or drug candidates with specific enhancer elements. Then a plasmid expressing the gene of interest was cotransfected into mammalian cells along with a cis-reporter plasmid to indicate transcription activation.

Testing the pNFAT-Luc Plasmid in Jurkat Cells:

Pharmacology studies have established that NFAT proteins can be activated by the protein kinase C activator phorbol ester (PMA) in combination with the calcium ionophore ionomycin, reagents that raise free intracellular calcium. When Jurkat cells, a mature human T-cell line, or CHO cells were transfected with the pNFAT-Luc plasmid and treated with 60 ng/ml of PMA and 1μg/ml of inomycin, luciferase activity increased by 13- and 16-fold, respectively. Therefore, the enhancer element in the pNFAT-Luc plasmid is responsive to calcium mobilization. Cells transfected with pNFAT-Luc and then treated with either PMA or ionomycin alone did not show a significant increase in luciferase activity.

Cyclosporin inhibits the activity of calcineurin, a protein phosphatase regulated by intracellular calcium mobilization. All the isoforms of NFAT protein contain a calcineurin-binding domain and are activated by calcineurin. The inhibition of luciferase expression from pNFAT-Luc in the present model, in both Jurkat and CHO cells induced by PMA and ionomycin, was monitored for cyclosporin (as a positive control) and the cyclosporin analogs of the present invention.

In another set of experiments, rat basophilic leukemia cells stably transfected with chemokine receptors were transfected with pNFAT-Luc and then treated with their respective ligands (data not shown). When both luciferase expression and calcium levels were monitored in these cells, luciferase expression correlated very well with calcium mobilization. Therefore, luciferase expression from pNFAT-Luc indeed reflects the activation of endogenous NFAT proteins by calcium immobilization.

Example 11. Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is

added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

Mishell-Dutton Test

Ca. 10^7 lymphocytes from the spleen of OFI, female mice are co-cultured with ca. $3x10^7$ sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

Delayed-type Hypersensitivity Resonse

On Day 0 groups of ten mice (having BALB/cByJ or any other acceptable strain) are dosed with test compound (1 to 10%), vehicle or the positive control, cyclophosphamide (Cyclosporin A), and monitored from Day-2 to 7. The mice are anesthetized and their abdomens shaved. 100 µl of a 3% solution of ovalbumin are applied to the abdomen and dried. Seven days later, the mice are challenged by applying 5 µl of ovalbumin to each side of the right ear. After 24 hours, both the right and left ear thickness are measured using a micrometer caliper.

Popliteal Lymph Node Assay

First, an inducer (phenytoin) is injected into the mice footpad (having BALB/cByJ or any other acceptable strain). Then the mice are challenged (subcutaneously or po) with ester and control agent using graded doses, for example, 2.5, 10, 20 mg/Kg (based on cyclosporine A data). On day 7 the popliteal lymph nodes are excised from the dosed mice and the lymph nodes are weighed. Then single cell suspensions of each lymph node are prepared and enumerated. The weight index for each animal is calculated (for example, a mean weight index <2 would indicate suppression of immune response).

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 µg/ml) with $Al(OH)_3$ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies, the procedure is repeated 1x after 2 weeks and the animals are used one

week later. For inhalation studies, the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

Challenge is effected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered (a) inhalation and/or (b) orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hrs. prior to and 6 hrs. after OA challenge. For inhalation studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 mins. prior to OA challenge.

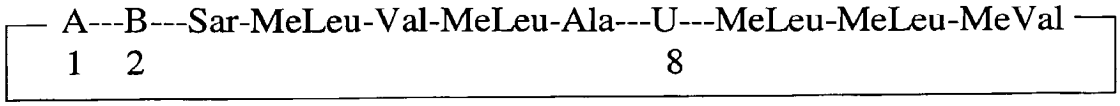
Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliqots of Ca^2 + and Mg^2 + free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 μl of this cell suspension is added to 190 μl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per smear are counted and the total population of each cell type is calculated.

In untreated animals, OA challenge induces increase of all cell types in BAL fluid 24 hours after challenge. Prior administration of cyclosporin analogs in accordance with the present invention by inhalation at dosages of the order of from 1.0 to 15.0 mg/kg reduces eosinophil count in BAL in a dose dependent manner as compared with untreated controls. Cell counts for other leucocytes (macrophages, neutrophils etc.) are also reduced.

Claims

What is claimed is:

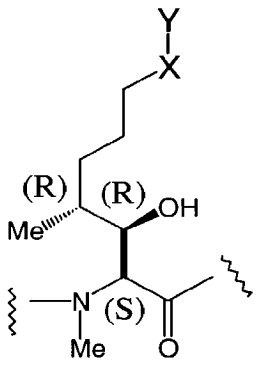
1. A cyclosporin analog of formula (I) or a pro-drug or a pharmaceutically acceptable salt thereof:



(I)

wherein,

(a) A is of the formula:



wherein

X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-;

Y is selected from the group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

iii. -C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl, optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

iv. -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

v. C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio.

(b) B is –αAbu-, -Val-, -Thr- or –Nva-; and

(c) U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-; or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

2. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula (I), B is -αAbu-, and U is -(D)Ala-.

3. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula I:

(i) A is of the formula A1 or A2, wherein:

X is absent; and

Y is selected from a group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

iii. C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

(ii) B is -αAbu-; and

(iii) U is -(D)Ala-.

4. A cyclosporin analog according to claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, selected from the group consisting of:

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOH;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOEt;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CH_2CH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Ph$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2F$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCHF_2$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Cl$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_2CH_2O\ CH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$C(=O)SCH_2Ph$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is -$CH_2CH_2CH_2$-, Y = -$COOCH_3$; and

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOFmoc.

5. A chemical process for preparing a cyclosporin analog of formula I as claimed in Claim 1, comprising:
   a. reacting a compound of formula I, wherein A= -MeBmt- with:
      i. an olefin of formula CH_2=CH-X-Y, wherein X and Y are as defined in Claim 1; and
      ii. a catalyst;
      in the presence of a lithium salt in an organic solvent; and
   b. hydrogenating the product of step a in an organic solvent under hydrogen with a catalyst;
      and optionally converting the product of said reaction into a pharmaceutically acceptable salt.

6. The chemical process as claimed in Claim 5, wherein the catalyst in step (a) (ii) is Grubb's ruthenium alkylidene, Nolan's catalyst, a benzylidene catalyst or a molybdenum catalyst.

7. The chemical process as claimed in Claim 5, wherein step (b) is performed at room temperature.

8. The chemical process as claimed in Claim 7, wherein the catalyst in step (b) is Palladium on carbon.

9. A pharmaceutical composition, said composition comprising at least one cyclosporin analog of formula 1 as claimed in Claim 1, said cyclosporin analog being present alone or in combination with a pharmaceutically acceptable carrier or excipient.

10. A method for treating diseases characterized by airflow obstruction in a subject in need of treatment which comprises the step of administering to said subject a therapeutically effective amount of at least one cyclosporin analog of formula I as claimed in Claim 1.

11. The method of Claim 10, wherein said disease is asthma.

12. The method of Claim 10, wherein the step of administering the cyclosporin analog of formula I is done by topical administration.

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(54) Title: METHOD AND COMPOSITION FOR DRY EYE TREATMENT

(57) Abstract: A method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil, a non-toxic emulsifying agent and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres thereto and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

METHOD AND COMPOSITION FOR DRY EYE TREATMENT

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the treatment of a dry eye condition, an in particular to a method and composition for this purpose which forms an artificia tear film on the surface of the eye acting to inhibit evaporation therefrom an delivering to the eye surface an efficacious medicament to treat an eye disease.

STATUS OF PRIOR ART

The main concern of the present invention is with the treatment of a dry ey condition by a method and composition that acts to lubricate the eye and to reduc evaporation of fluid from the cornea surface. The cornea normally functions t maintain this surface in a moist and lubricated state which is impaired when the ey suffers from a dry eye condition.

Dehydration of moisture from the eye gives rise to various discomforts suc an ocular dryness as well as burning and scratching sensations. But the mor serious consequence of a dry eye condition is a loss of visual acuity which if i persists and is not corrected, may result in permanent damage. Dry eye disease act to degrade the exposed ocular surface and may cause a complete breakdown c corneal tissues. In an extreme case, this may necessitate a corneal transplant.

Symptoms accompanying a dry eye condition are exacerbated when the ey is covered by a contact lens. The rate of evaporation of liquid from the eye i accelerated by the contact lens whose presence results in a meniscii formation tha promotes evaporation even when the eye has an adequate natural tear film.

The usual treatment prescribed for a dry eye condition is to alleviate it symptoms by the topical application of a tear film substitute that adds a substantia

volume of liquid to the anterior surface of the eye. A typical composition functioning as a tear film substitute includes soluble polymer solutions. Of prior art interest in this regard is the US patent to Trager 4,421,740 which discloses an artificial tear composition formed by an aqueous hypotonic solution of lecithin, a phospholipid, and a viscosity-adjusting agent.

Of particular prior art interest are the following US patents in each of which Korb is a co-inventor. Hence these patents will hereinafter be referred to as Korb patents:

I. 4,914,088 (1990)

II. 5,278,151 (1994)

III. 5,371,108 (1994)

IV. 5,294,607 (1994)

The Korb patents point out that a normal eye has an ocular surface coated with a tear film composed of:

(a) a mucous inner layer in contact with the ocular surface of the eye

(b) an aqueous middle layer which is the source of moisture, and

(c) a lipid outer layer which minimizes evaporation of the moisture from the film.

"Dry eye" is experienced when the outer layer (c) of the tear film is defective. The dry eye treatment disclosed and claimed in Patents I to IV involves the topical application to the eye of phospholipids which form an artificial film over the eye that replicates a normal outer lipid layer and maintains the eye in moist condition.

Patent I is directed to an artificial tear film formed by:

"a layer of a complex phospholipid having a net positive or negative charge".

According to this Korb patent, the significance of a net positive or negative net charge is that in either case, the charged molecules in the film coating the surface of the eye *"repel each other"* and in doing so, maintain *"the integrity of the phospholipid therein"* so that it acts *"as a barrier reducing*

evaporation." Hence it is a negative or positive repelling charge that the inventor regards to be the crux of his invention.

Patent I fails to take into account that the surface of the eye being treated is anionic and therefore will interact electrostatically with a charged coating in a way that depends on the polarity of the charge. According to Patent I, the polarity of the charge doesn't matter, for in either polarity the charged molecules in the film repel each other.

An important aspect of the present invention is not only that it has a positive net charge, but also that the strength and distribution of the charge is such as to cause the film to adhere electrostatically to the entire anionically-charged eye surface to provide an effective moisture barrier. A weak positive charge would not achieve this result. Inasmuch as in present invention, the positively-charged molecules in the film covering the eye surface electrostatically engage the negatively-charged molecules on this surface, the resultant electrostatic couple is neutral and the couples do not repel each other.

Korb patent II discloses an eye treatment composition comprising

"a layer of a complex phospholipid having a net charge" and

"a layer of an essentially non-polar oil over said phospholipid layer", the phospholipid and oil layers being in an amount *"below that amount that would result in significant prolonged blurring of vision"*.

According to Patent II, the preferred phospholipids are those "carrying a net negative charge because the negatively-charged molecules would be repelled by the negatively-charged ocular surface, thereby permitting the maintenance of a relatively thick aqueous layer".

In contradistinction, the present invention which resides in a positively-charged composition, exploits the fact that the eye surface is negatively charged (anionic) so that the composition is electrostatically attracted to this surface to create a coating which prevents the escape of moisture from the eye surface for a prolonged retention period.

Korb patent III also discloses a composition in which the phospholipid has a net negative or positive charge causing molecules in the tear film coating the eye surface to repel each other to maintain the integrity of the film. In Korb patent IV, the eye treated composition is a mixture of a charged phospholipid and a non-polar oil in a meta-stable water emulsion.

Essential to the present invention is that the emulsion coating the eye surface to form a film thereon carries a net positive charge which is distributed uniformly throughout the film so that it is electrostatically attracted to the entire anionic eye surface whereby the molecules on the film surface do not repel each other but are attracted to the eye surface.

Also of particular prior art interest is PCT patent publication WO 95/31211 (25 Nov. 1995) of Allergan, Inc. This publication discloses an emulsion for topical application to ocular tissue which includes cyclosporin admixed with castor oil. As noted in this publication, cyclosporin comprises a group of cyclic oligopeptides, the major component of which is cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$), Clyclosporin has been found to be effective in the treatment of a dry eye condition.

SUMMARY OF THE INVENTION

In view of the foregoing, the main object of this invention is to provide an improved method and composition for treating a dry eye condition by topically applying to the eye surface an emulsion forming a tear film that adheres electrostatically to the entire surface of the eye and acts to lubricate the eye and to inhibit evaporation of moisture therefrom.

Among the significant advantages of a method and composition in accordance with the invention are the following:

A. The tear film derived from the emulsion carries a strong net positive charge that is uniformly distributed throughout the film surface whereby the film is electrostatically attracted to the entire area of the negatively-charged eye surface and there is no uncoated zone.

B. The electrostatic attraction between the artificial tear film and the eye surface maintains adhesive contact therebetween for a prolonged retention period and the tear film cannot be readily washed away.

C. The tear film coating the eye surface has no adverse effects, for the film includes no toxic or other harmful agents.

Also an object of this invention is to provide a composition of the above type which incorporates therein a therapeutic agent for treating an eye disease, such as cyclosporin A which when the composition is topically applied then delivers the agent to the eye. The release of the agent from the coating film to the surface of the eye is maintained for a prolonged period in that the film is held electrostatically in contact therewith.

Briefly stated, these objects are attained in a method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres to the eye and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

DETAILED DESCRIPTION OF THE INVENTION

Cyclosporin A (CsA), a lipid-soluble cyclic endecapeptide, is a potent and well established immunomodulator drug mainly for oral use. With oral formulations, CsA bioavailability is limited because of the drug's insolubility in water and its tendency to separate immediately as a solid after coming into contact with water. Moreover, the bioavailability is highly dependent on complex

interactions occurring between the formulation and the physiological environment of the lumen.

CsA has been found to be effective in treating the immune-mediated keratoconjunctivitis sicca (KCS or dry eye disease) by the enhancement or restoration of lachrymal gland tearing in patient suffering from this syndrome. Dry eye disease is characterized by chronic drying of the conjunctiva and cornea, as well as by decreased tear production and changes in the composition of the tear film. In order to enhance the efficiency of CsA treatment, it becomes necessary to increase the absorption of the drug in the lachrymal gland as well as the conjunctiva and cornea target tissues, using for the purpose a suitable dosage of the drug to suppress ocular inflammation without significant systemic CsA exposure.

Since the aqueous solubility of CsA is between about 20 to 30 μg/ml, there is no adequate aqueous formulations available for ocular administration of the drug. Moreover, if cyclosporin is administered orally for the treatment of KCS, the accompanying side effects due to systemic circulation may cause adverse reactions such as hypertrichosis or renal dysfunction. In addition, the concentration of CsA present in oral formulations is limited due to the drug's hydrophobic nature.

Studies on ocular CsA penetration in animals were carried out using CsA formulations based on olive oil and corn oil. Local toxic effects on the cornea attributable to topical CsA formulations or the intrinsic solvent were observed. Upon using CsA in olive oil, in an *ex vivo* examination on bovine cornea, histological study revealed that the corneal epithelium was keratinized with some necrotic cells and rare pycnotic nuclei. Moreover, several researchers have confirmed that the probable toxic effect was due to topically administered CsA dissolved in olive oil. The conclusion reached is that olive oil, rather than CsA was responsible for the surface epithelial defects developing in the cornea. Hence, because of its high hydrophobicity, it is necessary to formulate CsA with compatible vehicles. These are not always biocompatible with ophthalmic administration, and may present some problems of stability such as the rancidity of olive oil. The drawback of corn-oil concentrated ointment formulations is that they

may exacerbate the symptoms (early-burning, redness and itching) of a dry eye condition.

It is possible to minimize problems related to unpleasant sensations and syndrome exacerbation by reducing the oil content and dispersing the oil phase in a water phase, resulting in an emulsion. We have found that when castor oil is used in developing the emulsion dosage, there are additional benefits to patients with dry eye disease arising from the long ocular retention time of the emulsion vehicle. The castor oil droplets in the emulsion form a lipid layer over the tear film, reducing the evaporation of the limited natural tears produced while the emulsion remains in the eye of a patient.

Our investigation of a positively-charged submicron emulsion containing a phospholipid having Zeta potential values ranging from 34 – 45 mV and a mean droplet size of around 150-250 nm supports the significant advantages which are gained when the emulsion vehicle carries a net-positive charge, rather than either a negative or neutral charge.

The resultant electrostatic attraction between the positively-charged submicron oil droplets in the emulsion and the corneal eye surface, which is negatively-charged results in a more prolonged residence or retention time conducive to topical drug flux enhancement.

Hence a positively-charged submicron emulsion of CsA enhances the local concentration of this medicament in conjunctiva and cornea which are the target ocular tissues. A positively-charged emulsion in accordance with the invention is therefore far more efficacious therapeutically than a negative charge emulsion having a similar composition.

The Composition The following represent formulations for a composition in accordance with the invention for treating a dry eye condition and other eye diseases.

Formulation (1) is a positive blank emulsion to be applied topically to an eye surface to create on the surface an artificial tear film. Formulation (2) which is for a CsA positive emulsion has the same ingredients as formulation (1), to which is

added cyclosporin. The resultant film serves as a vehicle to deliver the medicament to the eye surface.

Cyclosporin A	0.00	0.20
Castor oil	2.50	2.50
Lipoid E-80	0.50	0.50
Stearylamine	0.12	0.12
Vitamin E	0.01	0.01
Pluronic F-68	0.42	0.42
Glycerol	2.25	2.25
Benzalkonium chloride	0.01	0.01
Distilled water to	100.00	100.00

Lipid E-8 is a non-polar phospholipid, stearylamine is a cationic lipid and therefore imparts to the emulsion which also includes a non-polar castor oil a net positive charge. Pluronic F-68 is the trademark for poloxamer 188, a polyoxyalkyline derived from polypropylene glycol. Poloxamer 188 is an emulsifying agent and the glycerol in the formulation functions as an osmotic agent. Benzalkonium chloride is a cationic surfactant antiseptic agent acting as a preservative of the emulsion and strengthening the positive charge imparted to the emulsion by the cationic lipid. Vitamin E acts as a lipophillic antioxidant and as an eye lubricant.

In practice a composition may include instead of the cationic lipid stearylamine, cationic lipid oleylamine. The relative percentages of the ingredients included in the composition are not limited to those set forth above. Thus the relative percentage of castor oil may be in the range of 0.5 to 10%, that of the phospholipid (Lipoid E-80) in the range of 0.1 to 2.0%, that of the cationic lipid in the range of 0.1 to 0.5%, and that of the emulsifying agent, (Pluronic F-68), in the range of 0.5 to 2.0%.

It is vital however that whatever are the relative ranges of these ingredients, that the emulsion carry a net positive charge of sufficient strength to cause the emulsion when forming a film on the anionic surface of an eye, that it be electrostatically attracted to the surface so that it adheres thereto and cannot be readily washed away.

Lipoid E80, Pluronic F-68 and stearylamine coact to improve the stability of the emulsion droplets which are preferably in the submicron range, by enhancing the mechanical strength of the interfacial films formed around the droplets

It is important to bear in mind that in a composition in accordance with the invention which is to be administered topically to the anionic surface of an eye, that the phospholipid and castor oil included in the formulation carry no charge and that the aggregate net positive charge imparted to the submicron droplets is derived from the cationic surfactant plus the cationic antiseptic agent.

The advantage of this formulation over a dry eye treatment composition in which the charge imparted to the droplets is derived only from the phospholipid, as in the Korb patents, is that with the present formulation the positive charge of the emulsion is uniformly distributed over the entire area of the artificial tear film which is produced when the emulsion coats the anionic surface of the eye.

This results in electrostatic attraction throughout the entire area of the eye surface so that no portion thereof remains uncoated and untreated. Hence the present invention affords a treatment for a dry eye condition in which evaporation moisture is inhibited over the entire eye surface and no moisture is permitted to escape therefrom.

Preparation of Composition

Poloxamer 188 (Pluronic F-68) the osmotic agent (glycerol), and benzalkonium chloride were dissolved in the aqueous phase. The lipid E-80 is first dissolved in ethanol (1:5) and then dispersed in the aqueous phase. The ethanol is evaporated during the heating process of the aqueous phase. An antioxidant (α-tocopherol), the cationic lipid stearylamine (or oleylamine) and the CsA were dissolved in the castor oil phase. Both phases were heated separately to 70°C. The water phase was slowly incorporated into the oily phase and mixed with a magnetic stirrer. The resulting mixture was further heated to a temperature of 85°C.

The coarse emulsion obtained was emulsified for 5 minutes, using a high shear Polytron mixer and then rapidly cooled to below 20°C. After cooling in an ice bath, the emulsion was homogenized using a two stage homogenizer valve

assembly for 5 minutes. After further rapid cooling below 20°C, the pH was adjusted to 7.0 using 0.1 N hydrochloric acid. The emulsion was then filtered through a TE membrane filter (Schleicher & Schuell, Dassel, Germany) with a pore size of 0.45 μm. Finally, the emulsion was packed under nitrogen atmosphere in siliconized glass bottles and then sterilized by autoclaving at 121°C for 15 minutes. It is desirable that the droplets of the emulsion be in the submicron range and it is vital that the emulsion which is to be applied topically to the eye surface be sterile.

Medicaments: In an emulsion in accordance with the invention which is to be applied topically to the surface of an eye to treat a dry eye condition can also function as a vehicle to deliver a therapeutic agent to the eye to treat an eye disease.

The common practice in treating an eye infection is to deposit drops of an antibiotic agent in the eye, the number of drops to be applied on any one occasion being prescribed by a physician. Since this number defines the dosage of the drug applied to the eye, one must be careful that the drops are limited to the eye and that none of the applied liquid escapes therefrom. But in practice, it is difficult to deposit a drop of liquid into the eye so that none of the liquid flows beyond the eye borders, for there is little to hold the liquid to the eye surface.

The advantage of using an emulsion in accordance with the invention as a vehicle to deliver a therapeutic agent to the eye is that the emulsion which coats the entire surface of the eye and spreads the agent over its anionic surface, adheres electrostatically to this surface so that all of the therapeutic agent in a predetermined dosage is delivered to the eye. And because the coating electrostatically adheres to the eye surface and cannot be washed away, the residence time of treatment is prolonged and the therapeutic agent is therefore more effective.

The fact that the droplets in the charged emulsion in accordance with the invention are of submicron size is significant. This results in a much greater charge density per unit area of the emulsion film than would be produced had the droplet size been in the micron range and therefore produces a more powerful electrostatic force.

We have in the foregoing disclosed cyclosporin A as a preferred medicament to be incorporated in the emulsion. But other water-insoluble medicaments may be used provided that they possess properties for the treatment of eye disease similar to those of cyclosporin and are non-polar. Should the medicament carry a negative charge, then the amount of the cationic ingredient included in the emulsion must be such as to provide a net positive charge.

Thus among suitable medicaments that can be incorporated in an emulsion in accordance with the invention are those in the family of compounds including tacrolimus disclosed in US Patent 4,894,366. Also suitable is Sirolimus (Rapamycin) disclosed in US Patent 3,993,749.

While there has been disclosed preferred embodiments of the invention, it is to be understood that many changes may be made therein without departing from the spirit of the invention.

CLAIMS:

1. An emulsion to be topically applied to the anionic surface of an eye to form a tear film thereon which lubricates the eye to inhibit evaporation of fluid therefrom; said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

2. An emulsion as set forth in Claim 1, to treat a dry eye condition, the emulsion being defined by droplets in the submicron range.

3. An emulsion as set forth in Claim 1, in which the oil is castor oil.

4. An emulsion as set forth in Claim 1, in which the phospholipid is Lipoid E-80.

5. An emulsion as set forth in Claim 1, in which the cationic lipid is stearylamine.

6. An emulsion as set forth in Claim 1, in which the cationic lipid is oleylamine.

7. An emulsion as set forth in Claim 4, in which the relative percentage of the phospholipid in the emulsion lies in the range of 0.1 to 0.5 percent.

8. An emulsion as set forth in Claim 1, in which included in the mixture is vitamin E.

9. An emulsion as set forth in Claim 1, in which the mixture further includes an emulsifying agent.

10. An emulsion as set forth in Claim 9, in which the emulsifying agent is poloxamer.

11. An emulsion as set forth in Claim 10, in which the relative percentage of the emulsifying agent in the emulsion lies in the range of 0.5 to 2.0 percent.

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

AMENDED CLAIMS

[Received by the International Bureau on 23 December 2002 (23.12.02) ; new claims 22-24 ; remaining claims unchanged]

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

22. A method of treating immune-mediated keratoconjunctivitis sicca comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a

non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge, and further includes cyclosporin A; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

23. Use of an emulsion for the preparation of a pharmaceutical composition for the treatment of a dry eye condition, said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

24. The use according to Claim 23 for the preparation of a pharmaceutical composition for the treatment of immune-mediated keratoconjunctivitis sicca, wherein said emulsion further comprises cyclosporin A.

Statement under Article 19(1)

New Claim 23 is a reformulation of claim 19 in a form acceptable to the European Patent Office. New claims 22 and 24 claim a particular embodiment of the invention and find support in the specification on page 5, lines 3-11 and page 7, lines 20-24.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/01015

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/107 A61K38/13 A61K31/436 A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 18852 A (YISSAM RESEARCH DEV COMPANY OF) 30 September 1993 (1993-09-30) page 1, line 2 - line 6 examples 1-14,16 claims 1,3,18 --- -/--	1,2, 4-12,15, 19-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2002	23/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Epskamp, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ABDULRAZIK M ET AL: "Effect of emulsion droplet surface charge on cyclosporine ocular tissue distribution." IOVS, vol. 42, no. 4, 15 March 2001 (2001-03-15), page S925 XP008005391 Annual Meeting of the Association for Research in Vision and Ophthalmology;Fort Lauderdale, Florida, USA; April 29-May 04, 2001 abstract	1-21
X	KLANG SH ET AL: "Physiochemical characterization and acute toxicity evaluation of a positively-charged submicron emulsion vehicle" JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 46, no. 12, 1994, pages 986-993, XP008005426 ISSN: 0022-3573	1,2,4,5, 7-12,15, 19-21
Y	abstract page 986, left-hand column, line 1 - line 9 page 987, left-hand column, last line -right-hand column, paragraph 3 page 991, left-hand column, paragraph 3 -right-hand column, paragraph 1 page 992, left-hand column, paragraph 3	1-21
X	KLANG SH ET AL: "Evaluation of a positively charged submicron emulsion of piroxicam on the rabbit corneum healing process following alkali burn" JOURNAL OF CONTROLLED RELEASE, vol. 57, no. 1, 1999, pages 19-27, XP004155636 ISSN: 0168-3659	1,2,4,5, 7-12,15, 19-21
Y	abstract paragraph '02.2! paragraph '0004!	1-21
X	KLANG S ET AL: "Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution" PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY, vol. 5, no. 4, 2000, pages 521-532, XP008005503 ISSN: 1083-7450	1,2,4,5, 7-12,15, 19-21
Y	abstract page 522, right-hand column, last paragraph -page 523, left-hand column, paragraph 2 page 531, left-hand column, paragraph 2	1-21

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 09667 A (PHARMASOL GMBH) 7 February 2002 (2002-02-07) page 19, line 1 - line 5; examples 22-24	1,2,4,5, 7-12,15, 16,19-21

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IL 01/01015

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ional Application No
PCT/IL 01/01015

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		WO	0209667 A2	07-02-2002

Electronic Acknowledgement Receipt

EFS ID:	18616509
Application Number:	14222478
International Application Number:	
Confirmation Number:	9616
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine/Ken Dinh
Filer Authorized By:	Laura Lee Wine
Attorney Docket Number:	17618CON6CON1 (AP)
Receipt Date:	28-MAR-2014
Filing Date:	
Time Stamp:	16:53:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	17618C6C1-IDS_03_27_2014.pdf	100632 e3c732425d699939c0954dc4c59d408637b7c237	no	25

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Information:	

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2	Foreign Reference	DE19810655A1.pdf	642493 a134d524e4c7505c5732a8cab9f7ff85e484f7b9	no	6
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3	Foreign Reference	EP-0471293.pdf	1658633 f4204d9aae9add3360e71d625b87af99e5fcbb41	no	7
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4	Foreign Reference	EP-0547229.pdf	8161789 81ccdbb5d595b1c2a663b402f22b952405b3ade6	no	17
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5	Foreign Reference	EP-0760237.PDF	364223 11ca6edfbedb8a6c1f617c247c539021b6d7c292	no	11
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6	Foreign Reference	EP0448856.pdf	750356 8b79ca2026d78f8b534de30a1a3826eadd24e75c	no	4
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7	Foreign Reference	EP-0480690.pdf	3237017 c925e59e7d776b37bfe8702e003d8589bd6f1fc5	no	18
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8	Foreign Reference	WO-1995-031211.pdf	609318 785816ee787dd5564887887c172789c0434e5316	no	28
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10	Foreign Reference	WO-2001-032142.pdf	393715 2363e2b822d3076474c793023490c20efaf9c7fa	no	19

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11	Foreign Reference	WO2001-041671.pdf	477723 a384ff47dd523974818c54030a51b37a8918ffbf	no	21
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Information:					
12	Foreign Reference	WO2002-009667.pdf	2610140 f18e7844afe73b52f294b3cde2c525461d8f6da8	no	47
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13	Foreign Reference	WO2002-049603.pdf	540840 206d73970083fdb8f2793db01373453fd9928caf	no	25
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Information:					
14	Foreign Reference	WO2003-030834.pdf	884924 73f73deb9297abb6844121ece2cf99d1170cce48	no	36
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Information:					
15	Foreign Reference	WO2003-053405.pdf	495859 0d8699e29563ce29b3373ac58605c6958ccbbfb1	no	22
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16	Non Patent Literature	Abdulrazik-2001.pdf	2020446 b968c117d8bcaecd650fd6654bae4ba3db2b0fef	no	6
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17	Non Patent Literature	Acheampong-1996.pdf	77280 fdcc9ecdd28fa37ec7a80c28fe04a96b186308e3	no	1
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18	Non Patent Literature	Acheampong-1998.pdf	143254 66c7df564002661f5af66d6c394f16f314f024f4	no	4
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19	Non Patent Literature	Acheampong_1999.pdf	3760656 56867109cd7e843043e532d47d09b7e2ef238663	no	13

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20	Non Patent Literature	AkpekOph111_3_476_482_2004.pdf	667675 749fbcc7440650cffe44ba25d09130c7ca7ffd2f	no	8
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25	Non Patent Literature	BFGoodrich-1987.pdf	2452032 677b03ff97ebd0a2f498dfc177a6227c36b0ee6e	no	25
Warnings:					
Information:					
26	Non Patent Literature	BFGoodrich-1995.pdf	517715 4a32f10eb088de77f57e1117881d32c0c376ec79	no	2
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Information:					
27	Non Patent Literature	BFGoodrich-1993.pdf	2194648 96133277635c2655043d559600e4043b2f165dd6	no	9
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Information:					
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Warnings:					
Information:					
29	Non Patent Literature	Brewster_1994.pdf	2611645 f8dd875c527914d4d072c7d0b00c7c10c30e04b0	no	7
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Information:					
30	Non Patent Literature	Brewster_1997.pdf	2034358 c8c310235391b73a2515b79e5b7e69614cc154a1	no	5
Warnings:					
Information:					
31	Non Patent Literature	Brewster_1995.pdf	2435845 a87c613e9945ed2cb3e12ca36fc33523b6a62f2d	no	6
Warnings:					
Information:					
32	Non Patent Literature	Brinkmeier_PyodermatitisActaDerm_4_2001.pdf	462833 060bc24a76f2e8db058262428845c9fb8b9f914b	no	4
Warnings:					
Information:					
33	Non Patent Literature	Castillo_1995.pdf	2056885 f717067b8ac69bd61564f4dd29c6d1b10f5005d6	no	8
Warnings:					
Information:					
34	Non Patent Literature	CheeksInfluenceofVehicle1992.pdf	2450001 42b02fe1b3a1aac46ca35d929021b4907a8823ca	no	9
Warnings:					
Information:					
35	Non Patent Literature	Database_200044.pdf	76280 a1aa73b3e4580a5dbf752ca3d23b9aa23e0f0fb3	no	2
Warnings:					
Information:					
36	Non Patent Literature	DingPharmacAn98040585_1997.pdf	48725 0b046fb716cb7285c9f07c53e6595d8370eaaa2e	no	1
Warnings:					
Information:					
37	Non Patent Literature	Donnenfeld_2003.pdf	1514822 7a86cc202c471cce379f185113a1353747c98e63	no	3

Warnings:					
Information:					
38	Non Patent Literature	Drosos_1998.pdf	1638443 0e40d7b9b2ae76200baa9068530b5f21eefeaefb	no	5
Warnings:					
Information:					
39	Non Patent Literature	Drosos_1986.pdf	1032892 820c8e084c1b12333b1f635ec09c8afefd19579e	no	5
Warnings:					
Information:					
40	Non Patent Literature	Durrani-1992.pdf	1598136 0fbf5592ddf9a659736568779 47fb6950f34dc83	no	7
Warnings:					
Information:					
41	Non Patent Literature	EisenTopicalcyclosporine6_1990.pdf	752102 32ad87759ef79c77880006a594495cd6b992032d	no	6
Warnings:					
Information:					
42	Non Patent Literature	Epstein_1996.pdf	1582082 f316d19218cefdc0cca0e9be34264fe6f7503daa	no	5
Warnings:					
Information:					
43	Non Patent Literature	ErdmannMeetingattheDeptofDerm4_1997.pdf	462543 9f3fbb7197e803def6b46260fd835c5aaa5eef07	no	4
Warnings:					
Information:					
44	Non Patent Literature	FDA_Concludes_Restasis_1999.pdf	93984 63cff26925570cb091fa44ee5b19b28a3ff6ab6b	no	1
Warnings:					
Information:					
45	Non Patent Literature	Gaeta_1994.pdf	1817635 70f11e97d1851a46d0c18bb53f489f7f7ac5a37e	no	9
Warnings:					
Information:					
46	Non Patent Literature	Gipson_vol2no2_18_2004.pdf	2242587 0dc513ea67454195d9aef8bd3a8703267cb04156	no	18

Warnings:					
Information:					
47	Non Patent Literature	Gremse_UlcerativeColitis_in_Children10.pdf	1041954 091237aaaaa18ec3e0d7b0639e55086a00c290a2	no	10
Warnings:					
Information:					
48	Non Patent Literature	Gunduz_TopicalcyclosporinActaOphth6_1994.pdf	618144 4e3e7164d3de6e5be5691d0d7c6e8bc52a76e871	no	6
Warnings:					
Information:					
49	Non Patent Literature	Polyethylene_Glycol_Ester_2001.pdf	459916 86544da882fd05b2012425cb171c42eb144c32ab	no	6
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Information:					
50	Non Patent Literature	Hunter_1981.pdf	3004241 774fdd0660b54d79c4de0be05f7a3f8afdd571da	no	5
Warnings:					
Information:					
51	Non Patent Literature	Jumaa_1999.pdf	1946168 f50e943dc61513d9fed6651eee99968f6d36a553	no	9
Warnings:					
Information:					
52	Non Patent Literature	KanaiEffectontheCornea1989.pdf	868139 f60019d56376bcecff6bb85e0eb3238f954590b4	no	3
Warnings:					
Information:					
53	Non Patent Literature	KanpolatPenetrationofCyclosporin1994.pdf	359650 43be2c9cd6ba4e612554d7dd526b1e2b4f343b4e	no	4
Warnings:					
Information:					
54	Non Patent Literature	Kaur_1979.pdf	906300 496c705d93f0d7baa2424db48fbbf424512f96e6	no	2
Warnings:					
Information:					
55	Non Patent Literature	KuwanoCyclosporineA_Pharma19_1_108_111_2002.pdf	1878659 4e9a19149a7164165e1106060b80c8485b150be7	no	4

Warnings:					
Information:					
56	Non Patent Literature	Lambert_2003.pdf	460126 a4c43396f9847fb7308e6c0741e187941cd8aca8	no	3
Warnings:					
Information:					
57	Non Patent Literature	Leibovitz_1983.pdf	1024735 2c50222cc641655f84228b313ee6df845bcb2761	no	5
Warnings:					
Information:					
58	Non Patent Literature	Lixin_2002.pdf	1887719 6552422b21a2e5430452221f662f614d7560ede7	no	4
Warnings:					
Information:					
59	Non Patent Literature	LopatinChemicalcompositions31pgs2001.pdf	13079081 55c1ea8f4cfcf4343e8d36c01dadadf7516e0c55	no	31
Warnings:					
Information:					
60	Non Patent Literature	LyonsInfluenceofThreeEmulsion2000.pdf	59547 ea3c95c367170b2d95ca78b32f69875baf2a4c35	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	94266360		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	17618CON6CON1 (AP)
First Named Inventor	Andrew Acheampong
Title	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COM
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [✓] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [✓] **Specification** [*Total Pages* 34] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [ ] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* ____]
6. **Inventor's Oath or Declaration** [*Total Pages* 3] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [✓] A copy from a prior application (37 CFR 1.63(d))
7. [✓] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO:

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P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee _____
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [✓] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [✓] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [ ] **Other:** _____

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[✓] The address associated with Customer Number: 051957 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Laura L. Wine/	Date	March 21, 2014
Name (Print/Type)	Laura L. Wine	Registration No. (Attorney/Agent)	68681

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- [x] The attached application, or
- [ ] United States application or PCT international application number _____ filed on _____.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Diane D. Tang-Liu Date (Optional): _____

Signature: _____

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 13/961,828 filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Andrew Acheampong Date (Optional): _____

Signature: Andrew Acheampong

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 13/961,828 filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: DAVID F. POWER Date (Optional): 8-12-2015

Signature: David F. Power

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA/01 form for each additional inventor.

Doc code: Oath
Document Description: Oath or declaration filed

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Methods of Providing Therapeutic Effects Using Cyclosporin Components Docket No.: 17618CON6(AP)

This statement is directed to:

☐ The attached application.

OR

☒ United States application or PCT international application number 13/961,828 filed on 8-7-13.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

James N. Chang

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Newport Beach	CA	US

Mailing Address (except for a deceased or legally incapacitated inventor):

36 Cervantes

City	State	Zip	County
Newport Beach	CA	92660	US

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

- ☐ Legal Representative (for deceased or legally incapacitated inventor only).
- ☒ Assignee.
- ☐ Person to whom the inventor is under an obligation to assign.
- ☐ Person who otherwise shows a sufficient proprietary interest in the matter (petition under 37 CFR 1.46 is required), or
- ☐ Joint Inventor.

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SUBSTITUTE STATEMENT

Circumstances permitting execution of this substitute statement:

- ☐ Inventor is deceased.
- ☐ Inventor is under legal incapacity.
- ☐ Inventor cannot be found or reached after diligent effort, or
- ☒ Inventor has refused to execute the oath or declaration under 37 CFR 1.63.

If there are joint inventors, please check the appropriate box below:

- ☒ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

- ☐ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

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PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Debra D. Condino TITLE: ASSISTANT SECRETARY (ASSIGNEE) COMPANY: ALLERGAN, INC. Date (Optional):

Signature: D Condino

Residence (unless provided in an application data sheet, PTO/AIA/14 or equivalent):

City	State	Country
Irvine	CA	US

Mailing Address (unless provided in an application data sheet, PTO/AIA/14 or equivalent)

2525 Dupont Drive-T2-7H

City	State	Zip	Country
Irvine	CA	92612	US

Note: Use an additional PTO/AIA/02 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (03-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Andrew Acheampong	Nonprovisional Application Number (if known):	
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Laura L. Wine/	Date March 21, 2014
Name (Print/Typed) Laura L. Wine	Practitioner Registration Number 68681

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Andrew		Acheampong	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Irvine	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	16 Wintergreen		
Address 2			
City	Irvine	State/Province	CA
Postal Code	92604	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Diane	D.	Tang-Liu	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Las Vegas	State/Province	NV	Country of Residence	US

Mailing Address of Inventor:

Address 1	3726 Las Vegas Blvd., S Unit 3303 W		
Address 2			
City	Las Vegas	State/Province	CA
Postal Code	89158-4397	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	James	N.	Chang	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

City	Newport Beach	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	36 Cervantes		
Address 2			
City	Newport Beach	State/Province	CA
Postal Code	92660	Country	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	David	F.	Power	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	San Clemente	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	869 Avenida Avenue		
Address 2			
City	San Clemente	State/Province	CA
Postal Code	92672	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	051957		
Email Address	Patents_ip@Allergan.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS		
Attorney Docket Number	17618CON6CON1(AP)	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Filing By Reference :

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	051957		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13961828	2013-08-07
Prior Application Status	Patented		Remove

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
13961828	Continuation of	11897177	2007-08-28	8618064	2013-12-31
Prior Application Status	Abandoned				Remove

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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
11897177	Continuation of	10927857	2004-08-27
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10927857	Claims benefit of provisional	60503137	2003-09-15

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Allergan, Inc.

Mailing Address Information For Applicant:

Address 1	2525 Dupont Drive, T2-7H		
Address 2			
City	Irvine	**State/Province**	CA
Country	US	Postal Code	92612
Phone Number	(714) 246-6996	Fax Number	(714) 246-4249

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

Email Address	Patents_ip@Allergan.com

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Laura L. Wine/			Date (YYYY-MM-DD)	2014-03-21
First Name	Laura	Last Name	Wine	Registration Number	68681

Additional Signature may be generated within this form by selecting the Add button.

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6CON1(AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Acheampong, *et al.*	Examiner: TBA
Serial No.: TBA	Group Art Unit: TBA
Filed: Herewith	Confirmation No. TBA
For: METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	Customer No.: 51957

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examining the above-referenced application, please amend the specification as described on page 2 of this paper, and please amend the claims as described on pages 3-7 of this paper. Remarks follow on page 8.

Amendments to the Specification

Please replace page 1, lines 6-10 of the specification filed herewith with the following amended paragraph:

This application is a continuation of copending U.S. Application Serial No. 13/961,828 filed August 7, 2013, which is a continuation of U.S. Application Serial No. 11/897,177, filed August 28, 2007, now issued as U.S. Patent No. 8,618,064, which is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, now abandoned, which claimsed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which is are incorporated in its their entirety herein by reference.

Amendments to the claims

The following list of claims will replace all previous versions of claims presented in this application:

1. – 36. (Canceled)

37. (New) A method of treating dry eye disease, the method comprising topically administering to a human eye in need thereof a first topical ophthalmic emulsion at a frequency of twice a day, wherein the first topical ophthalmic emulsion comprises cyclosporin A in an amount of about 0.05% by weight, polysorbate 80, acrylate/C10-30 alkyl acrylate cross-polymer, water, and castor oil in an amount of about 1.25% by weight;

wherein the method is therapeutically effective in treating dry eye disease;

wherein the method provides overall efficacy substantially equal to administration of a second topical ophthalmic emulsion to a human eye in need thereof at a frequency of twice a day, the second emulsion comprising cyclosporin A in an amount of about 0.1% by weight and castor oil in an amount of about 1.25% by weight; and

wherein the method results in substantially no detectable concentration of cyclosporin A in the blood of the human.

38. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion further comprises a tonicity agent or a demulcent component.

39. (New) The method of Claim 38, wherein the tonicity agent or the demulcent component is glycerine.

40. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion further comprises a buffer.

41. (New) The method of Claim 40, wherein the buffer is sodium hydroxide.

42. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion further comprises glycerine and a buffer.

43. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion comprises polysorbate 80 in an amount of about 1.0% by weight.

44. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion comprises acrylate/C10-30 alkyl acrylate cross-polymer in an amount of about 0.05% by weight.

45. (New) The method of Claim 37, wherein the first topical ophthalmic emulsion further comprises glycerine in an amount of about 2.2% by weight and a buffer.

46. (New) The method of Claim 45, wherein the buffer is sodium hydroxide.

47. (New) The method of Claim 42, wherein the first topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

48. (New) The method of Claim 37, wherein substantially no detectable concentration of cyclosporin A in the blood of the human means that the concentration of cyclosporin A in the blood of the human is less than about 0.1 ng/ml.

49. (New) A method of enhancing tearing in a human eye, the method comprising topically administering to a human eye in need thereof a first topical ophthalmic emulsion at a frequency of twice a day, wherein the first topical ophthalmic emulsion comprises cyclosporin A in an amount of about 0.05% by weight, polysorbate 80, acrylate/C10-30 alkyl acrylate cross-polymer, water, and castor oil in an amount of about 1.25% by weight;

wherein the method is therapeutically effective in treating dry eye disease and wherein the method achieves at least as much therapeutic efficacy as administration of a second topical ophthalmic emulsion to a human eye in need thereof at a frequency of twice a day, the second emulsion comprising cyclosporin A in an amount of about 0.1% by weight and castor oil in an amount of about 1.25% by weight; and

wherein the method results in a concentration of cyclosporin A in the blood of the human of less than about 0.1 ng/ml.

50. (New) The method of Claim 49, wherein the first topical ophthalmic emulsion comprises acrylate/C10-30 alkyl acrylate cross-polymer in an amount of about 0.05% by weight, polysorbate 80 in an amount of about 1.0% by weight, and wherein the first topical ophthalmic emulsion further comprises glycerine in an amount of about 2.2% by weight and a buffer.

51. (New) The method of Claim 50, wherein the first topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

52. (New) The method of Claim 49, wherein the method is effective in enhancing lacrimal gland tearing.

53. (New) A method of treating dry eye disease, the method comprising topically administering to a human eye in need thereof a first topical ophthalmic emulsion at a frequency of twice a day, wherein the first topical ophthalmic emulsion comprises cyclosporin A in an amount of about 0.05% by weight, polysorbate 80, acrylate/C10-30 alkyl acrylate cross-polymer, water, and castor oil in an amount of about 1.25% by weight; and

wherein the first topical ophthalmic emulsion breaks down more quickly in the human eye, once administered to the human eye, thereby reducing vision distortion in the human eye as compared to a second topical ophthalmic emulsion that contains only about 50% as much castor oil as the first topical ophthalmic emulsion.

54. (New) The method of Claim 53, wherein the first topical ophthalmic emulsion comprises acrylate/C10-30 alkyl acrylate cross-polymer in an amount of about 0.05% by weight, polysorbate 80 in an amount of about 1.0% by weight, and wherein the first topical ophthalmic emulsion further comprises glycerine in an amount of about 2.2% by weight and a buffer.

55. (New) The method of Claim 54, wherein the first topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

56. (New) The method of Claim 55, wherein the method results in a concentration of cyclosporin A in the blood of the human of less than about 0.1 ng/ml.

57. (New) A method of restoring tearing, the method comprising topically administering to a human eye in need thereof a first topical ophthalmic emulsion at a frequency of twice a day, wherein the first topical ophthalmic emulsion comprises cyclosporin A in an amount of about 0.05% by weight, polysorbate 80, acrylate/C10-30 alkyl acrylate cross-polymer, water, and castor oil in an amount of about 1.25% by weight;

wherein the method demonstrates a reduction in adverse events in the human, compared to administration of a second topical ophthalmic emulsion to a human eye in need thereof at a frequency of twice a day, the second topical ophthalmic emulsion comprising cyclosporin A in an amount of about 0.1% by weight and castor oil in an amount of about 1.25% by weight; and

wherein the method achieves at least as much therapeutic efficacy as administration of the second topical ophthalmic emulsion to a human eye in need thereof at a frequency of twice a day.

58. (New) The method of Claim 57, wherein the method results in a concentration of cyclosporin A in the blood of the human of less than about 0.1 ng/ml.

59. (New) The method of Claim 57, wherein the adverse events are selected from the group consisting of visual distortion and eye irritation.

60. (New) The method of Claim 57, wherein the first topical ophthalmic emulsion comprises acrylate/C10-30 alkyl acrylate cross-polymer in an amount of about 0.05% by weight, polysorbate 80 in an amount of about 1.0% by weight, and wherein the first topical ophthalmic emulsion further comprises glycerine in an amount of about 2.2% by weight and a buffer.

61. (New) The method of Claim 60, wherein the first topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

62. (New) The method of Claim 57, wherein the method is effective in restoring lacrimal gland tearing.

63 (New) The method of Claim 57, wherein the adverse events are selected from the group consisting of visual distortion and eye irritation and wherein the method results in a concentration of cyclosporin A in the blood of the human of less than about 0.1 ng/ml.

REMARKS

The applicants have canceled claims 1-36 and have added claims 37-63. Support for the limitations recited in the new claims may be found throughout the specification, and at least at page 4, line 25 – page 5, line 14, page 14, line 28 - page 15, line 1, page 26, lines 5-19, and page 27, lines 4-31 of the application specification filed herewith. The amendment contains no new matter.

The claims of the present application may vary in scope from the claims pursued in the parent applications. To the extent any prior amendments or characterizations of the scope of any claim, or the specification, or referenced art could be construed as a disclaimer of any subject matter supported by the present disclosure, the Applicants hereby rescind and retract such disclaimer. Specifically, the Applicants would like to bring to the Examiner's attention comments made in the Response filed on June 15, 2009 in U.S. Patent Application Serial No. 10/927,857 (now abandoned) and comments made in the Amendment filed on June 15, 2009 in U.S. Patent Application Serial No. 11/897,177 (currently pending) regarding U.S. Patent No. 5,474,979 and the present application specification. Since these comments have been filed, the Applicants have collected evidence that supports the patentability of the pending claims.

The Commissioner is hereby authorized to charge any fees required or necessary for the filing, processing or entering of this paper or any of the enclosed papers, and to refund any overpayment, to deposit account 01-0885.

Respectfully submitted,

/Laura L. Wine/

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D-3111CON

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Related Application

This application is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, which claimed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which is incorporated in its entirety herein by reference.

Background of the Invention

The present invention relates to methods of providing desired therapeutic effects to humans or animals using compositions including cyclosporin components. More particularly, the invention relates to methods including administering to an eye of a human or animal a therapeutically effective amount of a cyclosporin component to provide a desired therapeutic effect, preferably a desired ophthalmic or ocular therapeutic effect.

The use of cyclosporin-A and cyclosporin A derivatives to treat ophthalmic conditions has been the subject of various patents, for example Ding et al U.S. Patent 5,474,979; Garst U.S. Patent 6,254,860; and Garst U.S. 6,350,442, this disclosure of each of which is incorporated in its entiraly herein by reference. In addition, cyclosporin A compositions used in treating ophthalmic conditions is the subject of a number of publications. Such publications include, for example, "Blood concentrations of cyclosporin a during long-term treatment with cyclosporin a ophthalmic emulsions in patients with moderate to severe dry eye disease," Small et al, *J Ocul Pharmacol Ther, 2002 Oct, 18(5):411-8;* "Distribution of

cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs," Acheampong et al, *Curr Eye Res, 1999 Feb, 18(2):91-103b*; "Cyclosporine distribution into the conjunctiva, cornea, lacrimal gland, and systemic blood following topical dosing of cyclosporine to rabbit, dog, and human eyes," Acheampong et al, *Adv Exp Med Biol, 1998, 438:1001-4*; "Preclinical safety studies of cyclosporine ophthalmic emulsion," Angelov et al, *Adv Exp Med Biol, 1998, 438:991-5*; "Cyclosporin A Emulsion A Eye," Stevenson et al, *Ophthalmology, 2000 May, 107(5):967-74*; and "Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group," Sall et al, *Ophthalmology, 2000 Apr, 107(4):631-9*. Each of these publications is incorporated in its entirety herein by reference. In addition, cyclosporin A-containing oil-in-water emulsions have been clinically tested, under conditions of confidentiality, since the mid 1990's in order to obtain U.S. Food and Drug Administration (FDA) regulatory approval.

Examples of useful cyclosporin A-containing emulsions are set out in Ding et al U.S. Patent 5,474,979. Example 1 of this patent shows a series of emulsions in which the ratio of cyclosporin A to castor oil in each of these compositions was 0.08 or greater, except for Composition B, which included 0.2% by weight cyclosporin A and 5% by weight castor oil. The Ding et al patent placed no significance in Composition B relative to Compositions A, C and D of Example 1.

Over time, it has become apparent that cyclosporin A emulsions for ophthalmic use preferably have less than 0.2%

by weight of cyclosporin A. With cyclosporin A concentrations less than 0.2%, the amount of castor oil employed has been reduced since one of the functions of the castor oil is to solubilize the cyclosporin A. Thus, if reduced amounts of cyclosporin are employed, reduced amounts of castor oil are needed to provide effective solubilization of cyclosporin A.

There continues to be a need for providing enhanced methods of treating ophthalmic or ocular conditions with cyclosporin-containing emulsions.

Summary of the Invention

New methods of treating a human or animal using cyclosporin component-containing emulsions have been discovered. Such methods provide substantial overall efficacy in providing desired therapeutic effects. In addition, other important benefits are obtained employing the present methods. For example, patient safety is enhanced. In particular, the present methods provide for reduced risks of side effects and/or drug interactions. Prescribing physicians advantageously have increased flexibility in prescribing such methods and the compositions useful in such methods, for example, because of the reduced risks of harmful side effects and/or drug interactions. The present methods can be easily practiced. In short, the present methods provide substantial and acceptable overall efficacy, together with other advantages, such as increased safety and/or flexibility.

In one aspect of the present invention, the present methods comprise administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component

in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

It has been found that the relatively increased amounts of hydrophobic component together with relatively reduced, yet therapeutically effective, amounts of cyclosporin component provide substantial and advantageous benefits. For example, the overall efficacy of the present compositions, for example in treating dry eye disease, is substantially equal to an identical composition in which the cyclosporin component is present in an amount of 0.1% by weight. Further, a relatively high concentration of hydrophobic component is believed to provide for a more quick or rapid breaking down or resolving of the emulsion in the eye, which reduces vision distortion which may be caused by the presence of the emulsion in the eye and/or facilitates the therapeutic effectiveness of the composition. Additionally, and importantly, using reduced amounts of the active cyclosporin component mitigates against undesirable side effects and/or potential drug interactions.

In short, the present invention provides at least one advantageous benefit, and preferably a plurality of advantageous benefits.

The present methods are useful in treating any suitable condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome,

phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic keratoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

Employing reduced concentrations of cyclosporin component, as in the present invention, is advantageously effective to provide the blood of the human or animal under treatment with reduced concentrations of cyclosporin component, preferably with substantially no detectable concentration of the cyclosporin component. The cyclosporin component concentration of blood can be advantageously measured using a validated liquid chromatography/mass spectrometry-mass spectrometry (VLC/MS-MS) analytical method, such as described elsewhere herein.

In one embodiment, in the present methods the blood of the human or animal has concentrations of clyclosporin component of 0.1 ng/ml or less.

Any suitable cyclosporin component effective in the present methods may be used.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporin A, along with several other minor metabolites, cyclosporin B through I, have been identified. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different substituents or configurations of some of the amino acids.

The term "cyclosporin component" as used herein is intended to include any individual member of the

cyclosporin group and derivatives thereof, as well as mixtures of two or more individual cyclosporins and derivatives thereof.

Particularly preferred cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof. Cyclosporin A is an especially useful cyclosporin component.

Any suitable hydrophobic component may be employed in the present invention. Advantageously, the cyclosporin component is solubilized in the hydrophobic component. The hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions.

The hydrophobic component preferably is present in the emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount of up to about 1.0% by weight or about 1.5% by weight or more of the composition.

Preferably, the hydrophobic component comprises one or more oily materials. Examples of useful oil materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils and the like and mixtures thereof. In a very useful embodiment, the hydrophobic component comprises one or more higher fatty acid glycerides. Excellent results are obtained when the hydrophobic component comprises castor oil.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the compositions. Examples of such other components include, without limitation, emulsifier components, tonicity components,

polyelectrolyte components, surfactant components, viscosity inducing components, acids and/or bases to adjust the pH of the composition, buffer components, preservative components and the like. Components may be employed which are effective to perform two or more functions in the presently useful compositions. For example, components which are effective as both emulsifiers and surfactants may be employed, and/or components which are effective as both polyelectrolyte components and viscosity inducing components may be employed. The specific composition chosen for use in the present invention advantageously is selected taking into account various factors present in the specific application at hand, for example, the desired therapeutic effect to be achieved, the desired properties of the compositions to be employed, the sensitivities of the human or animal to whom the composition is to be administered, and the like factors.

The presently useful compositions advantageously are ophthalmically acceptable. A composition, component or material is ophthalmically acceptable when it is compatible with ocular tissue, that is, it does not cause significant or undue detrimental effects when brought into contact with ocular tissues.

Such compositions have pH's within the physiological range of about 6 to about 10, preferably in a range of about 7.0 to about 8.0 and more preferably in a range of about 7.2 to about 7.6.

The present methods preferably provide for an administering step comprising topically administering the presently useful compositions to the eye or eyes of a human or animal.

Each and every feature described herein, and each and

every combination of two or more of such features, is included within the scope of the present invention provided that the features included in such a combination are not mutually inconsistent.

These and other aspects and advantages of the present invention are apparent in the following detailed description, example and claims.

Detailed Description

The present methods are effective for treating an eye of a human or animal. Such methods, in general, comprise administering, preferably topically administering, to an eye of a human or animal a cyclosporin component-containing emulsion. The emulsion contains water, for example U.S. pure water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the emulsion. In addition, beneficial results have been found when the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

As noted above, the present administering step preferably includes topically administering the emulsion to the eye of a patient of a human or animal. Such administering may involve a single use of the presently useful compositions, or repeated or periodic use of such compositions, for example, as required or desired to achieve the therapeutic effect to be obtained. The topical administration of the presently useful composition may involve providing the composition in the form of eye drops or similar form or other form so as to facilitate such topical administration.

The present methods have been found to be very

effective in providing the desired therapeutic effect or effects while, at the same time, substantially reducing, or even substantially eliminating, side effects which may result from the presence of the cyclosporin component in the blood of the human or animal being treated, and eye irritation which, in the past, has been caused by the presence of certain components in prior art cyclosporin-containing emulsions. Also, the use of the present compositions which include reduced amounts of the cyclosporin components allow for more frequent administration of the present compositions to achieve the desired therapeutic effect or effects without substantially increasing the risk of side effects and/or eye irritation.

The present methods are useful in treating any condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

The frequency of administration and the amount of the presently useful composition to use during each administration varies depending upon the therapeutic effect to be obtained, the severity of the condition being treated and the like factors. The presently useful compositions are designed to allow the prescribing physician substantial flexibility in treating various ocular conditions to achieve the desired therapeutic effect or effects with

reduced risk of side effects and/or eye irritation. Such administration may occur on an as needed basis, for example, in treating or managing dry eye syndrome, on a one time basis or on a repeated or periodic basis once, twice, thrice or more times daily depending on the needs of the human or animal being treated and other factors involved in the application at hand.

One of the important advantages of the present invention is the reduced concentration of the cyclosporin component in the blood of the human or animal as a result of administering the present composition as described herein. One very useful embodiment of the present administering step provides no substantial detectable concentration of cyclosporin component in the blood of the human or animal. Cyclosporin component concentration in blood preferably is determined using a liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS/MS), which test has a cyclosporin component detection limit of 0.1 ng/ml. Cyclosporin component concentrations below or less than 0.1 ng/ml are therefore considered substantially undetectable.

The LC-MS/MS test is advantageously run as follows.

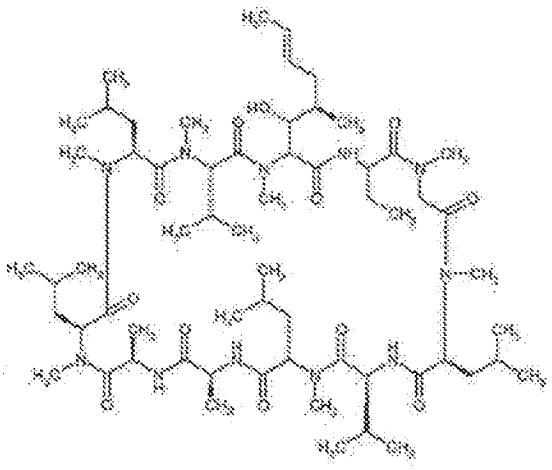
One ml of blood is acidified with 0.2 ml of 0.1 N HCl solution, then extracted with 5 ml of methyl t-butyl ether. After separation from the acidified aqueous layer, the organic phase is neutralized with 2 ml of 0.1 N NaOH, evaporated, reconstituted in a water/acetonitrile-based mobil phase, and injected onto a 2.1 x 50 mm, 3µm pore size C-8 reverse phase high pressure liquid chromatography (HPLC) column (Keystone Scientific, Bellefonte, PA). Compounds are gradient-eluted at 0.2 mL/min and detected using an API III triple quadrupole mass spectrometer with a

turbo-ionspray source (PE-Sciex, Concord, Ontario, Canada). Molecular reaction monitoring enhances the sensitivity and selectivity of this assay. Protonated molecules for the analyte and an internal standard are collisionally dissociated and product ions at m/z 425 are monitored for the analyte and the internal standard. Under these conditions, cyclosporin A and the internal standard cyclosporin G elute with retention times of about 3.8 minutes. The lower limit of quantitation is 0.1 ng/mL, at which concentration the coefficient of variation and deviation from nominal concentration is <15%.

As noted previously, any suitable cyclosporin component effective in the present methods may be employed. Very useful cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof.

The chemical structure for cyclosporin A is represented by Formula 1

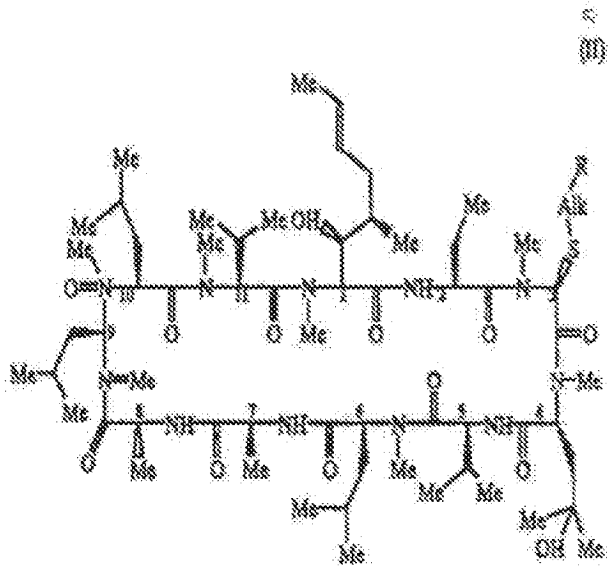
Formula I



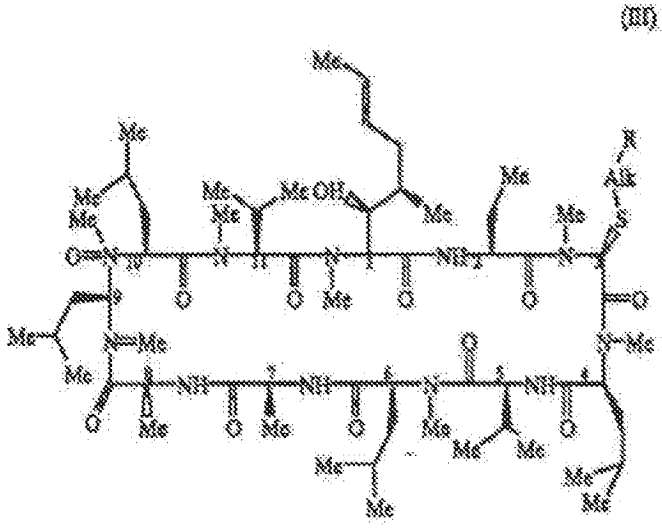
As used herein the term "derivatives" of a cyclosporin refer to compounds having structures sufficiently similar to the cyclosporin so as to function in a manner substantially similar to or substantially identical to the cyclosporin, for example, cyclosporin A, in the present methods. Included, without limitation, within the useful cyclosporin A derivatives are those selected from ((R)-methylthio-Sar)^3-(4'-hydroxy-MeLeu) cyclosporin A, ((R)-(Cyclo)alkylthio-Sar)^3-(4'-hydroxy-MeLeu)^4-cyclosporin A, and ((R)-(Cyclo)alkylthio-Sar)^3-cyclosporin A derivatives described below.

These cyclosporin derivatives are represented by the following general formulas (II), (III), and (IV) respectively:

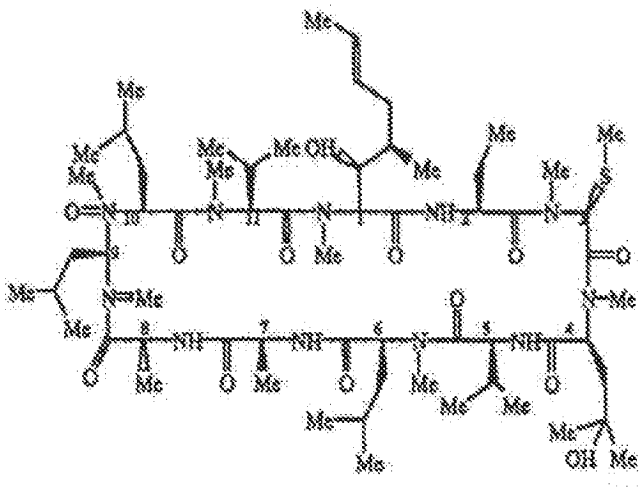
Formula II



Formula III



Formula IV



wherein Me is methyl; Alk is 2-6C alkylene or 3-6C cycloalkylene; R is OH, COOH, alkoxycarbonyl, $-NR_1R_2$ or $N(R_3)-(CH_2)-NR_1R_2$; wherein R_1,R_2 is H, alkyl, 3-6C cycloalkyl, phenyl (optionally substituted by halo, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino), benzyl or saturated or unsaturated heterocyclyl having 5 or 6 members and 1-3 heteroatoms; or NR_1R_2 is a 5 or 6 membered heterocycle which may contain a further N, O or S heteroatom and may be alkylated; R_3 is H or alkyl and n is 2-4; and the alkyl moieties contain 1-4C.

In one embodiment, the cyclosporin component is effective as an immunosuppressant. Without wishing to be limited to any particular theory of operation, it is believed that, in certain embodiments of the present invention, the cyclosporin component acts to enhance or restore lacrimal gland tearing in providing the desired

therapeutic effect.

One important feature of the present invention is that the presently useful compositions contain less than 0.1% by weight of the cyclosporin component. The advantages of such low-concentrations of cyclosporin components have been discussed in some detail elsewhere herein. Low concentrations of cyclosporin component, together with concentrations of the hydrophobic component such that the weight ratio of cyclosporin component to hydrophobic component is greater than 0.08, provides one or more substantial advantages in the present methods.

Any suitable hydrophobic component may be employed in the present invention. Such hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions, with the water or aqueous phase being considered the continuous phase in such emulsion. The hydrophobic component is preferably selected so as to solubilize the cyclosporin component, which is often substantially insoluble in the aqueous phase. Thus, with a suitable hydrophobic component included in the presently useful emulsions, the cyclosporin component is preferably solubilized in the emulsions.

In one very useful embodiment, the hydrophobic component comprises an oily material, in particular, a material which is substantially not miscible in water. Examples of useful oily materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils, and the like and mixtures thereof. Thus, the present hydrophilic components may comprise naturally occurring oils, including, without limitation refined naturally occurring oils, or naturally occurring oils which

have been processed to alter their chemical structures to some extent or oils which are substantially entirely synthetic. One very useful hydrophobic component includes higher fatty acid glycerides.

Examples of useful hydrophobic components include, without limitation, olive oil, arachis oil, castor oil, mineral oil, silicone fluid and the like and mixtures thereof. Higher fatty acid glycerides such as olive oil, peanut oil, castor oil and the like and mixtures thereof are particularly useful in the present invention. Excellent results are obtained using a hydrophobic component comprising castor oil. Without wishing to limit the invention to any particular theory of operation, it is believed that castor oil includes a relatively high concentration of ricinoleic acid which itself may be useful in benefitting ocular tissue and/or in providing one or more therapeutic effects when administered to an eye.

The hydrophobic component is preferably present in the presently useful cyclosporin component-containing emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount up to about 0.75% by weight or about 1.0% by weight or about 1.5% by weight or more of the presently useful emulsion compositions.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the present methods and/or the presently useful compositions. Examples of such other components include, without limitation, emulsifier components, surfactant components, tonicity components, poly electrolyte components, emulsion stability components, viscosity inducing components, demulcent components, acid

and/or bases to adjust the pH of the composition, buffer components, preservative components and the like.

In one very useful embodiment, the presently useful compositions are substantially free of preservatives. Thus, the presently useful compositions may be sterilized and maintained in a sterile condition prior to use, for example, provided in a sealed package or otherwise maintained in a substantially sterile condition.

Any suitable emulsifier component may be employed in the presently useful compositions, provided, that such emulsifier component is effective in forming maintaining the emulsion and/or in the hydrophobic component in emulsion, while having no significant or undue detrimental effect or effects on the compositions during storage or use.

In addition, the presently useful compositions, as well as each of the components of the present compositions in the concentration present in the composition advantageously are ophthalmically acceptable.

Useful emulsifier components may be selected from such component which are conventionally used and well known in the art. Examples of such emulsifier components include, without limitation, surface active components or surfactant components which may be anionic, cationic, nonionic or amphoteric in nature. In general, the emulsifier component includes a hydrophobic constituent and a hydrophilic constituent. Advantageously, the emulsifier component is water soluble in the presently useful compositions. Preferably, the emulsifier component is nonionic. Specific examples of suitable emulsifier components include, without limitation, polysorbate 80, polyoxyalkylene alkylene ethers, polyalkylene oxide ethers

of alkyl alcohols, polyalkylene oxide ethers of alkylphenols, other emulsifiers/surfactants, preferably nonionic emulsifiers/surfactants, useful in ophthalmic compositions, and the like and mixtures thereof.

The emulsifier component is present in an amount effective in forming the present emulsion and/or in maintaining the hydrophobic component in emulsion with the water or aqueous component. In one preferred embodiment, the emulsifier component is present in an amount in a range of about 0.1% to about 5%, more preferably about 0.2% to about 2% and still more preferably about 0.5% to about 1.5% by weight of the presently useful compositions.

Polyelectrolyte or emulsion stabilizing components may be included in the presently useful compositions. Such components are believed to be effective in maintaining the electrolyte balance in the presently useful emulsions, thereby stabilizing the emulsions and preventing the emulsions from breaking down prior to use. In one embodiment, the presently useful compositions include a polyanionic component effective as an emulsion stabilizing component. Examples of suitable polyanionic components useful in the presently useful compositions include, without limitation, anionic cellulose derivatives, anionic acrylic acid-containing polymers, anionic methacrylic acid-containing polymers, anionic amino acid-containing polymers and the like and mixtures thereof.

A particularly useful class of polyanionic components include one or more polymeric materials having multiple anionic charges. Examples include, but are not limited to:

metal carboxy methylcelluloses
metal carboxy methylhydroxyethylcelluloses

metal carboxy methylstarchs
metal carboxy methylhydroxyethylstarchs
hydrolyzed polyacrylamides and polyacrylonitriles
heparin
gucoaminoglycans
hyaluronic acid
chondroitin sulfate
dermatan sulfate
peptides and polypeptides
alginic acid
metal alginates
homopolymers and copolymers of one or more of:
acrylic and methacrylic acids
metal acrylates and methacrylates
vinylsulfonic acid
metal vinylsulfonate
amino acids, such as aspartic acid, glutamic acid and the like
metal salts of amino acids
p-styrenesulfonic acid
metal p-styrenesulfonate
2-methacryloyloxyethylsulfonic acids
metal 2-methacryloyloxethylsulfonates
3-methacryloyloxy-2-hydroxypropylsulonic acids
metal 3-methacryloyloxy-2-hydroxypropylsulfonates
2-acrylamido-2-methylpropanesulfonic acids
metal 2-acrylamido-2-methylpropanesulfonates
allylsulfonic acid
metal allylsulfonate and the like.

One particularly useful emulsion stabilizing component

includes crosslinked polyacrylates, such as carbomers and Pemulen® materials. Pemulen® is a registered trademark of B.F. Goodrich for polymeric emulsifiers and are commercially available from B.F. Goodrich Company, Specialty Polymers & Chemicals Division, Cleveland, Ohio. Pemulen® materials include acrylate/C10-30 alkyl acrylate cross-polymers, or high molecular weight co-polymers of acrylic acid and a long chain alkyl methacrylate cross-linked with allyl ethers of pentaerythritol.

The presently useful polyanionic components may also be used to provide a suitable viscosity to the presently useful compositions. Thus, the polyanionic components may be useful in stabilizing the presently useful emulsions and in providing a suitable degree of viscosity to the presently useful compositions.

The polyelectrolyte or emulsion stabilizing component advantageously is present in an amount effective to at least assist in stabilizing the cyclosporin component-containing emulsion. For example, the polyelectrolyte/emulsion stabilizing component may be present in an amount in a range of about 0.01% by weight or less to about 1% by weight or more, preferably about 0.02% by weight to about 0.5% by weight, of the composition.

Any suitable tonicity component may be employed in accordance with the present invention. Preferably, such tonicity component is non-ionic, for example, in order to avoid interfering with the other components in the presently useful emulsions and to facilitate maintaining the stability of the emulsion prior to use. Useful tonicity agents include, without limitation, glycerine, mannitol, sorbitol and the like and mixtures thereof. The presently useful emulsions are preferably within the range

of plus or minus about 20% or about 10% from being isotonic.

Ophthalmic demulcent components may be included in effective amounts in the presently useful compositions. For example, ophthalmic demulcent components such as carboxymethylcellulose, other cellulose polymers, dextran 70, gelatin, glycerine, polyethylene glycols (e.g., PEG 300 and PEG 400), polysorbate 80, propylene glycol, polyvinyl alcohol, povidone and the like and mixtures thereof, may be used in the present ophthalmic compositions, for example, compositions useful for treating dry eye.

The demulcent components are preferably present in the compositions, for example, in the form of eye drops, in an amount effective in enhancing the lubricity of the presently useful compositions. The amount of demulcent component in the present compositions may be in a range of at least about 0.01% or about 0.02% to about 0.5% or about 1.0% by weight of the composition.

Many of the presently useful polyelectrolyte/emulsion stabilizing components may also be effective as demulcent components, and vice versa. The emulsifier/surfactant components may also be effective as demulcent components and vice versa.

The pH of the emulsions can be adjusted in a conventional manner using sodium hydroxide and/or hydrochloric acid to a physiological pH level. The pH of the presently useful emulsions preferably is in the range of about 6 to about 10, more preferably about 7.0 to about 8.0 and still more preferably about 7.2 to about 7.6.

Although buffer components are not required in the presently useful compositions, suitable buffer components, for example, and without limitation, phosphates, citrates,

acetates, borates and the like and mixtures thereof, may be employed to maintain a suitable pH in the presently useful compositions.

The presently useful compositions may include an effective amount of a preservative component. Any suitable preservative or combination of preservatives may be employed. Examples of suitable preservatives include, without limitation, benzalkonium chloride, methyl and ethyl parabens, hexetidine, phenyl mercuric salts and the like and mixtures thereof. The amounts of preservative components included in the present compositions are such to be effective in preserving the compositions and can vary based on the specific preservative component employed, the specific composition involved, the specific application involved, and the like factors. Preservative concentrations often are in the range of about 0.0001% to about 0.05% or about 0.1% (w/v) of the composition, although other concentrations of certain preservatives may be employed.

Very useful examples of preservative components in the present invention include, but are not limited to, chlorite components. Specific examples of chlorite components useful as preservatives in accordance with the present invention include stabilized chlorine dioxide (SCD), metal chlorites such as alkali metal and alkaline earth metal chlorites, and the like and mixtures thereof. Technical grade (or USP grade) sodium chlorite is a very useful preservative component. The exact chemical composition of many chlorite components, for example, SCD, is not completely understood. The manufacture or production of certain chlorite components is described in McNicholas U.S. Patent 3,278,447, which is incorporated in its entirety by

reference herein. Specific examples of useful SCD products include that sold under the trademark Dura Klor by Rio Linda Chemical Company, Inc., and that sold under the trademark Anthium Dioxide® by International Dioxide, Inc. An especially useful SCD is a product sold under the trademark Bio-Cide® by Bio-Cide International, Inc., as well as a product identified by Allergan, Inc. by the trademark Purite®.

Other useful preservatives include antimicrobial peptides. Among the antimicrobial peptides which may be employed include, without limitation, defensins, peptides related to defensins, cecropins, peptides related to cecropins, magainins and peptides related to magainins and other amino acid polymers with antibacterial, antifungal and/or antiviral activities. Mixtures of antimicrobial peptides or mixtures of antimicrobial peptides with other preservatives are also included within the scope of the present invention.

The compositions of the present invention may include viscosity modifying agents or components, such as cellulose polymers, including hydroxypropyl methyl cellulose (HPMC), hydroxyethyl cellulose (HEC), ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose and carboxymethyl cellulose; carbomers (e.g. carbopol, and the like); polyvinyl alcohol; polyvinyl pyrrolidone; alginates; carrageenans; and guar, karaya, agarose, locust bean, tragacanth and xanthan gums. Such viscosity modifying components are employed, if at all, in an amount effective to provide a desired viscosity to the present compositions. The concentration of such viscosity modifiers will typically vary between about 0.01 to about 5 % w/v of the

total composition, although other concentrations of certain viscosity modifying components may be employed.

The presently useful compositions may be produced using conventional and well known methods useful in producing ophthalmic products including oil-in-water emulsions.

In one example, the oily phase of the emulsion can be combined with the cyclosporin component to solubilize the cyclosporin component in the oily material phase. The oily phase and the water may be separately heated to an appropriate temperature. This temperature may be the same in both cases, generally a few degrees to about 10°C above the melting temperature of the ingredient(s) having the highest melting point in the case of a solid or semi-solid oily phase for emulsifier components in the oily phase. Where the oily phase is a liquid at room temperature, a suitable temperature for preparation of a composition may be determined by routine experimentation in which the melting point of the ingredients aside from the oily phase is determined. In cases where all components of either the oily phase or the water phase are soluble at room temperature, no heating may be necessary. Non-emulsifying agents which are water soluble are dissolved in the water and oil soluble components including the surfactant components are dissolved in the oily phase.

To create an oil-in-water emulsion, the final oil phase is gently mixed into either an intermediate, preferably de-ionized water, phase or into the final water phase to create a suitable dispersion and the product is allowed to cool with or without stirring. In the case where the final oil phase is first gently mixed into an intermediate water phase, the resulting emulsion

concentrate is thereafter mixed in the appropriate ratio with the final aqueous phase. In such cases, the emulsion concentrate and the final aqueous phase may not be at the same temperature or heated above room temperature, as the emulsion may be already formed at this point.

The oil-in-water emulsions of the present invention can be sterilized after preparation using heat, for example, autoclave steam sterilization or can be sterile filtered using, for example, a 0.22 micron sterile filter. Sterilization employing a sterilization filter can be used when the emulsion droplet (or globule or particle) size and characteristics allows this. The droplet size distribution of the emulsion need not be entirely below the particle size cutoff of the 0.22 micron sterile filtration membrane to be sterile-filtratable. In cases wherein the droplet size distribution of the emulsion is above the particle size cutoff of the 0.22 micron sterile filtration membrane, the emulsion needs to be able to deform or change while passing through the filtration membrane and then reform after passing through. This property is easily determined by routine testing of emulsion droplet size distributions and percent of total oil in the compositions before and after filtration. Alternatively, a loss of a small amount of larger droplet sized material may be acceptable.

The present oil-in-water emulsions preferably are thermodynamicaly stable, much like microemulsions, and yet may not be isotropic transparent compositions as are microemulsions. The emulsions of the present invention advantageously have a shelf life exceeding one year at room temperature.

The following non-limiting examples illustrate certain aspects of the present invention.

EXAMPLE 1

Two compositions are selected for testing. These compositions are produced in accordance with well known techniques and have the following make-ups:

	Composition I	Composition II
	wt%	wt%
Cyclosporin A	0.1	0.05
Castor Oil	1.25	1.25
Polysorbate 80	1.00	1.00
Premulen®	0.05	0.05
Glycerine	2.20	2.20
Sodium hydroxide	qs	qs
Purified Water	qs	qs
pH	7.2-7.6	7.2-7.6
Weight Ratio of Cyclosporin A to Castor Oil	0.08	0.04

These compositions are employed in a Phase 3, double-masked, randomized, parallel group study for the treatment of dry eye disease.

The results of this study indicate that Composition II, in accordance with the present invention, which has a reduced concentration of cyclosporin A and a cyclosporin A to castor oil ratio of less than 0.08, provides overall efficacy in treating dry eye disease substantially equal to that of Composition I. This is surprising for a number of reasons. For example, the reduced concentration of cyclosporin A in Composition II would have been expected to result in reduced overall efficacy in treating dry eye disease. Also, the large amount of castor oil relative to the amount of cyclosporin A in Composition II might have been expected to cause increased eye irritation relative to

Composition I. However, both Composition I and Composition II are found to be substantially non-irritating in use.

Using relatively increased amounts of castor oil, with reduced amounts of cyclosporin component, as in Composition II, is believed to take advantage of the benefits, for example the ocular lubrication benefits, of castor oil, as well as the presence of ricinoleic acid in the castor oil, to at least assist in treating dry eye syndrome in combination with cyclosporin A.

In addition, it is found that the high concentration of castor oil relative to cyclosporin component, as in Composition II, provides the advantage of more quickly or rapidly (for example, relative to a composition which includes only 50% as much castor oil) breaking down or resolving the emulsion in the eye, for example, as measured by split-lamp techniques to monitor the composition in the eye for phase separation. Such rapid break down of the emulsion in the eye reduces vision distortion as the result of the presence of the emulsion in the eye, as well as facilitating the therapeutic effectiveness of the composition in treating dry eye disease.

Using reduced amounts of cyclosporin A, as in Composition II, to achieve therapeutic effectiveness mitigates even further against undesirable side effects and potential drug interactions. Prescribing physicians can provide (prescribe) Composition II to more patients and/or with fewer restrictions and/or with reduced risk of the occurrence of adverse events, e.g., side effects, drug interactions and the like, relative to providing Composition I.

While this invention has been described with respect to various specific examples and embodiments, it is to be

understood that the invention is not limited thereto and that it can be variously practiced within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating an eye of a human or animal comprising:

administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition, the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

2. The method of claim 1 wherein the administering step is effective in treating a condition selected from the group consisting of dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic keratoconjunctivitis and corneal graft rejection.

3. The method of claim 1 wherein the administering step is effective in treating dry eye syndrome.

4. The method of claim 1 wherein the blood of the human or animal has substantially no detectable concentration of the cyclosporin component.

5. The method of claim 1 wherein the blood of the human or animal has substantially no detectable concentration of the cyclosporin component as measured using a validated liquid chromatography/mass spectrometry-mass spectrometry analytical method.

6. The method of claim 1 wherein the blood of the human or animal has a concentration of the cyclosporin component of 0.1 ng/ml or less.

7. The method of claim 1 wherein the cyclosporin component comprises a material selected from cyclosporin A, derivatives of cyclosporin A and mixtures thereof.

8. The method of claim 1 wherein the cyclosporin component comprises cyclosporin A.

9. The method of claim 1 wherein the cyclosporin component is solubilized in the hydrophobic component present in the composition.

10. The method of claim 1 wherein the hydrophobic component is present in the composition in an amount greater than 0.625% by weight of the composition.

11. The method of claim 1 wherein the hydrophobic component comprises an oily material.

12. The method of claim 1 wherein the hydrophobic component comprises an ingredient selected from the group consisting of vegetable oils, animal oils, mineral oils, synthetic oils and mixtures thereof.

13. The method of claim 1 wherein the hydrophobic component comprises castor oil.

14. The method of claim 1 wherein the administering step comprises topically administering the composition to the eye of the human.

15. The method of claim 1 wherein the composition comprises an effective amount of an emulsifier component.

16. The method of claim 1 wherein the composition comprises an effective amount of a tonicity component.

17. The method of claim 1 wherein the composition comprises an effective amount of an organic tonicity component.

18. The method of claim 1 wherein the composition comprises a polyelectrolyte component in an amount effective in stabilizing the composition.

19. The method of claim 1 wherein the composition has a pH in the range of about 7.0 to about 8.0.

20. The method of claim 1 wherein the composition has a pH in the range of about 7.2 to about 7.6.

21. A composition for treating an eye of a human or animal comprising an emulsion comprising water, a hydrophobic component, and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight, the weight ratio of the cyclosporin component to the hydrophobic component being less than 0.08.

22. The composition of claim 21 having a make-up so that when the composition is administered to an eye of a

human in an effective amount in treating dry eye syndrome, the blood of the human has substantially no detectable concentration of the cyclosporin component.

23. The composition of claim 21 wherein the cyclosporin component comprises a material selected from cyclosporin A, derivatives of cyclosporin A and mixtures thereof.

24. The composition of claim 21 wherein the cyclosporin component comprises cyclosporin A.

25. The composition of claim 21 in the form of an emulsion.

26. The composition of claim 21 wherein the hydrophobic component is present in an amount greater than 0.625% by weight of the composition.

27. The composition of claim 21 wherein the hydrophobic component is an oily material.

28. The composition of claim 21 wherein the hydrophobic component comprises an ingredient selected from the group consisting of vegetable oils, animal oils, mineral oils, synthetic oils, and mixtures thereof.

29. The composition of claim 21 wherein the hydrophobic component comprises castor oil.

30. The composition of claim 21 wherein the administering step comprises topically administering the composition to the eye of the human.

31. The composition of claim 21 wherein the composition comprises an effective amount of an emulsifier component.

32. The composition of claim 21 wherein the composition comprises an effective amount of a tonicity component.

33. The composition of claim 21 wherein the composition comprises an effective amount of an organic tonicity component.

34. The composition of claim 21 wherein the composition comprises a polyelectrolytic component in an amount effective in stabilizing the composition.

35. The composition of claim 21 wherein the composition includes water and has a pH in the range of about 7.0 to about 8.0.

36. The composition of claim 21 wherein the composition includes water and has a pH in the range of about 7.2 to about 7.6.

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Abstract of the Disclosure

Methods of treating an eye of a human or animal include administering to an eye of a human or animal a composition in the form of an emulsion including water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.8.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Filer:	Laura Lee Wine
Attorney Docket Number:	17618CON6CON1 (AP)

Filed as Large Entity

Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Claims in Excess of 20	1202	7	80	560
Independent claims in excess of 3	1201	1	420	420

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**6580**

Electronic Acknowledgement Receipt

EFS ID:	18554507
Application Number:	14222478
International Application Number:	
Confirmation Number:	9616
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine
Filer Authorized By:	
Attorney Docket Number:	17618CON6CON1 (AP)
Receipt Date:	21-MAR-2014
Filing Date:	
Time Stamp:	19:44:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$6580
RAM confirmation Number	5436
Deposit Account	010885
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		17618CON6CON1_FilingPapers.pdf	6860066 48cb58cbbec4716d37a097dbb4b2298ec1f21e4b	yes	63

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal of New Application	1	2
Oath or Declaration filed	3	8
Power of Attorney	9	10
TrackOne Request	11	12
Application Data Sheet	13	20
Preliminary Amendment	21	29
Specification	30	57
Claims	58	62
Abstract	63	63

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	40034 ee78d12a320ba71b7a096d64da2eb42ed5acddbb	no	2

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Electronic Acknowledgement Receipt

EFS ID:	18554507
Application Number:	14222478
International Application Number:	
Confirmation Number:	9616
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine
Filer Authorized By:	
Attorney Docket Number:	17618CON6CON1 (AP)
Receipt Date:	21-MAR-2014
Filing Date:	
Time Stamp:	19:44:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$6580
RAM confirmation Number	5436
Deposit Account	010885
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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		17618CON6CON1_FilingPapers.pdf	6860066 48cb58cbbec4716d37a097dbb4b2298ec1f21e4b	yes	63

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal of New Application	1	2
Oath or Declaration filed	3	8
Power of Attorney	9	10
TrackOne Request	11	12
Application Data Sheet	13	20
Preliminary Amendment	21	29
Specification	30	57
Claims	58	62
Abstract	63	63

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	40034 ee78d12a320ba71b7a096d64da2eb42ed5acddbb	no	2

Warnings:

Information:

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Doc Code: PA.
Document Description: Power of Attorney

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I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

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☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above:

51957

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I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Debra D. Condino/	Date (Optional)	March 21, 2014
Name	Debra D. Condino, Reg. No. 31,007		
Title	Vice President, Chief Intellectual Property Counsel, and Assistant Secretary, Allergan, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ Total of forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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I hereby appoint:

☒ Practitioners associated with Customer Number: 51957

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

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☐	Firm or Individual Name			
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Assignee Name and Address:

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	/Debra Condino/	Date	March 21, 2014
Name	Debra D. Condino, Reg. No. 31,007	Telephone	(714)246-2388
Title	Vice President, Chief Intellectual Property Counsel, and Assistant Secretary, Allergan, Inc.		

Document code: WFEE

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PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/222,478	Filing Date 03/21/2014	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☒ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	**280**
☒ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	**600**
☒ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	**720**
TOTAL CLAIMS (37 CFR 1.16(i))	27 minus 20 =	* 7	x $80 =	**560**
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x $420 =	**420**
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	**2580**

APPLICATION AS AMENDED – PART II

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

AMENDMENT

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x $ =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x $ =	
☐ Application Size Fee (37 CFR 1.16(s))						
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
					TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LDRC
/EVA GILLIS/

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ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	03/21/2014	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 27	Minus	** 27	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LDRC
/EVA GILLIS/