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Electronic Acknowledgement Receipt

EFS ID:	18616805
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:	16:55:06
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	Non Patent Literature	PedersenExpertOpin1415_1436_2001.pdf	4262912 0ffbacdd587d316724d2c00a7e64fcdbc6ed49e4	no	22

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Warnings:					
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New Applications Under 35 U.S.C. 111

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National Stage of an International Application under 35 U.S.C. 371

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Doc code: IDS
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14222478
	Filing Date	2014-03-21
	First Named Inventor	ANDREW ACHEAMPONG
	Art Unit	1621
	Examiner Name	TBD
	Attorney Docket Number	17618-US-CN6CN1-AP

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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	Attorney Docket Number	17618-US-CN6CN1-AP

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	Art Unit	1621
	Examiner Name	TBD
	Attorney Docket Number	17618-US-CN6CN1-AP

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Application Number	14222478
Filing Date	2014-03-21
First Named Inventor	ANDREW ACHEAMPONG
Art Unit	1621
Examiner Name	TBD
Attorney Docket Number	17618-US-CN6CN1-AP

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	19810655	DE		1999-09-16	Eberhard-Karls-Universitat Tubingen Universitatskl		☐
	2	0448856	EP		1991-10-02	Chatfield Pharmaceuticals		☐
	3	0471293	EP		1992-02-19	ABBOTT LABORATORIES		☐
	4	0480690	EP		1992-04-15	IOLAB CORPORATION		☐
	5	0547229	EP		1993-01-07	LLT Institute Co., Ltd.		☐
	6	0760237	EP		1997-03-05	Cipla Limited		☐
	7	1995-031211	WO		1995-11-23	Allergan, Inc.		☐
	8	2000-000179	WO		2000-01-06	Won Jin Biopharma Co., Ltd		☐
	9	2001-032142	WO		2001-05-10	Cipla Limited		☐
	10	2001-041671	WO		2001-06-14	Transneuronix, Inc.		☐

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	11	2002-009667	WO		2002-02-07	Pharmasol GMBH		☐
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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ None

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Laura L. Wine/	Date (YYYY-MM-DD)	2014-03-27
Name/Print	Laura L. Wine	Registration Number	68,681

This collection of information is required by 37 CFR 1.97 and 1.98. 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 1 hour 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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(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

(12) **Offenlegungsschrift**

(10) **DE 198 10 655 A 1**

(51) Int. Cl.[6]: **A 61 K 38/13**

(21) Aktenzeichen: 198 10 655.6
(22) Anmeldetag: 12. 3. 98
(43) Offenlegungstag: 16. 9. 99

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(56) Entgegenhaltungen:

US	54 72 706 A
US	53 42 625 A
EP	06 96 452 A1

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(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.

DE 198 10 655 A 1

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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 Phosphatidylserin 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 Neurodermitis.

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.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 471 293 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **91113327.0**

(22) Date of filing: **09.08.91**

(51) Int. Cl.[5]: **G01N 33/50**, //G01N33/68, C07K7/64

(30) Priority: **15.08.90 US 567940**

(43) Date of publication of application: **19.02.92 Bulletin 92/08**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI NL SE**

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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.

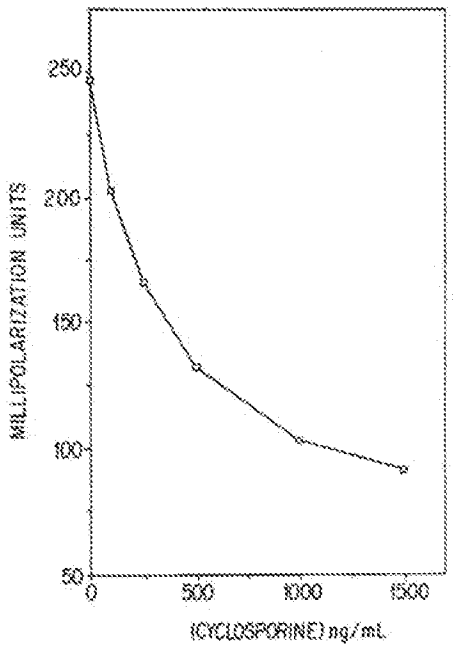

FIG. 1

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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 includs, 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 lymphpocytes, 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 erythrocyles, 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 background 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 background 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 background 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.

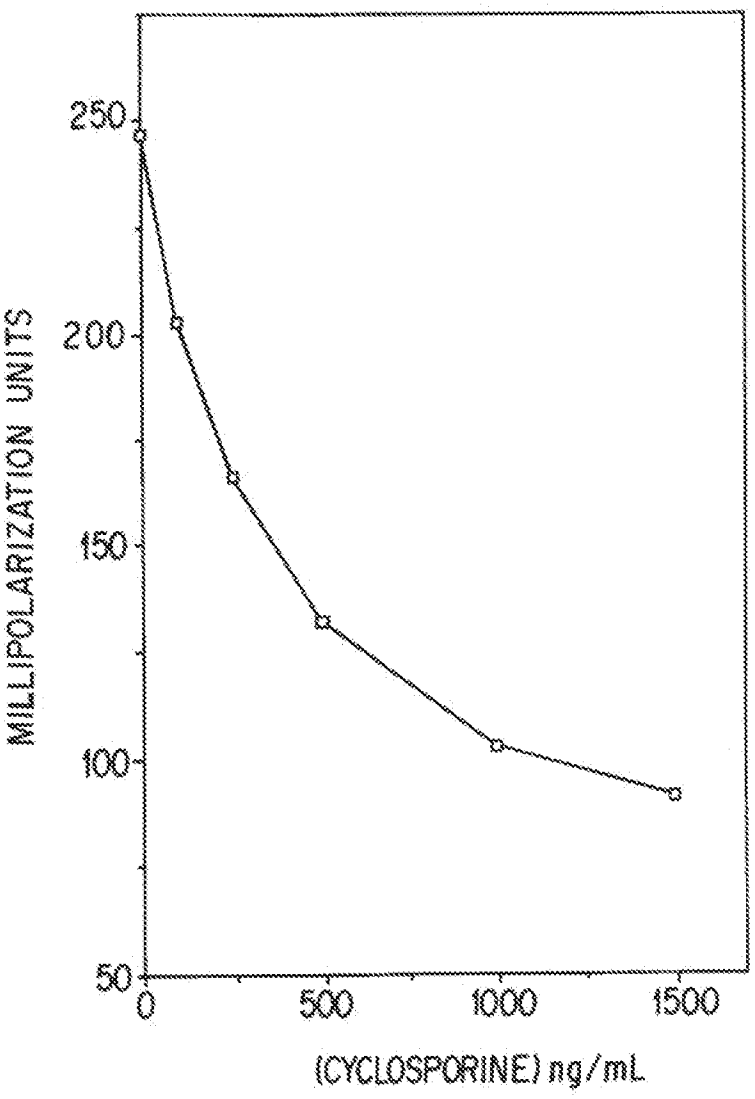
250
200
150
100
50
MILLIPOLARIZATION UNITS
0
500
1000
1500
(CYCLOSPORINE) ng/mL

FIG. 1

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 547 229 A1**

(12) **EUROPEAN PATENT APPLICATION published in accordance with Art. 158(3) EPC**

(21) Application number: 92911973.3

(51) Int. Cl.5: A61K 37/02, A61K 9/06

(22) Date of filing: 22.06.92

(86) International application number:
PCT/JP92/00798

(87) International publication number:
WO 93/00106 (07.01.93 93/02)

(30) Priority: 27.06.91 JP 252696/91
17.01.92 JP 27396/92
10.03.92 JP 108089/92

(43) Date of publication of application:
23.06.93 Bulletin 93/25

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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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.

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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, 155-158 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	38	38
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 sorbitol 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% dinitrofurobenzene (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]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] A61K37/02, 9/06

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: [14]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
September 14, 1992 (14. 09. 92)	October 6, 1992 (06. 10. 92)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

Form PCT/ISA/210 (second sheet) (January 1985)

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 760 237 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
05.03.1997 Bulletin 1997/10

(51) Int. Cl.[6]: **A61K 9/107**, A61K 38/13

(21) Application number: **95306022.5**

(22) Date of filing: **30.08.1995**

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI NL PT SE

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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.

EP 0 760 237 A1

Printed by Rank Xerox (UK) Business Services
2.13.16/3.4

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
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

EPO FORM 1503 03.82 (P04C01)

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 448 856 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 90303231.6

(51) Int. Cl.5: A61K 31/535, A61K 9/06

(22) Date of filing: 27.03.90

(43) Date of publication of application:
02.10.91 Bulletin 91/40

(84) Designated Contracting States:
DE ES FR IT NL

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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 8; page 9, lines 1-7 *	1,2,6	A 61 K 31/535 A 61 K 9/06
X	GB-A-1 258 502 (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
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A: technological background
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D: document cited in the application
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&: member of the same patent family, corresponding document

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : **0 480 690 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **91309243.3**

(22) Date of filing : **09.10.91**

(51) Int. Cl.[5] : **A61K 9/107**, A61K 9/06, A61K 31/415

(30) Priority : **10.10.90 US 595337**

(43) Date of publication of application :
15.04.92 Bulletin 92/16

(84) Designated Contracting States :
CH ES FR GB LI

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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, 1989, 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 pharmacolocically 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), polyoxyethylene 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 (64.08:1 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 80) 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_4 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 Na2HPO4 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 P-127	6%	0.035
	Tween 80	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. Cavalic, 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=0.9998 (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	3	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 6, 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 5 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)

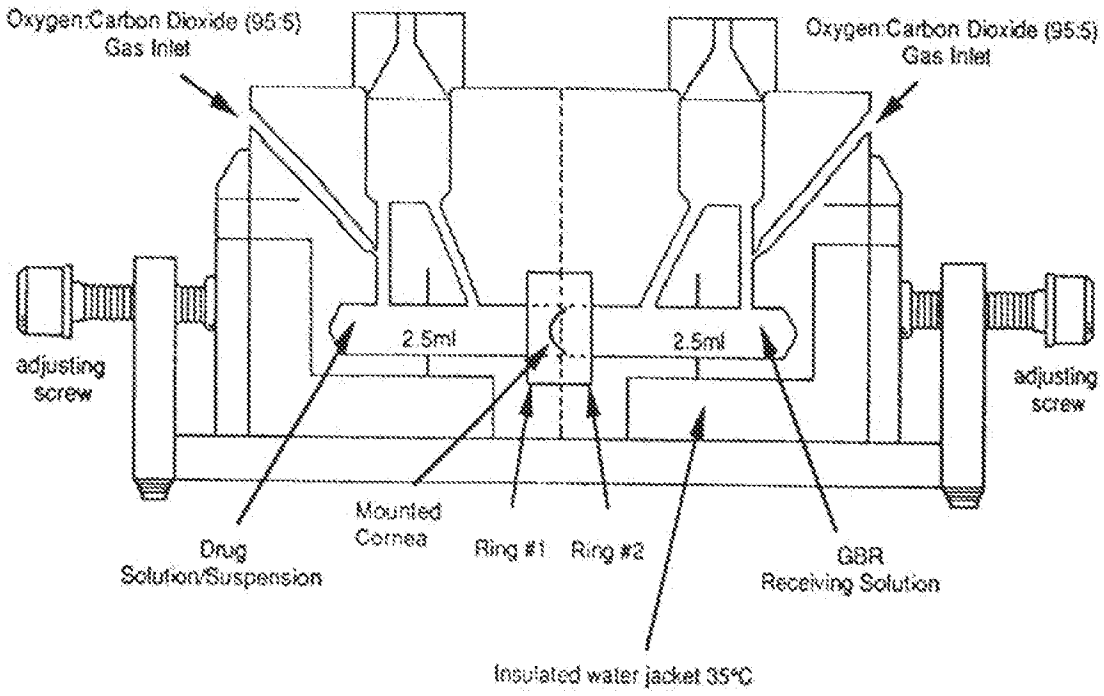

Figure 1.

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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.

CATEGORY OF CITED DOCUMENTS

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/13, 9/00

A1

(11) International Publication Number: **WO 95/31211**

(43) International Publication Date: 23 November 1995 (23.11.95)

(21) International Application Number: PCT/US95/06302

(22) International Filing Date: 17 May 1995 (17.05.95)

(30) Priority Data:
08/243,279 17 May 1994 (17.05.94) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/243,279 (CIP)
Filed on 17 May 1994 (17.05.94)

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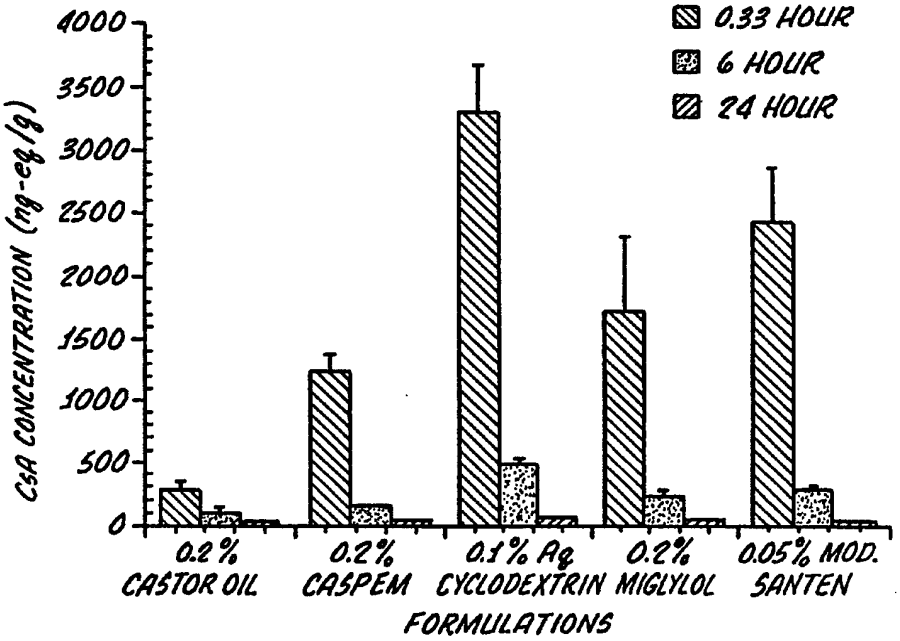
(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LACRIMAL GLAND SPECIFIC EMULSIONS FOR TOPICAL APPLICATION TO OCULAR TISSUE



(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.

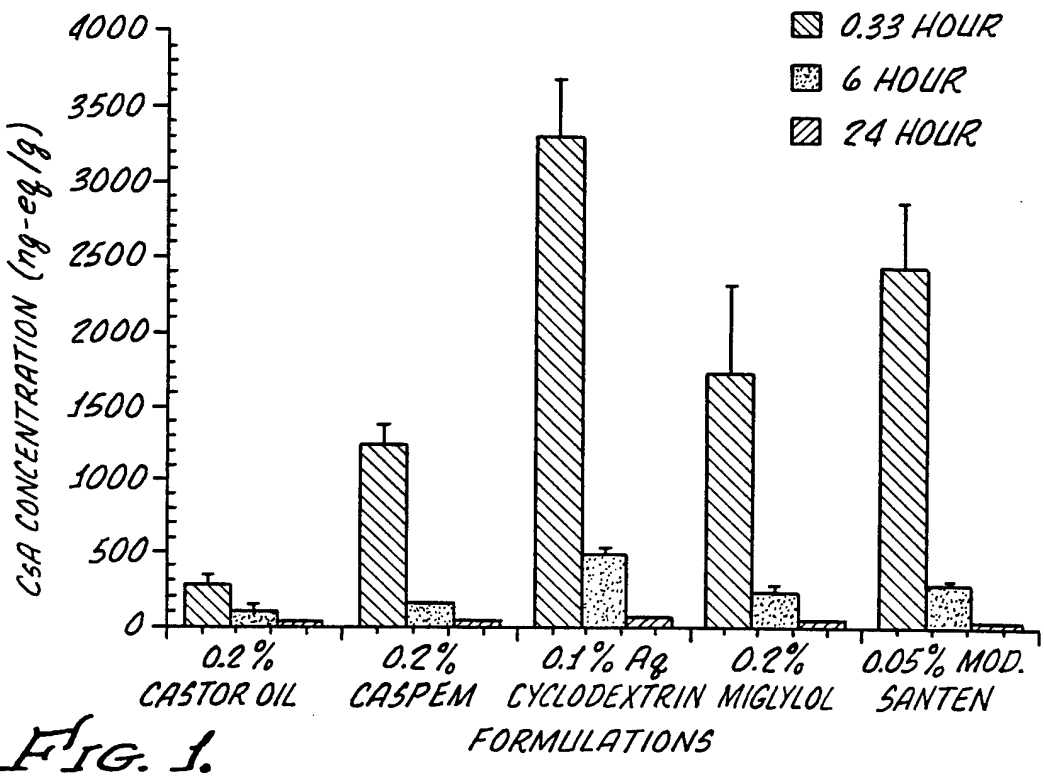
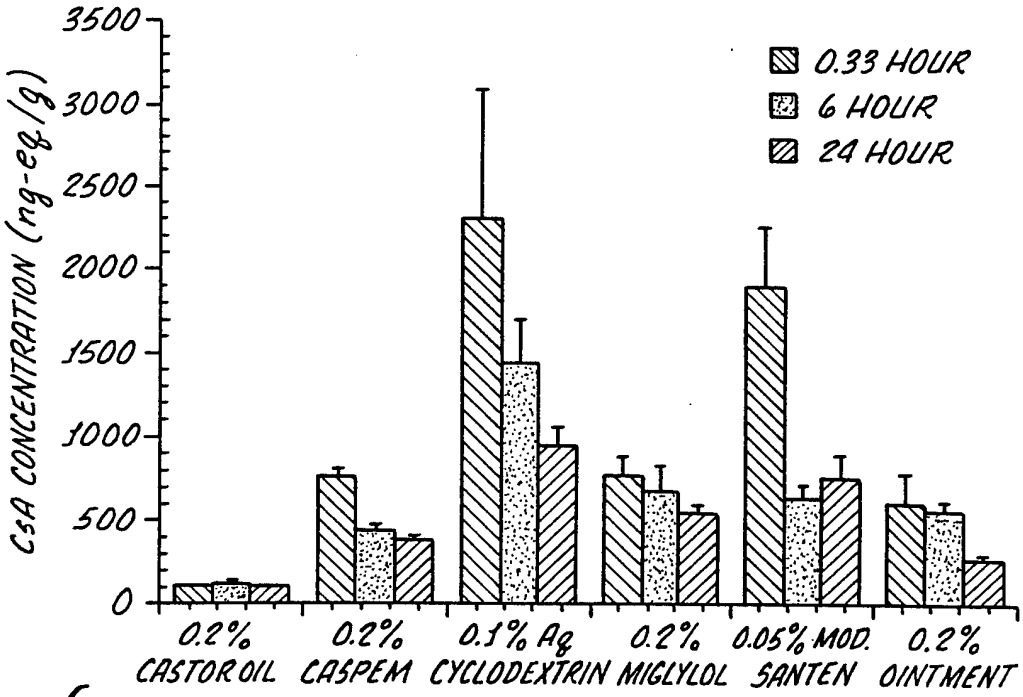

0.33 HOUR
6 HOUR
24 HOUR
CsA CONCENTRATION (ng-eq/g)
4000
3500
3000
2500
2000
1500
1000
500
0
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
FORMULATIONS

FIG. 1.


0.33 HOUR
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CsA CONCENTRATION (ng-eq/g)
3500
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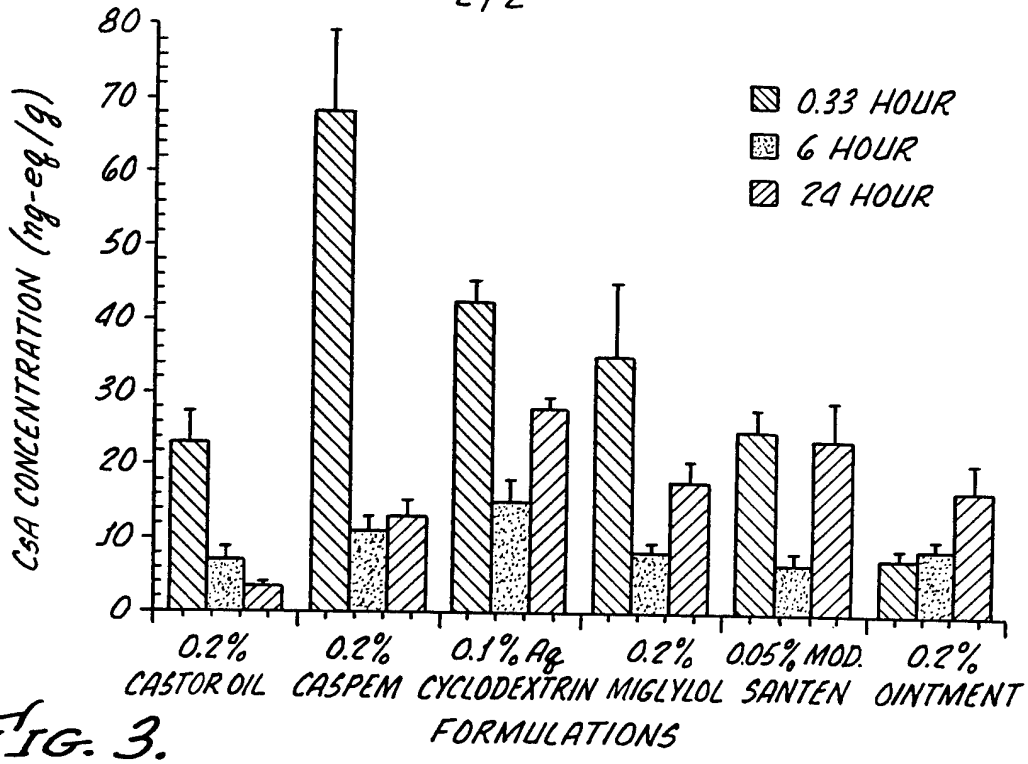
0
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

FIG. 2.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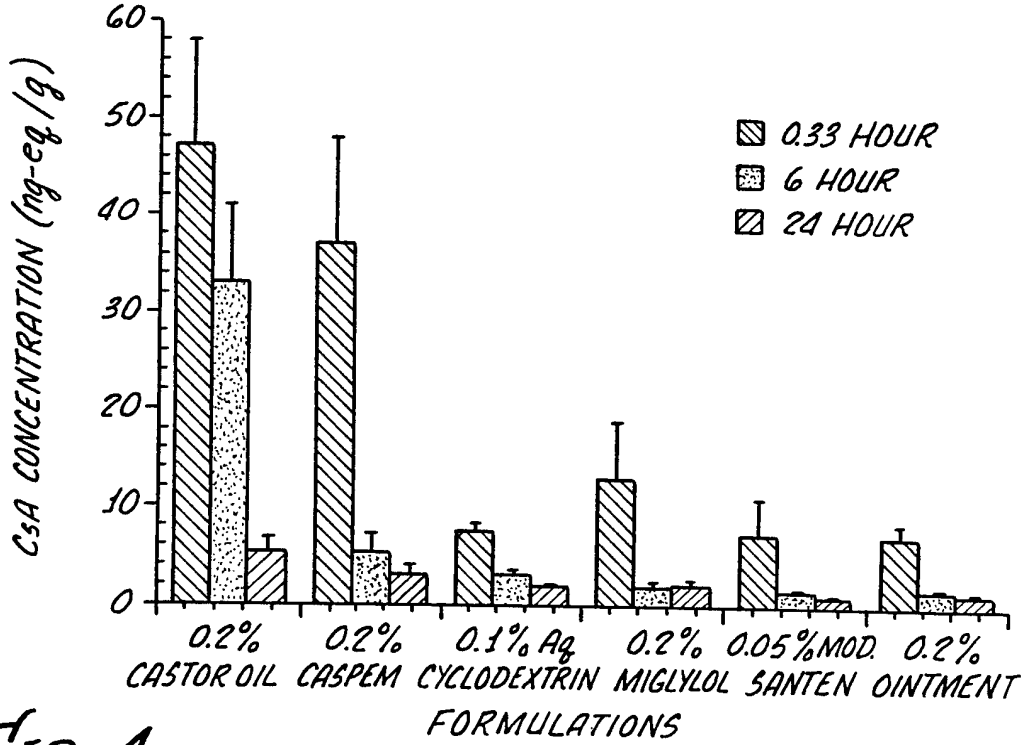
CsA CONCENTRATION (ng-eq/g)
0.33 HOUR
6 HOUR
24 HOUR
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

FIG. 3.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CsA CONCENTRATION (ng-eq/g)
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24 HOUR
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

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

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"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

1

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 1995	26.09.95

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	Ventura Amat, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ..al Application No
PCT/US 95/06302

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2228198	22-08-90	BE-A-	1005236	08-06-93
		CH-A-	680650	15-10-92
		DE-A-	4005190	23-08-90
		FR-A,B	2643262	24-08-90
		IT-B-	1240765	17-12-93
		JP-A-	2255623	16-10-90
		JP-B-	6011703	16-02-94
WO-A-8901772	09-03-89	US-A-	4839342	13-06-89
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		AU-B-	2421388	31-03-89
		DE-D-	3851152	22-09-94
		DE-T-	3851152	26-01-95
		EP-A-	0391909	17-10-90
		GR-B-	1000558	26-08-92
		JP-B-	6067850	31-08-94
		US-A-	5411952	02-05-95
		CA-A-	1335566	16-05-95

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/14, 9/16, 9/20, 9/48, 31/20, 9/107, 38/00 | A1

(11) International Publication Number: **WO 00/00179**

(43) International Publication Date: 6 January 2000 (06.01.00)

(21) International Application Number: PCT/KR99/00341

(22) International Filing Date: 28 June 1999 (28.06.99)

(30) Priority Data:

1998/24563	27 June 1998 (27.06.98)	KR
1999/24437	26 June 1999 (26.06.99)	KR

(81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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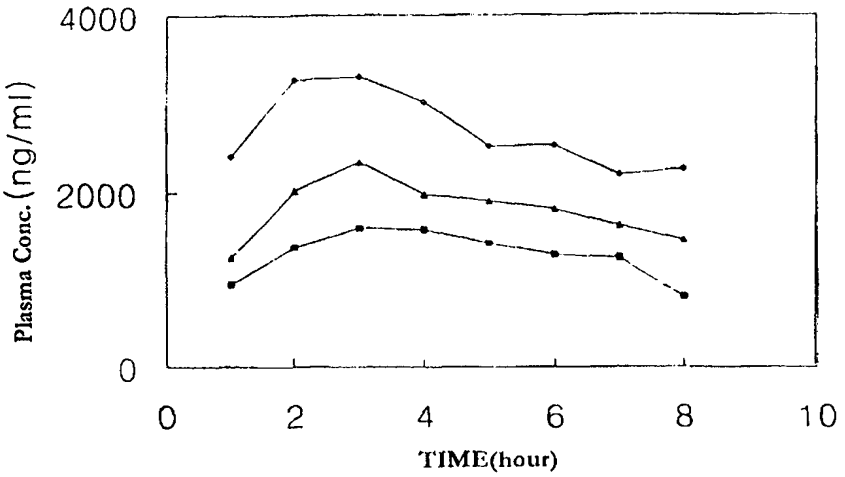
(54) Title: SOLID DISPERSED PREPARATION OF POORLY WATER–SOLUBLE DRUG CONTAINING OIL, FATTY ACID OR MIXTURES THEREOF



(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_3; 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

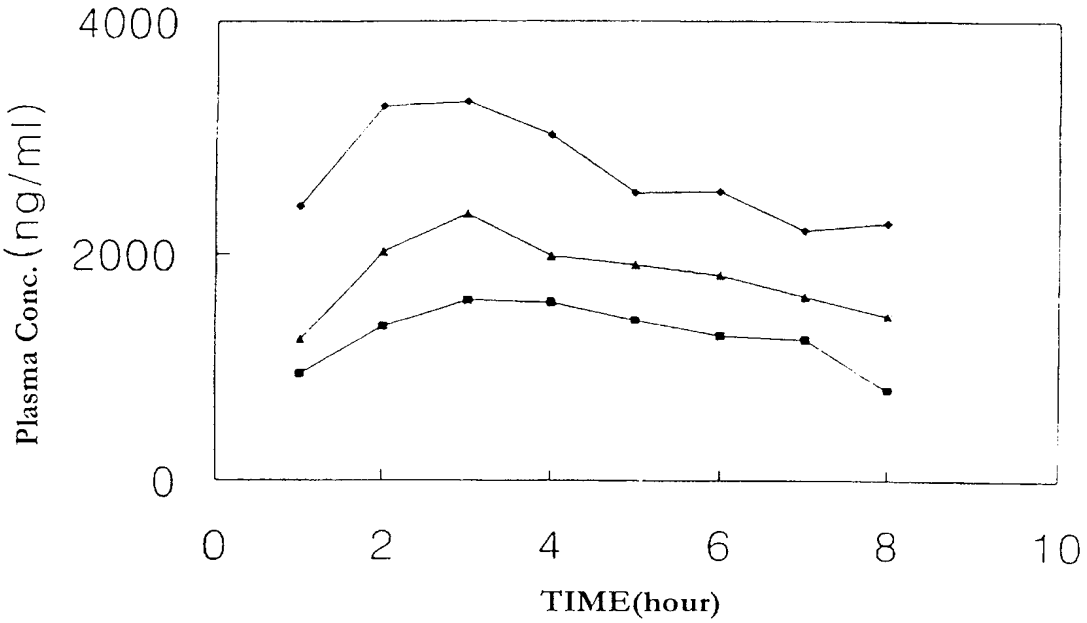
from the group comprising ketoconazole, itraconazole and its derivatives, cyclosporine, cisapride, acetaminophen, aspirin, acetylsalicylic acid, indomethacin, naproxen, warfarin, papaverine, thiabendazole, miconazole, cinnarizine, doxorubicin, omeprazole, cholecalciferol, melphalan, nifedipine, digoxin, 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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2000
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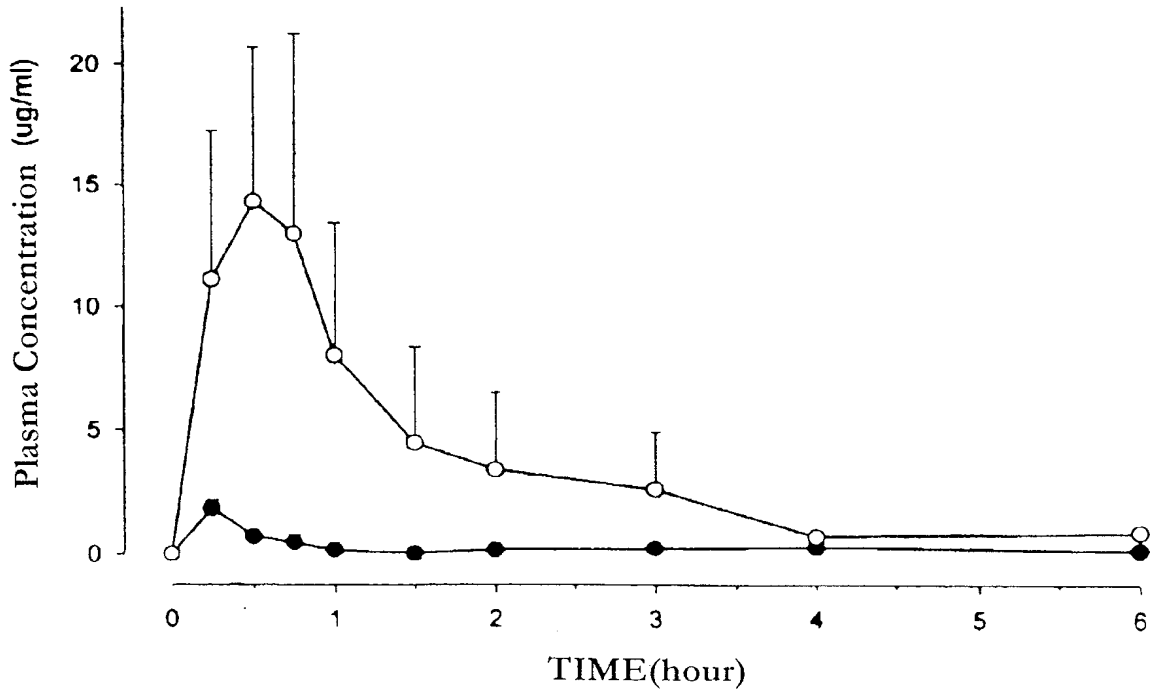
Plasma Conc. (ng/ml)
0
2
4
6
8
10
TIME(hour)

FIG. 2


20
15
10
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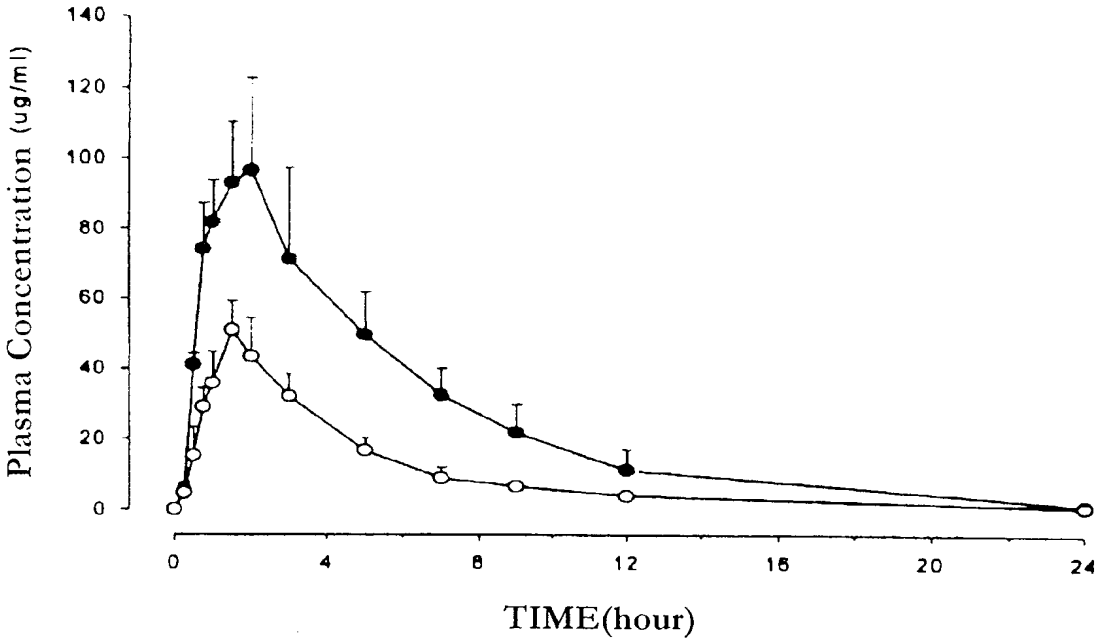
0
Plasma Concentration (ug/ml)
0
1
2
3
4
5
6
TIME(hour)

FIG. 3


Plasma Concentration (ug/ml)
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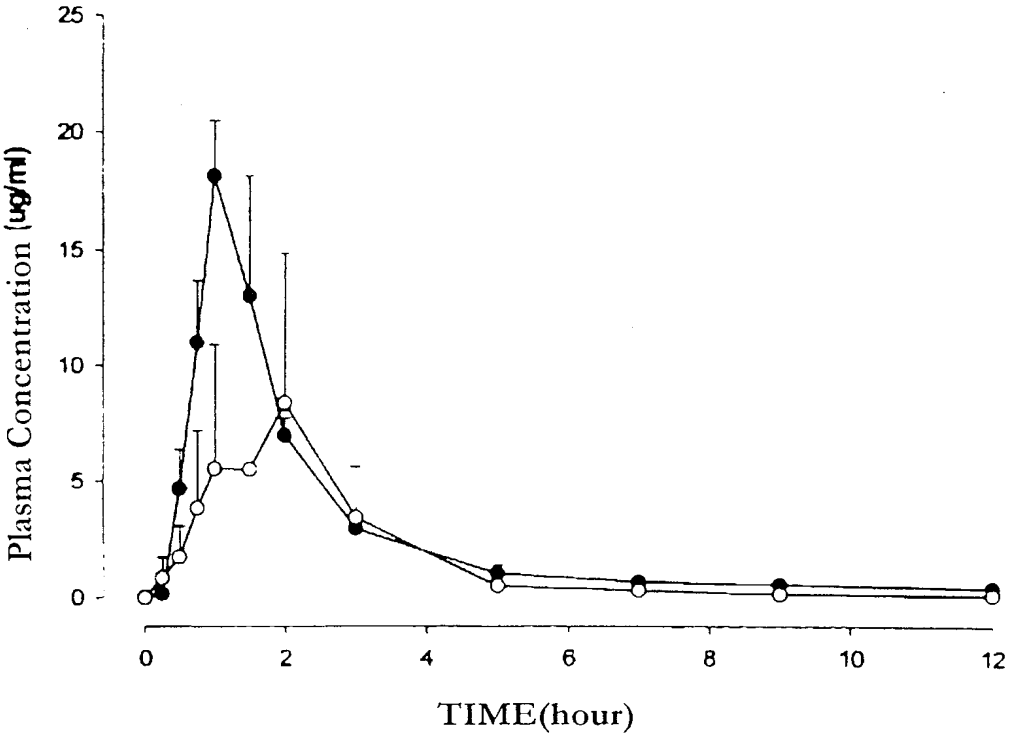
120
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME(hour)

FIG. 4


Plasma Concentration (ug/ml)
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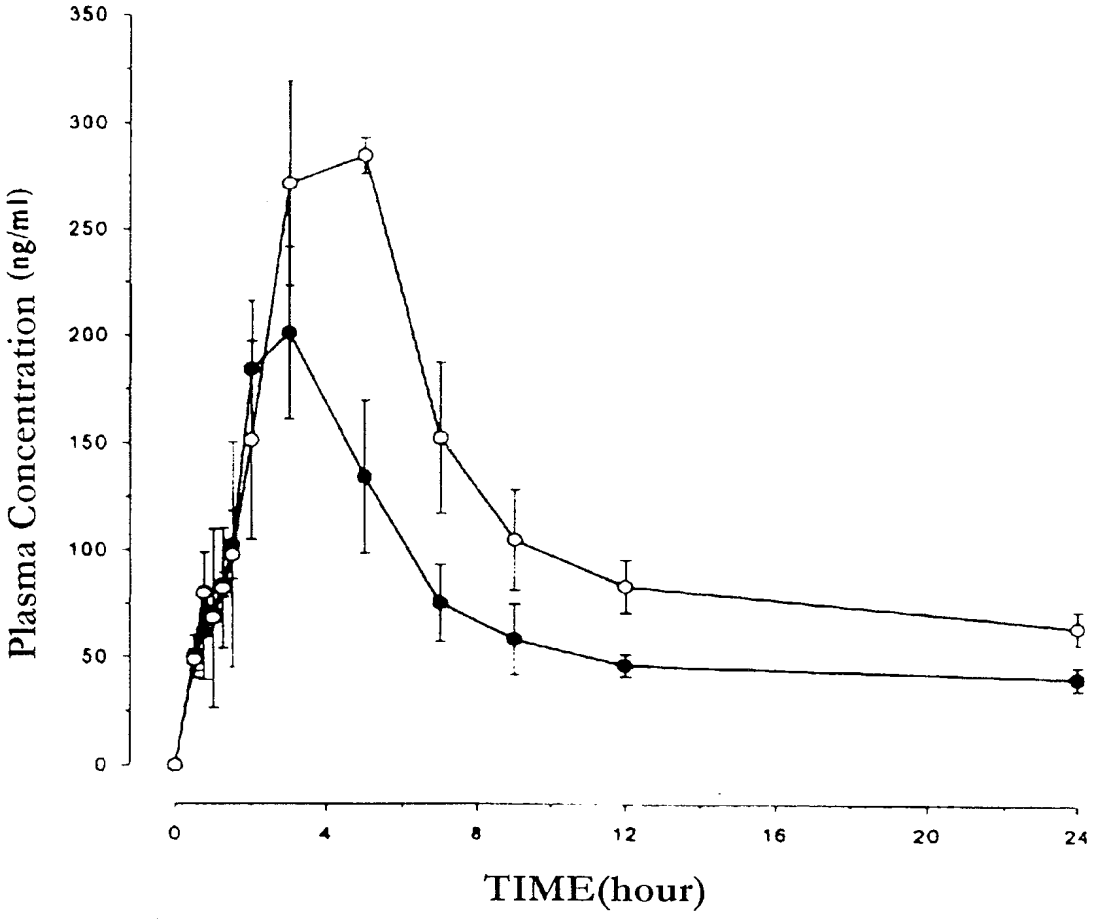
20
15
10
5
0
0
2
4
6
8
10
12
TIME(hour)

FIG. 5


Plasma Concentration (ng/ml)
350
300
250
200
150
100
50
0
0
4
8
12
16
20
24
TIME(hour)

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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„&" document member of the same patent family

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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Information on patent family members

International application No.
PCT/KR 99/00341

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Form PCT/ISA/210 (patent family annex) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00341

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Form PCT/ISA/210 (patent family annex) (July 1998)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32142 A1

(51) International Patent Classification[7]: A61K 9/107, 9/14, 9/48

(21) International Application Number: PCT/GB00/04143

(22) International Filing Date: 27 October 2000 (27.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/IN99/00062 2 November 1999 (02.11.1999) IN
0012816.5 25 May 2000 (25.05.2000) GB

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/32142 A1

(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