cyclosporins, e.g. cyclosporin A. dihydrocyclosporin C, cyclosporin D and dihydrocyclosporin D. It is also useful with other water-insoluble substances such as, for example, water-insoluble peptides, or water-insoluble antimicrobial or antineoplastic substances. Examples include desmopresin, calcitonin, insulin, lenprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol, etoposide or mixtures thereof.

In the compositions of the invention, component (a) is in solution in component (b). Component (b) can be a propylene glycol ester of a fatty acid or a mixture of any two or more such esters. The fatty acids may optionally be derived from a vegetable oil and are preferably C_8 - C_{20} residues. Particular preferred compounds are propylene glycol monocaprylate (Caprgol 90) and propylene glycol monolaurate (Lauroglycol 90). We prefer to formulate the composition such that the weight ratio of component (a) to component (b) is from about 1:1 to about 1:10 but ratios outside this range can be used if desired.

These compounds, which increase the bioavailability of the active material can be used alone or in combination with one or more of the glycerides described in EP 0760237. For example, oleoyl macrogol-6 glycerides (Labrafil M 1944 CS), linoleoyl macrogol-6 glycerides (Labrafil M 2125 CS), and caprylocaproyl macrogol-8 glycerides (Labrasol) are particularly preferred compounds for use with the oils employed in the present invention.

Component (c) is a surfactant to provide the preconcentrate mixture and, where employed, the fully formed microemulsion with stability. Those skilled in the art will be aware of many surfactants which can be used, but we prefer to use polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene sorbitan monostearate. If desired, the surfactant can be mixed with a phospholipid, such as lecithin. We prefer to use a weight ratio of component (a) to surfactant of about 1:1 to about 1:50, but ratios outside this range can also be employed if desired. When a phospholipid is included in the

composition, we prefer to use a weight ratio of component (a) to phospholipid of about 1:05 to about 1:5.0, but, again, other ratios can be used.

In the case where the composition of the invention is provided as a microemulsion, component (d) is a hydrophilic phase. The preferred material is propylene glycol or diethylene glycol monoethyl ether (transcutol) but other substances can be used. Ethanol cannot be present. Water can of course also be present but it is not preferred. Despite the use of propylene glycol, component (a) remains wholly dissolved in the oil phase (component (b)).

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

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

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

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

Component	General	Usual	Preferred
Active pharmaceutical	1-12%	2.5-10%	7 -1 0%
Oil phase	20-80%	30-60%	25-40%
Surfactant	20-40%	25-60%	40-50%
Hydrophilic phase	10-60%	20-50%	25-30%

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

In the case where the composition of the present invention is provided as a blend of preconcentrate and solid carrier, component (e) is employed instead of component (d). Preferred solid carriers include colloidal silicon dioxide and polyvinyl pyrrolidone (cross Povidone) but other suitable inert solid substances can also be used, as will be clear to those skilled in the art. Typically, the solid carrier will be in the form of a dry powder. Generally, the preconcentrate mixture (comprising active material, oil and surfactant) is simply blended with the solid material such that the oily preconcentrate is absorbed by the material. Preferably, the blended mixture is provided in the form of a free-flowing powder. Such a powder can then be easily coated, for example, into a hard gelatin capsule or, alternatively, compressed into tablets, for instance. The technique of absorbing an oily phase (in this case an oily preconcentrate) on to a solid phase such as colloidal silicon dioxide followed by formulation into a final dosage form is a technique well known by those skilled in the art of formulation, so further details are considered unnecessary.

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

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

Examples 1-3

Examples of compositions comprising a blend of preconcentrate and solid carrier are:

Example 1

Imusporin-25

Component	**mg/capsule**
Cyclosporin USP	25
Glyceryl Monolinoleate (Maisine 33-1)	17.25
Propylene glycol monocaprylate (Capryol 90)	17.25
Polyoxyl 35 Castor Oil NF (Cremophor EL)	50.00
Colloidal silicon dioxide	52.50
Crospovidone USP (PVP CL-M)	13.00
Net Fill Wt/cap (mg)	175.00

Example 2

Imusporin-50

Component	**mg/capsule**
Cyclosporin USP	50.00
Glyceryl Monolinoleate (Maisine 33-1)	34.50
Propylene glycol monocaprylate (Capryol 90)	34.50
Polyoxyl 35 Castor Oil NF (Cremophor EL)	100.00
Colloidal silicon dioxide	105.00
Crospovidone USP (PVP CL-M)	26.00
Net Fill Wt/cap (mg)	350.00

Example 3

Imusporin-100

Component	**mg/capsule**
Cyclosporin USP	100.00
Glyceryl Monolinoleate (Maisine 33-1)	69.00
Propylene glycol monocaprylate (Capryol 90)	69.00
Polyoxyl 35 Castor Oil NF (Cremophor EL)	200.00
Colloidal silicon dioxide	210.00
Crospovidone USP (PVP CL-M)	52.00
Net Fill Wt/cap (mg)	700.00

The blended preparations were made as follows:

1 Mix Maisine 35-1, Capryol 90 and Cremophor EL in a clean jacketed vessel.

2 Add Cyclosporin to the above vessel under stirring, continue stirring for about 70-75 mins. If required, heat the blend to not more than 50°C till the drug dissolves completely.

4 Cool the above blend to room temperature and strain through 150#.

5 Sift Aerosil and Crospovidone through 20# and 40# respectively. Mix in a suitable mixer.

6 Adsorb the above blend (step 4) over the mixture of Aerosil and Crospovidone.

7 Pass the powder blend of Cyclosporin through 20#.

8 Fill this blend in hard gelatin capsules or compressed with tablets.

The blends were then either fill into hard gelatin capsules or compressed into tablets.

Examples 4 - 8

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

(a) dissolve the cyclosporin A in the mixture of oils with slight warming and under stirring to obtain a clear yellow liquid. Confirm the complete dissolution of the drug by microscopy.

(b) add the surfactant with stirring.

(c) add the hydrophilic phase with stirring

(d) add the alpho tocopherol and mix thoroughly.

Example 4

Preparation of microemulsion for administration in Soft Gelatin capsules:

Component	**mg/capsule**
Capryol 90	130
Castor oil	130
Polyoxyl-40 hydrogenated	400
Castor oil	-
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 5

Preparation of microemulsion for administration as oral solution:

Component	**mg/capsule**
Capryol 90	150
Maisine	125

Polysorbate-80 (Tween 80)	425
α-tocopherol	10
Transcutol	225
Cyclosporin A	100

Example 6

Preparation of microemulsion for administration as oral solution

Component	**mg/capsule**
Capryol 90	275
Polyoxyl-40 hydrogenated castor oil	425
α-tocopherol	10
Propylene glycol	225
Cyclosporin A	100

Example 7

Preparation of microemulsion for administration as oral solution:

Component	**%**
Capryol 90	130
Lauroglycol 90	130
Polysorbate 80 (Tween 80)	400
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 8

Preparation of microemulsion for administration as oral solution:

Component	mg/capsule
Capryol 90	14
Maisine	15
Polyoxyl-40 hydrogenated castor oil	45
α-tocopherol	1
Transcutol	25
Cyclosporin A	10

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

The compositions described in Examples 4 to 8 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C). Ref 40°C-80% RH and 45°C, after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Neoral capsules containing 100mg cyclosporin A per capsule was also examined. It was observed from the above examination that the stability of solutions prepared according to the process of invention did not differ from the stability of the commercially available composition.

CLAIMS:

1. A pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, which composition is substantially free from ethanol and comprises:

a) a water-insoluble pharmaceutically active material;

b) one or more propylene glycol esters of a fatty acid;

c) surfactant; and either

d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or

e) a solid carrier.

2. A composition according to claim 1, which composition is a microemulsion comprising components (a), (b), (c) and (d).

3. A composition according to claim 1, which composition is a blend of said preconcentrate and said solid carrier comprising components (a), (b), (c) and (e).

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

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

6. A composition according to any preceding claim, wherein component (b) is a propylene glycol ester of C_{12} to C_{18} fatty acids.

7. A composition according to any preceding claim, wherein said surfactant is polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate or mixtures thereof.

8. A composition according to any preceding claim, wherein component (c) further comprises a phospholipid.

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

10. A composition according to claim 8 or 9, wherein the weight ratio of component (a) to said phospholipid is from 1:0.5 to 1:5.0.

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

12. A composition according to any of claims 1-9 and containing component (e), wherein component (e) is colloidal silicon dioxide, polyvinyl pyrrolidone or a mixture thereof.

13. A soft gelatin capsule or oral administration fluid which comprises a composition as claimed in any of claims 1 to 11

14. A tablet or hard gelatin capsule which comprises a composition as claimed in any of claims 1 to 12 when in said solid form.

15. A process for making a composition according to claim 1, which comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution either with component (d) or with component (e) and component (c) if not included earlier.

16. A process according to claim 150, wherein a preconcentrate composition is mixed with component (d).

17. A process according to claim 15, wherein a preconcentrate composition is mixed with component (e).

18. A method of making a pharmaceutical composition according to any of claims 1 to 11, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c) but being free from hydrophilic phase, and then mixing the preconcentrate with the hydrophilic phase, to form said stable oil-in-water microemulsion, the composition being free from ethanol.

19. A method of making a pharmaceutical composition according to any of claims 1-12, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c), and then mixing the preconcentrate with the solid carrier, to form a solid, stable composition if preconcentrated and carrier, the composition being free from ethanol.

INTERNATIONAL SEARCH REPORT

Interr ,nal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"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

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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
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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 01/41671 A2

(54) Title: REMOVABLE GASTRIC BAND

(57) Abstract: A removable gastric band is provided which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present removable gastric band comprises an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises at least one aperture in the first zone and a button in the second zone, and where the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach. The removable gastric band can be easily paired with the use of a gastric electrostimulator and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. Such electrostimulation devices may either be incorporated into the removable gastric band or located at a distance from the removable gastric band.

REMOVABLE GASTRIC BAND

Related Application

This application claims priority from Italian Patent Application Number MI99A002641, filed December 7,1999.

Field of the Invention

The present invention relates to a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach.

Background of the Invention

Laparoscopic banding systems are available which provide for the use of an elongated main part that is placed around the stomach and closed over the stomach so as to reduce the diameter of the stomach to be able to treat the patient's obesity. Such currently available bands, however, present some drawbacks essentially due to the difficulty of application and/or removal of the gastric band. In fact, normally, the current bands' means of closing the elongated main part are almost always hard to manipulate; moreover, their connection entails the use of additional instruments and/or devices that further complicate the application and/or later removal of the gastric band for the surgeon.

Furthermore, to be able to remove the known bands, which must necessarily be done after a more or less long time interval, it is necessary to execute an additional surgical intervention and, consequently, to administer more anesthesia to the patient. The application and/or removal of the known bands also require the application of suture stitches, in addition to another intervention and more anesthesia. In particular, the bands used today are also hard to remove because they present little resistance to tissue adhesions and lack sufficient mechanical integrity to withstand tensile forces, both of which hinder their removal unless the patient is undergoing surgery.

It is desirable, therefore, to provide an improved gastric band which is both easier to implant within the patient and, when necessary, to remove from the patient.

Summary of the Invention

The present invention provides a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present invention provides a removable gastric band comprising an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach.

The present invention provides a removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The present invention also provides a method for treatment of obesity or for reducing weight in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The task proposed by the present invention is the realization of a removable gastric band that eliminates the above-noted drawbacks of the known gastric bands. Within the scope of this task, one important purpose of the invention is to realize a removable gastric band that can be removed without having to subject the patient to further intervention and, consequently, to additional general anesthesia.

Yet another purpose of the invention is to realize a removable gastric band that is easy to remove because it is highly resistant to adhesion to the tissue and has sufficient mechanical integrity to withstand tensile forces during removal. Yet another purpose of the invention is to realize a removable gastric band that can be applied without necessarily having to use suture stitches.

Yet another purpose of the invention is to realize a removable gastric band that can be applied and/or removed by the surgeon very simply and without having to use additional instruments or devices for that purpose. Another purpose of the invention is to realize a removable gastric band that is extremely easy to manipulate, so that it can be easily placed in and/or removed from the patient.

Brief Description of the Drawings

Figure 1 illustrates the gastric band according to the invention.

Figure 2 shows schematically the gastric band according to the invention being applied to the proximal tract of a patient's stomach;

Figure 3 shows the gastric band according to the invention applied to the proximal tract of the patient's stomach;

Figure 4 shows the gastric band according to the invention inflated so as to compress a portion of the patient's stomach;

Figure 5A shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band compressing the patient's stomach before the gastric band has been inflated;

Figure 5B shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band in relation to the patient's stomach after the gastric band has been inflated;

Figure 5C shows in cross-section sectional view a reinforcing member or element located within the elongated perimeter (i.e., the rib connecting the inner and outer surfaces, thereby forming an inflatable chamber or cavity) of the gastric band which reduces the tendency of the gastric band to twist around its longitudinal axis;

Figure 6A is a view of the gastric band attached to the patient's stomach with the inflation mechanism positioned to allow for inflation; and

Figure 6B is an expanded view of the inflation mechanism.

Detailed Description of the Invention

With reference to the figures described above, the removable gastric band according to the invention, indicated as a whole with reference number 1, comprises an elongated body 3 having a first or distal zone 30, a second or middle zone 32, a third or proximal zone 34, and a closure mechanism 2 for closing the elongated body 3 back upon itself so as to surround a portion, preferably the proximal tract, of the patient's stomach 4. The closure mechanism 2 preferably comprises a button 6 in the first zone 30 and a corresponding aperture 5 in the second zone 32 whereby the button 6 can fit

through the aperture 5 and fix or lock the elongated body 3 back onto to itself. Once locked into place, the gastric band 1 completely encircles and compresses a portion of the patient's stomach (see, e.g., Figures 3 and 4). Although only one aperture 5 is shown within the second zone 32, a plurality of such apertures can be provided if desired; using such a plurality of apertures allows the surgeon to more closely adjust the diameter of the encircling portion of the gastric band to the particular patient's situation and needs.

Appropriately, button 6 is suitably shaped and sized to allow it to be internally introduced into aperture 5, as well as to close, in an extremely simple but secure manner, the elongated main part 3 around stomach 4 and keep it in place. Although the button 6 and aperture 5 are preferably circular as shown in Figure 1, other shapes can be used so long as they provide the desired closing/locking action. Elongated body 3 presents at least an inner surface 7 and an outer surface 8 as more clearly shown in Figure 5A (deflated state) and Figure 5B (inflated state). Preferably, the elongated body 3 has an inflatable portion or internal cavity 9 formed by inner surface 7, outer surface 8, and ribs, welds, or closures 22 at the edges of the elongated body 3. Ribs 22 essentially form a closed space or internal cavity 9 in combination with the inner and outer surfaces 7 and 8 (see, e.g., Figure 5B). Such an inflatable member allows the elongated body 3 to be expanded when a physiological inflation medium (i.e., liquid or gas) 9 is introduced between inner surface 7 and outer surface 8. (Both the inflatable portion or internal cavity and the inflation medium, which effectively defines the size of the internal cavity, are referred to by common reference number 9 in the figures.) Preferably, button 6 is fluid-dynamically connected to inner surface 7 of elongated body 3 in such a way that, as the latter inflates, button 6 also inflates, as can be seen, for example, in Figure 3; this provides a more secure locking of the elongated body back upon itself. Preferably, the inner surface 7 is more easily expandable relative to outer surface 8 so that inflation of the elongated body 3 allows further compression, and thus more control of the compression, of the stomach. Generally, therefore, it is preferred that outer surface 8

undergoes little, if any, expansion when the physiological inflation liquid or gas 9 is introduced between inner surface 7 and outer surface 8. Compression of the stomach using the gastric band of the present invention allows for a reduction of the stomach volume as desired. The degree of compression can be modified as desired throughout the course of treatment by adding or removing inflation medium 9.

Furthermore, button 6 and aperture 5 are preferably sized relative to one another that once button 6 is passed through aperture 5 and inflated, the closure mechanism is securely activated but, once button 6 is deflated, the closure mechanism can easily be deactivated by simply pulling on one end of the gastric band (preferably by pulling on tube 20) to remove the gastric band from the abdomen. Furthermore, button 6 is preferably located outside of elongated main part 3 by a distance that can allow a substantial alignment of the first and second zones of elongated body 3, when the latter is closed around the stomach. Thus, when the elongated body 3 is inflated (and preferably button 6 is also inflated), there is no unsuitable and/or harmful superposition of two parts of the elongated body 3 that would provide an undesired enlargement at the zone where they are superposed. In other words, the inflatable portions of the gastric band do not overlap; such overlapping might result in undesirable and/or additional stomach compression in the area of overlap.

Preferably, button 6 is equipped with flap 10 that makes it easier to catch and insert the button 6 into aperture 5 using appropriate instruments. Flap 10 is appropriately made with no internal cavity and, therefore, is not inflatable. Flap 10 can be grasped quickly and simply by surgical endoscopic forceps 11 that is passed first through aperture 5 (see Figure 2). Once grasped, flap 10 and button 6 are pulled back through aperture 5 to lock the gastric band in place (see Figure 3).

As noted above, it is preferred that the button 6 expands at the same time as inner surface 7 of the elongated body 3. The expansion of button 6 should, however, be limited so that, once the gastric band 1 is locked firmly in place, the button 6 does not under go significant further expansion. For

example, the relative thicknesses of the walls of the button 6 and inner surface 7 can be controlled such that the inflation of the button will reach a definite value without expanding any further, independently of the inflation of inner surface 7 of elongated body 3. Thus, preferably the button 6 expands to a size sufficient to lock the closure mechanism 2 in place but not significantly larger.

The elongated body 3 is preferably designed so as to prevent or reduce the tendency of the elongated body 3 to rotate around its long axis as it is being placed in the proper position around the patient's stomach. For example, one or both of the ribs 22 at the edges of the elongated body 3 can contain stiffening elements 12 (see Figure 5C) which will reduce the tendency of the elongated body 3 to rotate or twist about its long axis without effecting the ability of the elongated body to fold back on itself and encircle the patient's stomach. Such stiffening elements 12 will reduce the tendency to twist as the gastric band is being positioned within the patient. Such stiffening or antirotation elements 12 will tend to stabilize the prosthesis and make the insertion easier. The ribs 22 at the edges of the elongated body 3 are preferably gently curved so as not to create problems either at the time of the implant or during removal by pulling of elongated body 3 from the outside; in other words, the ribs, as well as other portions of the gastric band, preferably present smooth and gently curved surfaces to allow the gastric band to slide easily around organs during implantation and removal.

The gastric band preferably has an inflation mechanism 15 comprising a reservoir 16 for receiving the inflation medium, preferably a physiological liquid or gas, for inflating both elongated body 3 and button 6. Preferably, the reservoir 16 has several concentric layers 17 to allow it to be pierced, for example with needle 18, without the inflation medium 9 being able to escape from the perforation. Preferably, reservoir 16 is constructed with multiple layers of material (preferably elastomeric or plastic materials) that, when pricked with needle 18, allows the hole to be made without skewing or leakage between the different layers 17. Such skewing or leakage would generally be mainly noticeable or chiefly accentuated during the expansion of

reservoir 16 when the inflation medium 9 would tend to leak. The external layer of reservoir 16, preferably constructed of biocompatible materials, is generally thicker than the other, internal layers and can even be rigid, since it preferably remains adjacent to the abdominal wall, more preferably within the subcutis, and presents such dimensions as to permit easy introduction through a surgical laparoscopic trocar. By maintaining the reservoir 16 near the abdominal wall, the compression of the stomach can more easily be modified as desired by addition or removal of the inflation medium 9. In some instances, it may be desired for the reservoir 16 to remain outside the abdominal wall.

The elongated body 3 can be inflated using the inflation medium introduced into the reservoir 16 using, for example, a syringe 18 as shown in Figures 6A and 6B. The elongated body is inflated until the desired degree of compression of the stomach occurs. The inflation of the gastric band is generally performed under the control of the endoscopist, who can observe, preferably using an endoscope from inside the stomach, the diameter of the gastric restriction induced by the inflation of the gastric band, particularly by inner surface 7. Preferably, essentially the entire length of the gastric band 3 encircling the stomach can be inflated using the inflation medium 9.

Reservoir 16 is preferably located in the third or proximal zone 34 of elongated body 3 and is connected to the second or middle zone 32 containing aperture 5 is present via tube 20. The length of tube 20 can be varied as needed for particular patients; preferably, tube 20 does not significantly expand when inflation medium 9 is added to the gastric band. In operation, the reservoir 16 is preferably not secured and remains in the subcutis of the abdominal wall. It may be located, using, for example, feel or ultrasound, for introduction of the inflation medium in order to inflate or deflate the gastric band. Using such a technique, the diameter of the gastric constriction provided by the gastric band can be modified or adjusted as desired. Preferably, reservoir 16 has a flap 21 which can be grasped using appropriate instruments to assist in the inflation or deflation operation.

Preferably, both the main portion of the elongated body 3 and the tube 20 have stiffening or antirotation elements 12 within the ribs 22 as shown in Figure 5C. For example, the stiffening elements 12 could be a thin steel, other metal, or other type wire that is fused into the plastic material of the rib 22. Such a stiffening element 12 reduces the tendency of the gastric band to rotate about its long axis before the closure mechanism is activated. Additionally, it makes the gastric band considerably stronger (i.e., acting as a reinforcing element); this added strength may be especially important when the gastric band is removed from the patient by pulling on the proximal end 34 from the outside. The stiffening element 12, when formed using a steel or other suitable metal wire, can also be observed using X-rays, thereby determining the exact position of the band inside the patient's abdomen. Preferably, such stiffening element 12 extends essentially the entire length of the elongated body 3 (i.e., through the first, second, and third zones, including tube 20).

When it is desired to remove the gastric band from the abdomen, it is generally preferred to remove at least a portion of the inflation medium 9 so that the closure mechanism 2 can more easily be disengaged. A significant portion of the inflation medium 9 can be removed using, for example, a syringe using essentially the same procedures as used for the initial inflation process. Alternatively, tube 20 can be cut using cutting device 11a to separate reservoir 16, as represented in Figure 3, to release inflation medium 9. Preferably, at least a portion of inflation medium 9 is removed prior to cutting tube 20 so as to minimize release of inflation medium 9 into the abdominal cavity. For this purpose, under local anesthesia, a small cutaneous incision is made in the abdominal wall to access reservoir 16, at which time tube 20 is cut and the reservoir 16 is removed from the abdominal cavity. After the closure mechanism 2 is disengaged, the gastric band 3 can be removed from the abdominal cavity by pulling on the tube 20 through the small cutaneous incision.

Preferably, the limit of expandability of inner surface 7 is linked to the limit of compressibility of the gastric walls and the two ends of the elongated

body must be blunted enough to allow sliding between the patient's tissues in the phase of removal from the abdomen. In the removal phase, the gastric band will behave as an abdominal drainage tube. Preferably, the materials of construction and the surface smoothness are such that they will impede the production of fibrotic scar adhesions, as normally occurs with drainage tubes or prostheses of silicone materials. Such a smooth surface helps to prevent tissue adhesion to the gastric band. Thus, once deflated and unbuttoned, the gastric band can be removed easily by pulling on one end through a small incision. Preferably, the gastric band will have sufficient strength to withstand the forces associated with removal by this technique.

The gastric band of the present invention can be easily paired with the use of a gastric electrostimulator 100 and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. The electrostimulator 100 may be incorporated into the design of the gastric band as shown in Figure 1 (i.e., attached to the inner surface 7) such that the electrostimulator 100 is in contact with the stomach when the gastric band is properly positioned. Alternatively, it may be separately implanted elsewhere within the abdominal cavity as shown in Figure 2 (e.g., attached to the antrum). If incorporated into the gastric band design, the electrostimulator 100 is implanted at the same time as, and held in place by, the gastric band, thereby eliminating separate attachment of the electrostimulator 100. In such a unitary design, however, the electrostimulator 100 must be removed at the same time as the gastric band. If such an electrostimulator 100 is separately placed at a distance from the gastric band, it may remain within the abdominal cavity after removal of the gastric band. The selection of the preferred location of such an electrostimulator 100 relative to the gastric band will depend largely on the particular patient's requirements and planed treatment regime. Both the electrostimulator 100 and the gastric band are preferably installed and/or removed at the same time, thereby reducing the extent of surgical intervention and anesthesia.

Conventional electrostimulation devices 100 may be used in the practice of this invention in combination with the gastric band 3. Such

devices include, for example, those described in U.S. Patent 5,423,872 (June 3, 1995) (an implantable gastric electrical stimulator at the antrum area of the stomach which generates sequential electrical pulses to stimulate the entire stomach, thereby artificially altering the natural gastric motility to prevent emptying or to slow down food transit through the stomach); U.S. Patent 5,690,691 (November 25, 1997) (a portable or implantable gastric pacemaker employing a number of electrodes along the greater curvature of the stomach for delivering phased electrical stimulation at different locations to accelerate or attenuate peristaltic movement in the gastrointestinal tract); U.S. Patent 5,836,994 (November 17, 1998) (an implantable gastric stimulator which incorporates direct sensing of the intrinsic gastric electrical activity by one or more sensors of predetermined frequency bandwidth for application or cessation of stimulation based on the amount of sensed activity); U.S. Patent 5,861,014 (January 19, 1999) (an implantable gastric stimulator for sensing abnormal electrical activity of the gastrointestinal tract so as to provide electrical stimulation for a preset time period or for the duration of the abnormal electrical activity to treat gastric rhythm abnormalities); U.S.Patent 6,041,258 (March 21, 2000) (electrostimulation device with improved handle for laparoscopic surgery); U.S. Patent Application Serial Number 09/640,201(filed August 16, 2000) (eletrostimulation device attachable to enteric or endo-abdominal tissue or viscera which is resistance to detachment); PCT Application Serial Number PCT/US00/09910 (filed April 14, 2000; Attorney Docket No. 3581/006 PCT) entitled "Gastric Stimulator Apparatus and Method for Installing" based on United States Provisional Application Serial Numbers 60/129,198 and 60/129,199 (both filed April 14, 1999); PCT Application Serial Number PCT/US00/10154 (filed April 14, 2000; Attorney Docket No. 3581/004 PCT) entitled "Gastric Stimulator Apparatus and Method for Use" based on United States Provisional Application Serial Numbers 60/129,209 (filed April 14, 1999) and 60/466,387 (filed December 17, 1999); and U.S. Provisional Patent Application Serial Number 60/235,660 (filed September 26, 2000) entitled "Method and Apparatus for Intentional Impairment of Gastric Motility and/or

Efficiency by Triggered Electrical Stimulation of the Gastric Tract with Respect to the Intrinsic Gastric Electrical Activity." All of these patents, patent applications, provisional patent applications, and/or publications are hereby incorporated by reference.

Moreover, the gastric band of the invention is of great clinical interest, especially in relation to problems inherent to prolonged permanence in the abdomen, that is, intragastric decubitus, perforation, strangulation, and the like. In practice it has been confirmed that the removable gastric band according to the invention is particularly advantageous because it can be removed without having to perform an additional surgical intervention and additional anesthesia on the patient, thanks especially to its qualities of resistance to pulling.

The invention thus conceived is susceptible to numerous modifications and variations, all falling within the scope of the inventive concept; furthermore, all of the details can be substituted with technically equivalent elements. In practice, other materials and dimensions can be used, depending on the demands and on the state of the technique.

Claims

That which is claimed is:

1. A removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

2. The removable gastric band of claim 1, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

3. The removable gastric band of claim 2, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be more securely closed around the section of the stomach when the cavity is inflated.

4. The removable gastric band of claim 3, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

5. The removable gastric band of claim 3, wherein the button has a flap for catching and easy introduction into the aperture.

6. The removable gastric band of claim 5, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

7. The removable gastric band of claim 6, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

8. The removable gastric band of claim 7, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

9. The removable gastric band of claim 8, wherein the reinforcing elements are radiopaque.

10. The removable gastric band of claim 7, wherein the reservoir has a flap for easy holding.

11. The removable gastric band of claim 8, wherein the reservoir has a flap for easy holding.

12. The removable gastric band of claim 2, wherein essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in placed around the stomach.

13. The removable gastric band of claim 7, wherein the essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in place around the stomach.

14. A method for treatment of obesity in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

15. The method of claim 14, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

16. The method of claim 15, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be

more securely closed around the section of the stomach when the cavity is inflated.

17. The removable gastric band of claim 16, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

18. The method of claim 16, wherein the button has a flap for catching and easy introduction into the aperture.

19. The method of claim 18, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

20. The method of claim 19, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

21. The method of claim 20, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

22. The method of claim 21, wherein the reinforcing elements are radiopaque.

23. The method of claim 20, wherein the reservoir has a flap for easy holding.

24. The method of claim 21, wherein the reservoir has a flap for easy holding.

25. The method of claim 15 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

26. The method of claim 25, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

27. The method of claim 25, wherein the electrostimulator is located separately from the gastric band.

28. The method of claim 20 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

29. The method of claim 28, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

30. The method of claim 28, wherein the electrostimulator is located separately from the gastric band.

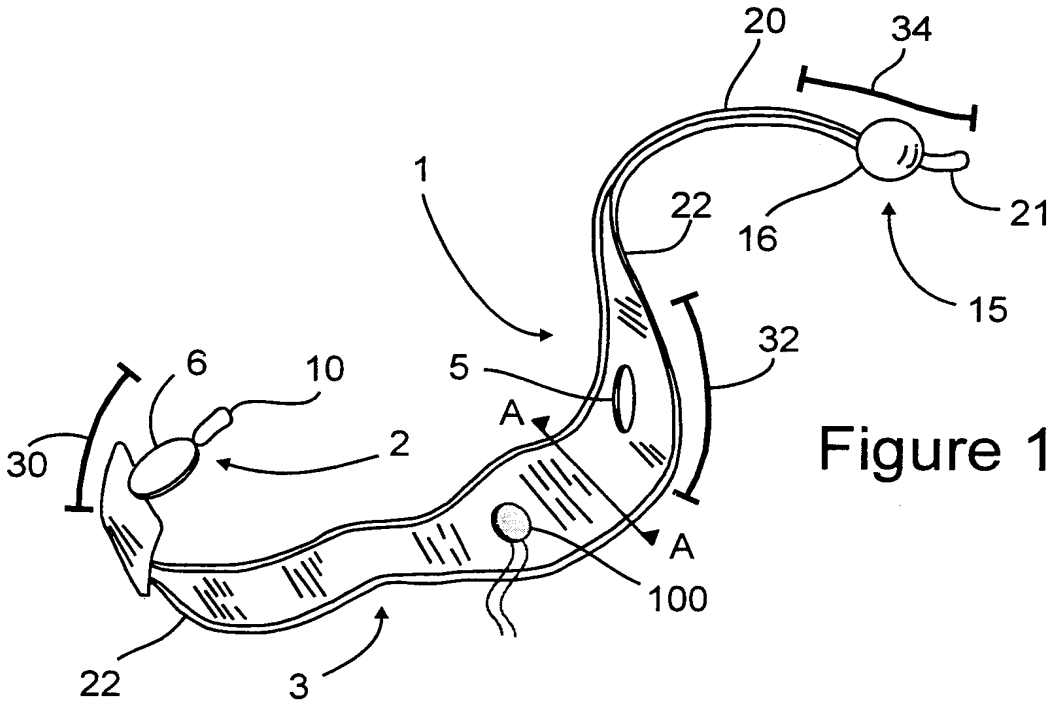


Figure 1

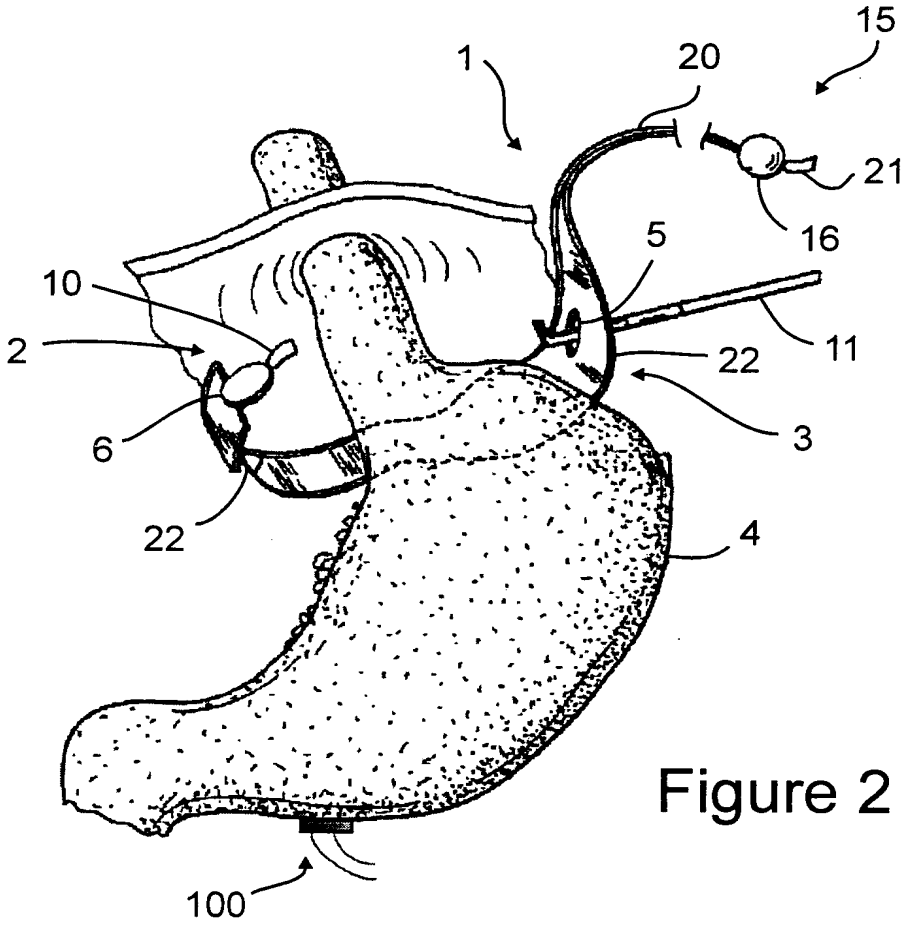
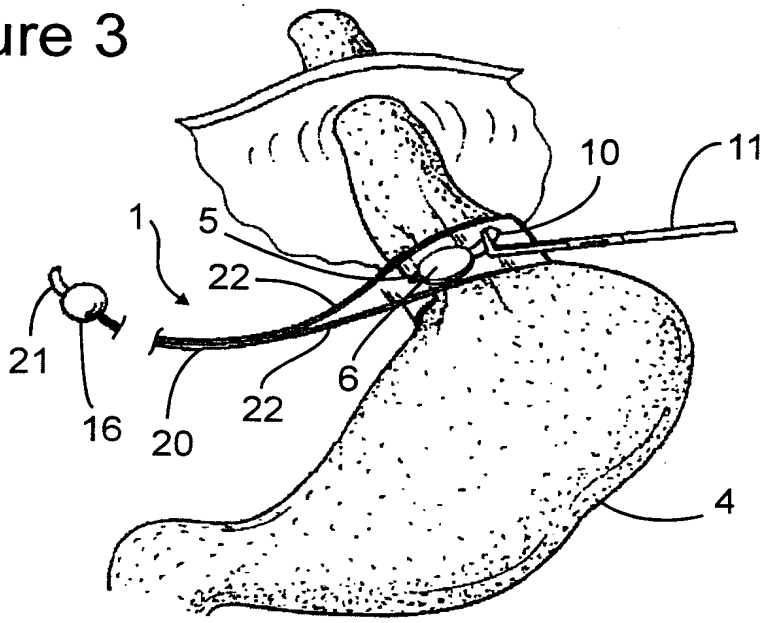


Figure 2

Figure 3



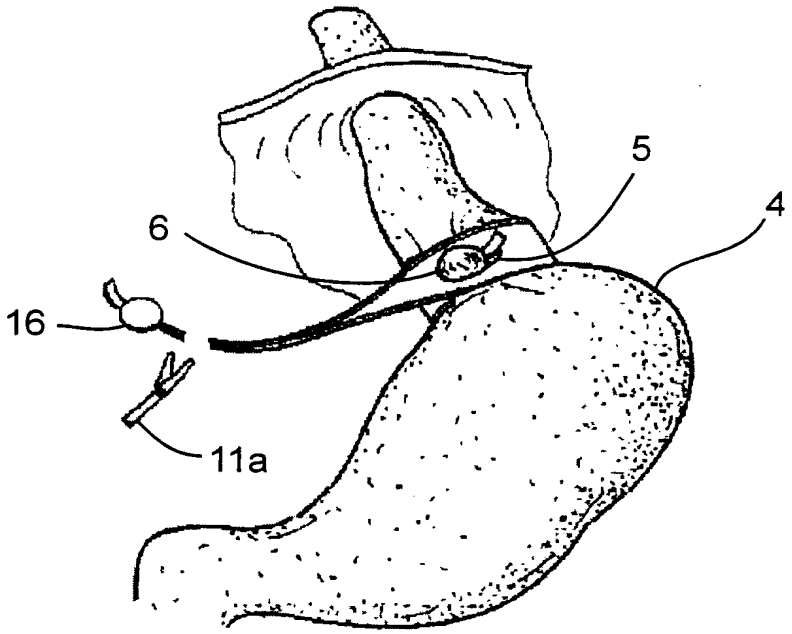


Figure 4

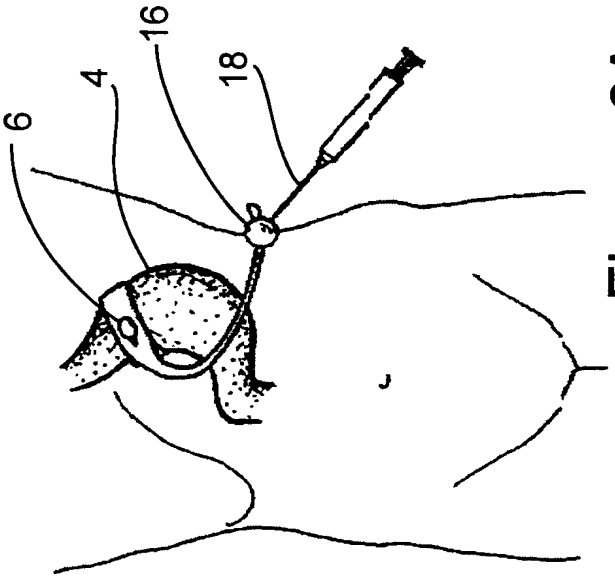


Figure 6A

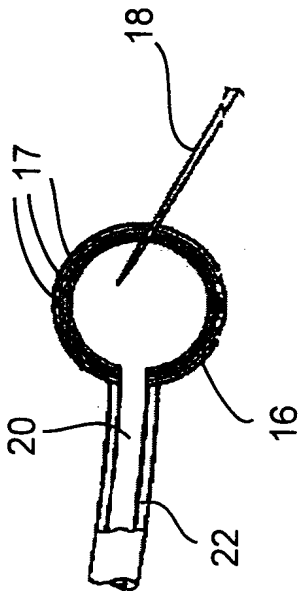


Figure 6B

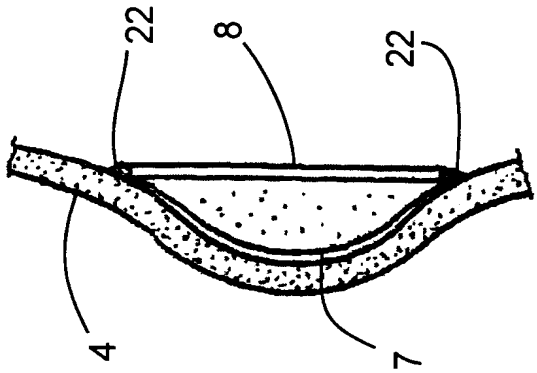


Figure 5B

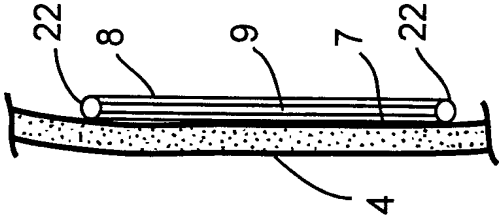


Figure 5A

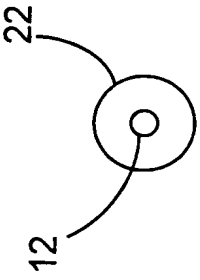


Figure 5C

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

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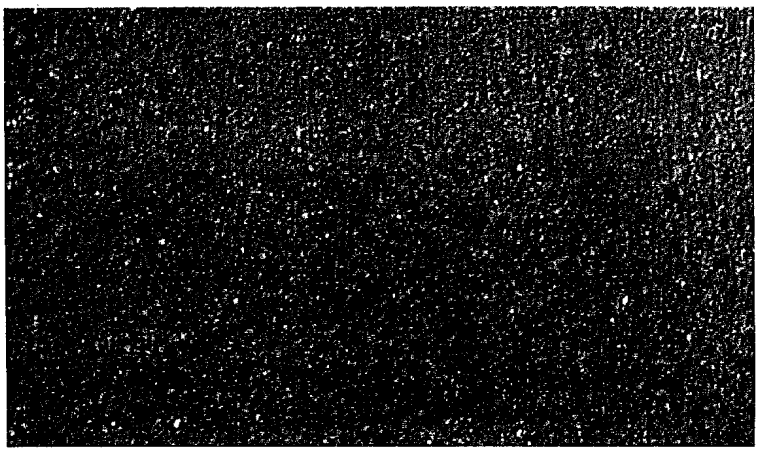
(81) Bestimmungsstaaten *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,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

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

[Fortsetzung auf der nächsten Seite]

(54) **Title:** DISPERSIONS FOR FORMULATING SLIGHTLY OR POORLY SOLUBLE ACTIVE INGREDIENTS

(54) **Bezeichnung:** DISPERSIONEN ZUR FORMULIERUNG WENIG ODER SCHWER LÖSLICHER WIRKSTOFFE



1 Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

1 OPTICAL MICROSCOPE PHOTO OF THE EMULSION CONTAINING 5mg/mL AMPHOTERICIN B. FROM EXAMPLE 19

(57) **Abstract:** The invention relates to a dispersion, comprising an oily phase and an aqueous phase in the form of an O/W emulsion or a W/O emulsion, at least one active ingredient which is slightly or poorly soluble in the oily and the aqueous phases, in addition to optionally one or more emulsifiers and/or stabilisers. The dispersion is devoid of toxicologically questionable organic solvents and contains a dissolved quantity of said active ingredient that is higher than the additive quantity obtained by its maximum solubility in both the oily and the aqueous phase of the emulsion.

[Fortsetzung auf der nächsten Seite]

WO 02/09667 A2

Erklärung gemäß Regel 4.17:

— *Erfindererklärung (Regel 4.17 Ziffer iv) nur für US*

Veröffentlicht:

— *ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts*

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(57) Zusammenfassung: Die Erfindung betrifft eine Dispersion, die eine ölige Phase und eine wässrige Phase in Form einer O/W-Emulsion oder W/O-Emulsion, mindestens einen in der öligen und der wässrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfasst, wobei die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wässrigen Phase der Emulsion ergibt.

Dispersionen zur Formulierung wenig oder schwer löslicher Wirkstoffe

Die Erfindung betrifft Dispersionen, die eine ölige Phase, eine wäßrige Phase und in diesen beiden Phasen wenig löslichen, schwer löslichen bis zu unlöslichen Arzneimittelwirkstoff umfassen.

Wirkstoffe mit geringer Löslichkeit haben sehr oft das Problem einer unzureichenden Bioverfügbarkeit. Der generelle Lösungsansatz für dieses Problem ist die Erhöhung der Löslichkeit dieser Wirkstoffe. Beispiele hierfür sind die Lösungsvermittlung über Solubilisation, Bildung von Einschlußverbindungen (z. B. mit Cyclodextrinen) sowie die Verwendung von Lösungsmittelgemischen (K. H. Bauer, K.-H. Frömming, C. Führer, Pharmazeutische Technologie, Georg Thieme Verlag Stuttgart, 1991). Für viele Wirkstoffe führt dies jedoch nicht zu einer ausreichenden Erhöhung der Löslichkeit, insbesondere wenn Wirkstoffe gleichzeitig schwerlöslich in wäßrigen Medien und gleichzeitig schwerlöslich in organischen Medien sind. Hier scheiden z. B. Lösungsmittelgemische als Lösung für das Problem aus. Alternativ können gering wasserlösliche Wirkstoffe in Ölen gelöst werden, eine O/W-Emulsion hergestellt und diese dann oral oder parenteral (in der Regel i.v.) appliziert werden. Sehr viele Wirkstoffe, insbesondere Wirkstoffe mit gleichzeitig geringer Löslichkeit in wäßrigen und organischen Medien, sind jedoch nicht ausreichend in Ölen löslich. Nicht ausreichend bedeutet, daß aufgrund zu geringer Löslichkeit bei erforderlicher Dosis das zu applizierende Volumen der Emulsion zu groß wird.

In Wasser und in Ölen gering lösliche Wirkstoffe wie Amphotericin B können trotzdem in Emulsionen eingearbeitet werden (Seki et al. US 5 534 502). Um dies zu erreichen müssen jedoch zusätzliche organische Lösungsmittel eingesetzt werden. Diese Lösungsmittel müssen dann in Zwischenschritten der Emulsionsherstellung oder dem Produkt wieder entzogen werden (Davis, Washington, EP 0 296 845 A1) wobei jedoch ein gewisser Restlösungsmittelgehalt im Produkt verbleibt. Zusätzlich ist diese Herstellung sehr zeitaufwendig und kostenintensiv, so daß Produkte basierend auf dieser Technologie praktisch auf dem Markt nicht vertreten sind. Eine alternative Methode ist die Einlagerung von derartigen Substanzen wie Amphotericin B in die Phospholipid-Doppelmembran von Liposomen, Handelsprodukt ist beispielsweise Ambisome® (Janknegt et al., Liposomal and lipid formulations of amphotericin B, Clin. Pharmacokinet., 23, 279-291 [1992]). Nachteilig ist aber auch hier die sehr teure Herstellung, so daß es in der Regel nur in Notfällen eingesetzt wird, wenn eine andere Behandlung nicht zum Ziel führt bzw. nur bei Patienten eingesetzt wird, die finanziell in der Lage sind, die Behandlung zu bezahlen. Somit besteht eindeutig ein Bedarf an einer kostengünstigen Formulierung, die gleichzeitig möglichst einfach herzustellen ist, im Gegensatz zu Liposomen lagerstabil ist und eine Lyophilisation nicht erfordert sowie nicht von Restlösungsmitteln belastet ist.

Der vorliegenden Erfindung liegt daher die Aufgabe zugrunde, eine Dispersion zur Verfügung zu stellen, die einen wenig, schwer oder sogar bisher unlöslichen Wirkstoff in einer bisher nicht möglichen Menge gelöst enthält, wobei gleichzeitig die oben beschriebenen Nachteile der Verwendung zusätzlicher zur Formulierung bisher notwendiger organischer Lösungsmittel entfällt.

Gegenstand der vorliegenden Erfindung ist daher eine Dispersion auf der Basis einer O/W-Emulsion oder einer W/O-Emulsion beladen mit Wirkstoff, der in Wasser und gleichzeitig auch in Ölen wenig löslich oder schwer löslich bis hin zu unlöslich ist, wobei diese Dispersion frei von toxikologisch bedenklichen organischen

Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der Wasser- und der Ölphase der Emulsion ergibt.

Insbesondere ist die erfindungsgemäß gelöste Menge um den Faktor 2, bevorzugter 5, noch bevorzugter 10 oder noch größer als die additive Menge.

Die "additive Menge" wird durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen), wobei keine weiteren zusätzlichen organischen Lösungsmittel zum Einsatz kommen. Die erfindungsgemäße Dispersion enthält zusätzlich zu der additiven Menge ein überadditive Menge an gelöstem Wirkstoff.

Ein wichtiges erfindungsgemäßes Merkmal ist, daß bei gleicher Zusammensetzung hochenergetisch homogenisiert wird, im Vergleich zu niederenergetischem Dispergieren (Schütteln oder Blattrührer).

Die Herstellung der erfindungsgemäßen Dispersion erfolgt insbesondere unter Ausschluß von toxikologisch bedenklichen organischen Lösungsmitteln wie z.B. Methylenchlorid und Ethanol. Die Wirkstoffe werden unter Umgehung eines Zwischenschrittes direkt aus der festen Substanz in die Emulsion eingearbeitet.

Detaillierte Beschreibung der Erfindung

Generell ist es anerkannter Stand der Wissenschaft, daß die Moleküle eines schwerlöslichen oder gering löslichen Wirkstoffes aus dem festen Aggregatzustand (Pulver) über mindestens einen Zwischenschritt (z. B. molekulardisperse Verteilung in einem Lösungsmittel) in eine Emulsion als Trägersystem eingearbeitet werden müssen. Die Erfahrung zeigt, daß bei in Wasser und Öl gleichzeitig sehr gering löslichen Substanzen es nicht genügt,

eine Emulsion mit Kristallen des Wirkstoffes zu versetzen. So führt die teilweise praktizierte Zumischung von Amphotericin B-Lösung (Lösungsmittelgemisch) zu einer handelsüblichen O/W-Emulsion wie Intralipid oder Lipofundin zur Präzipitation des Wirkstoffes, es entstehen Amphotericin B-Kristalle, die sedimentieren und sich nicht mehr in der Emulsion auflösen.

Überraschender Weise wurde jedoch nun gefunden, daß die Herstellung eines Emulsionssystems mit gelöstem Wirkstoff auch direkt aus dem festen Aggregatzustand des Wirkstoffes möglich ist. Zur Herstellung der erfindungsgemäßen Dispersion wird der Wirkstoff in partikulärer Form der Wasserphase oder der Ölphase zugesetzt und anschließend alle Komponenten einem höher energetischen oder hochenergetischen Prozeß wie z. B. der Homogenisation, insbesondere der Hochdruckhomogenisation unterzogen. Der hochenergetische Prozeß der Hochdruckhomogenisation führt dazu, daß der Wirkstoff in die Emulsion molekulardispers eingearbeitet wird und keine Wirkstoffkristalle mehr im Polarisationsmikroskop detektierbar sind. Die erhaltenen Emulsionen sind überraschender Weise ähnlich stabil wie Systeme, die unter Einsatz von organischen Lösungsmitteln erzeugt worden sind.

Eine sehr einfache Art der Einarbeitung der Wirkstoffkristalle ist die Verreibung des Wirkstoffes mit einer handelsüblichen O/W-Emulsion (z. B. Lipofundin, Intralipid). Nach Anreiben befindet sich der Wirkstoff primär in der Wasserphase, es ist ein disperses System entstanden, das als innere Phase gleichzeitig Öltropfen und Wirkstoff-Kristalle enthält. Dieses disperse System wird dann homogenisiert oder hochdruckhomogenisiert (z. B. 1.500 bar und 5 – 20 Homogenisationszyklen). Es wird eine feindisperse Emulsion erhalten (Beispiel 1), in der am Ende des Homogenisationsprozesses keine Wirkstoff-Kristalle mehr nachweisbar sind. Die Kristalle haben sich daher nahezu vollständig oder vollständig aufgelöst, d.h. daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10

Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

Falls es gewünscht ist, kann der Wirkstoff jedoch auch in einer solchen Menge eingesetzt werden, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

Alternativ kann eine wäßrige Suspension des Wirkstoffes mit einer O/W-Emulsion gemischt werden. Es handelt sich wieder um ein disperses System mit einer dispergierten Phase aus Öltropfen und Wirkstoff-Kristallen. Dieses wird ebenfalls einem höher oder hochenergetischem Prozeß wie der Hochdruckhomogenisation unterzogen. Die Zumischung einer wäßrigen Suspension des Wirkstoffes eignet sich insbesondere dann, wenn die Wirkstoffkonzentration relativ gering ist. Zusätzlich kann die wäßrige Suspension des Wirkstoffes vor der Zumischung einem in den Lehrbüchern beschriebenem Mahlprozeß unterzogen werden, z. B. Naßmahlung mit einer Kolloidmühle, einer Kugelmühle oder einer Perlmühle oder durch Hochdruckhomogenisation vorzerkleinert werden.

Generell ist es günstig, den Wirkstoff in der Form sehr feiner Kristalle zu verwenden, d. h. in mikronisierter Form mit einer Teilchengröße im Bereich von ca. 0,1 µm - 25 µm (Kolloidmühle, Gasstrahlmühle).

Alternativ kann der Wirkstoff auch im Öl dispergiert werden. Das Öl mit den Wirkstoff-Kristallen wird dann in der Wasserphase dispergiert, wobei das dafür notwendige Tensid entweder der Wasserphase zugesetzt wird oder in der Ölphase gelöst wird bzw. jeweils dispergiert wird. Im Falle von Lecithin kann das Lecithin im Wasser dispergiert werden oder in der Ölphase unter leichtem Erwärmen gelöst werden.

Bei Einarbeitung der Wirkstoff-Kristalle in die Ölphase kann dies ohne Zusatz eines Tensids erfolgen. Das Tensid, z. B. Lecithin, wird anschließend zugesetzt. Alternativ können auch die Wirkstoff-Kristalle in eine Ölphase eingearbeitet werden, die bereits Tensid enthält.

Nach Einarbeitung der Wirkstoff-Kristalle in das Öl wird die Ölphase in Wasser dispergiert (z. B. mit einem hochtourigen Rührer) und die erhaltene Rohemulsion anschließend hochdruckhomogenisiert. Auch hier ist es günstig, die Wirkstoff-Kristalle möglichst klein einzusetzen. Zur weiteren Zerkleinerung der in die Ölphase eingearbeiteten Wirkstoff-Kristalle kann diese ölige Suspension vor dem Herstellen der Rohemulsion zunächst einer Mahlung unterzogen werden. Die Wirkstoff-Kristalle in der Ölphase werden durch diese Naßmahlung weiter zerkleinert, teilweise bis in den Nanometerbereich. Übliche Verfahren der Naßmahlung, die eingesetzt werden können, sind z. B. die Kolloidmühle und die Hochdruckhomogenisation der Ölphase. Generell ist die Kavitation einer wäßrigen Phase das anerkannte Prinzip der Zerkleinerung bei der Hochdruckhomogenisation, d. h. die Anwesenheit von Wasser ist zur Kavitation erforderlich. Öle mit einem zu Wasser extrem geringen Dampfdruck sind zur Kavitation nicht fähig. Trotzdem wurde überraschender Weise gefunden, daß eine zur Herstellung des neuen Trägersystems ausreichende Zerkleinerung auftritt.

Charakteristisch für die erfindungsgemäße Dispersion ist, daß der in der Emulsion eingearbeitete Wirkstoff in höherer Menge gelöst vorliegt als es sich additiv aus seiner maximalen Löslichkeit in der Wasser- und Ölphase der Emulsion ergibt und gleichzeitig zur Herstellung keine toxikologisch bedenklichen organischen Lösungsmittel eingesetzt wurden. Zu solchen toxikologisch bedenklichen organischen Lösungsmitteln gehören insbesondere Chloroform, Methylenchlorid, längerkettige Alkohole wie Hexanol und Octanol, aber auch ethanol in höheren Konzentrationen.

In der Regel handelt es sich bei den erfindungsgemäßen Wirkstoffen um Wirkstoffe, die wenig löslich (1 Teil löst sich in 30-100 Teilen Lösungsmittel) oder schwer löslich (1 Teil in 100-1000 Teilen Lösungsmittel), insbesondere aber sehr schwer löslich (1 Teil löst sich in 1.000 bis 10.000 Teilen Lösungsmittel) oder sogar unlöslich sind (> 10.000 Teile Lösungsmittel).

So beträgt die Löslichkeit von Amphotericin B in Wasser weniger als 0,001% (< 0,01 mg/ml) bei pH 6-7, das heißt dem pH-Wert der Emulsion. Die Löslichkeit von Amphotericin ist zwar höher bei pH 2 und pH 11 (0,1 mg/ml), jedoch sind diese Lösungen nicht intravenös applizierbar.

Die Löslichkeit von Amphotericin in Sojaöl (Long Chain Triglycerides – LCT) und in Miglyol 812 (Medium Chain Triglycerides – MCT), den Standardölen für die meisten auf dem Markt befindlichen Emulsionen zur parenteralen Infusion ist kleiner als 0,0001 mg/ml.

40g Emulsion aus Beispiel 1 bestehen zu 20% aus Öl (8g) und ca. 80% aus Wasser (32g). Somit lassen sich aufgrund der Löslichkeiten 8 x 0,0001 mg/ml plus 32 x 0,01 mg, d.h. insgesamt 0,3208 mg Amphotericin in 40g Emulsionsbestandteilen Öl und Wasser auflösen, d.h. 0,008 mg/ml. In der vorliegen erfindungsgemäßen Emulsion konnten 0,2 mg/ml Emulsion eingearbeitet werden (Beispiel 1) ohne daß mikroskopisch Kristalle von ungelöstem Arzneistoff detektierbar waren (Beispiel 12). Auch höhere Konzentration wie 1 mg/ml Emulsion konnten eingearbeitet werden (Beispiel 2), mit Laserdiffraktometrie waren keine der zur Herstellung eingesetzten Arzneistoffpartikel mehr detektierbar (Beispiel 11).

Bei einer gewünschten Dosis von z.B. 100 mg Amphotericin B ergibt sich bei den erfindungsgemäßen Dispersionen mit 1 bzw. 0,2 mg/ml Emulsion ein intravenös zu applizierendes Volumen von 100 bis 500 ml Emulsion. Somit werden mit der erfindungsgemäßen Emulsion

wenig lösliche und schwer lösliche Wirkstoffe erst in einem ausreichend kleinen Applikationsvolumen bei verträglichen pH-Werten applizierbar.

Gelöster Wirkstoff ist schnell verfügbar. Zur Erzeugung eines Depots kann mehr Wirkstoff in die Dispersion eingearbeitet werden als sich darin löst, d h. man erzeugt Kristalle, die als Depot wirken. Die Löslichkeit in Wasser und Ölphase betragen z.B. für Amphotericin B 0,008 mg/ml, die erfindungsgemäße Emulsion löst ohne detektierbare Kristalle z.B. 0,2 mg/ml (Beispiel 1). Arbeitet man 5 mg/ml Dispersion ein, so ist die Löslichkeit überschritten (übersättigtes System). Nach Hochdruckhomogenisation erhält man zusätzlich zum gelösten Wirkstoff noch hochfeine Arzneistoffkriställchen (Beispiel 15).

Die durch Mischung von Arzneistoff (Beispiel 15) oder einer Arzneistoffsuspension (analog Beispiel 6) mit einer Emulsion und anschließende Homogenisation hergestellten heterogenen, übersättigten Dispersionen sind dadurch gekennzeichnet, daß separat nebeneinander Öltropfen und hochfeine Kriställchen existieren, d.h. die Kristalle sind primär außerhalb der Öltropfen.

Die Bestimmung der Partikelgröße erfolgt mit Lichtmikroskopie unter Ermittlung der Anzahlverteilung. Alternativ erfolgt die Bestimmung mit Laserdiffraktometrie (Gerät: Coulter LS 230, Coulter Electronics, Krefeld, Germany), wobei die erhaltene Volumenverteilung in die Anzahlverteilung umgerechnet wird.

Sind in der Dispersion bei hoher Beladung mit Wirkstoff neben den Emulsionstropfen noch Arzneistoffkristalle vorhanden, so sind direkt nach der Herstellung mindestens 90%, bevorzugt 95% der Anzahl der Wirkstoffkristalle in der Anzahlverteilung kleiner als 5 µm. Bei Anwendung von hohen Drücken (z.B. 1000 bar) und einer ausreichenden Anzahl an Homogenisationszyklen erhält man hochdisperse Systeme. In Abhängigkeit von Druck und Zyklenzahl erhält man Dispersionen mit mindestens 90%, teilweise 95% und

insbesondere 99% der Anzahl der Kristalle in der Anzahlverteilung kleiner als 1 µm.

Oben wurde die in situ Erzeugung des Wirkstoff-Depots aus Kriställchen durch Herstellung der erfindungsgemäßen Dispersion mit einer Wirkstoffmenge oberhalb der Sättigungslöslichkeit des Systems beschrieben. Alternativ kann auch eine erfindungsgemäße Dispersion mit ausschließlich gelöstem Wirkstoff hergestellt werden, der man nachträglich Wirkstoffkristalle definierter Größe zumischt, z.B. mikronisierter Wirkstoff.

Zur Herstellung der erfindungsgemäßen Dispersion können handelsübliche O/W-Emulsionen eingesetzt werden (z.B. Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid und Salvilipid) oder es wird eine Emulsion aus Ölphase, Emulgator / Stabilisator und äußerer Phase (z.B. Wasser) hergestellt.

Beispiele für Bestandteile der Ölphase der Emulsionen sind: Sojaöl, Safloröl (Distelöl), langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT) wie z.B. Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride wie Stesolid, einzeln oder in Mischungen.

Zur Stabilisierung der Dispersionen können Emulgatoren und Stabilisatoren eingesetzt werden. Diese sind gegebenenfalls bereits in der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten Emulsion enthalten, Zusatz weiterer Emulgatoren und Stabilisatoren bei der Herstellung der Dispersion kann vorteilhaft sein.

Beispiele für Emulgatoren sind z.B. Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Tween 80, Natriumglykocholat und Natriumlaurylsulfat (SDS). Alternativ kann Stabilisierung durch Zusatz von Substanzen erfolgen die über andere Mechanismen als Emulgatoren stabilitätserhöhend wirken, z.B. über sterische

Stabilisierung oder Erhöhung der Zetapotentials. Solche Stabilisatoren sind z.B. Block-Copolymere wie z.B. Poloxamere (z.B. Poloxamer 188 und 407) und Poloxamine (z.B. Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide wie Hyaluronsäure und Chitosan und ihre Derivate, Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester wie Saccharosemonostearat und Antiflokkulatien wie Natriumcitrat. Emulgatoren und Stabilisatoren können einzeln oder in Mischungen verwendet werden. Typische Konzentrationen sind 0,1% bis 20%, insbesondere 0,5% bis 10%.

Als wäßrige äußere Phase der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten O/W-Emulsion können dienen: Wasser, Mischungen von Wasser mit anderen wassermischbaren organischen Flüssigkeiten, flüssige Polyethylenglykole (PEG, insbesondere PEG 400 und 600).

Die wäßrige äußere Phase kann auch Zusätze enthalten, z.B. Elektrolyte, Nichtelektrolyte (z.B. Glycerol, Glucose, Mannit, Xylit zur Isotonisierung), Gelbildner wie Cellulosederivate und Polysaccharide wie Xanthan und Alginate (z.B. zur Viskositätserhöhung).

Für die topische Applikation können der Dispersion Penetrationsverstärker (z.B. Azone, Laurinsäure) und für die Applikation zum Gastrointestinaltrakt Absorptionsverstärker (z.B. Gallensäuren, Lysophospholipide) zugesetzt werden.

Wirkstoffe zur Einarbeitung in die Emulsion sind neben Amphotericin B z.B. Ciclosporin, Buparvaquon und Atovaquon. Weitere Wirkstoffe sind Hormone (z.B. Estradiol), Antioestrogene und Kortikoide (z.B. Prednicarbat).

Die Applikation der Emulsion kann auf verschiedenen Wegen erfolgen, z.B. parenteral aber auch oral oder topisch. Bei

parenteraler Applikation sind alle gängigen Wege möglich, z.B. intravenös, intra- und subkutan, intramuskulär, intraartikulär, intraperitoneal etc.

Topische Emulsionen mit Ciclosporin können die Wirkstoffpenetration in die Haut verbessern aufgrund des hohen gelösten Anteils an Arzneistoff (erhöhter Konzentrationsgradient). Orale Applikation der Ciclosporin-Emulsion kann die Bioverfügbarkeit erhöhen da im Gegensatz zu mikronisiertem Ciclosporin ein erhöhter gelöster Anteil vorliegt.

Die Bioverfügbarkeit von oral appliziertem Amphotericin B ist aufgrund seiner geringen Löslichkeit nahezu Null. Orale Applikation der Amphotericin-Emulsion kann aufgrund des erhöhten gelösten Anteils ebenfalls die Bioverfügbarkeit erhöhen.

Die erfindungsgemäßen Emulsionen (z.B. mit Buparvaquon und Atovaquon) können nach intravenöser Injektion auch durch Anlagerung einer Targeting-Einheit (z.B. Apolipoprotein E in Kombination mit Apolipoprotein AI und AIV) für eine gewebsspezifische Arzneistoffapplikation eingesetzt werden (Targeting zum Gehirn). Erreger lokalisieren bei bestimmten Erkrankungen des monozytären phagocytierenden Systems (MPS) auch im Gehirn und sind bisher schwer einer Therapie zugänglich (z.B. Leishmaniosen, Toxoplasmose).

Die oben beschriebenen Systeme sind vom Typ O/W, d. h. Öltropfen sind dispergiert in einer Wasserphase. Es ist jedoch auch möglich, Dispersionen auf der Basis von W/O-Emulsionen zu produzieren. Ein grundsätzlicher Vorteil ist, daß die äußere Ölphase als eine Diffusionsbarriere fungiert und die Freigabe des Arzneistoffes verzögert. Derartige Dispersionen können nicht intravenös appliziert werden, aber sie können zum Beispiel intramuskulär oder subkutan als Depotformulierung injiziert werden. Applikation dieser W/O-Systeme am Auge erhöht die Verweilzeit aufgrund der erhöhten Viskosität und gleichzeitig

wird eine verlängerte Arzneistofffreisetzung erreicht. Bei topischer Applikation auf die Haut hat die Ölphase einen okklusiven Effekt, der zu einer erhöhten Arzneistoffpenetration führt. Daher besitzen diese W/O-Typ-Systeme einen Vorteil für spezielle Anwendungen. Bevorzugte Form der Erfindung ist jedoch die Dispersion auf der Basis des O/W-Typs.

Bei Öl-in-Wasser Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie 5 bis 99,5 Gew.-% wäßrige Phase, vorzugsweise 10 bis 95 Gew.-% wäßrige Phase, besonders bevorzugt 60 bis 95 Gew.-% wäßrige Phase und speziell 70-95% wäßrige Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Bei Wasser-in-Öl Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie aus 5 bis 30 Gew.-% wäßriger Phase, vorzugsweise 10 bis 25 Gew.-% wäßriger Phase, besonders bevorzugt 10 bis 20 Gew.-% wäßriger Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Die Bestandteile der Ölphase der Emulsionen sind – wie oben ausgeführt - insbesondere ausgewählt aus der Gruppe bestehend aus Sojaöl, Safloröl (Distelöl), langkettigen Triglyceriden (LCT), mittelkettigen Triglyceriden (MCT), wie z. B. Miglyole, Fischölen und Ölen mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierten Partialglyceriden, wie in Stesolid®, einzeln oder in Mischungen. Die mittelkettigen Triglyceride enthalten vorzugsweise wenigstens 90 % Triglyceride der Capryl-Säure (C8) und der Caprin-Säure (C10). Als Ölphase sind im Rahmen der Erfindung Gemische aus Sojaöl und MCT, vorzugsweise im Gewichtsverhältnis 5:1 bis 1:5, besonders bevorzugt zwischen 2:1 und 1:2 oder 1:1 geeignet.

Die Fettphase der erfundenen Dispersion kann aus Ölen bestehen, d.h. die Lipide sind bei einer Raumtemperatur von 20°C flüssig. Es besteht weiterhin die Möglichkeit, daß diese Öle mit Lipiden gemischt werden, die bei einer Raumtemperatur von 20°C fest sind.

Die Mischungsanteile von Öl zu festem Lipid können von 99 + 1 bis 1 + 99 (Gewichtsanteile) variieren. Bevorzugt sind Mischungen, die mindestens 10 Teile flüssiges Öl enthalten, speziell mindestens 30 Teile flüssiges Öl und insbesondere mindestens 50 Anteile flüssiges Öl.

In speziellen Fällen kann die Lipidphase der Dispersion zu 100% Lipide enthalten, die bei einer Raumtemperatur von 20°C fest sind. Schmelzen die Lipide nahe der Raumtemperatur, können Dispersionen erhalten werden, deren Lipidtröpfchen sich in einem Zustand einer "Unterkühlten Schmelze" befinden. Liegen sehr hochschmelzende Lipide vor, können – ungeachtet der durch die Thomson-Gleichung beschriebenen Schmelzpunktionsdepression – die Partikel der Dispersion aushärten. Die Thomson-Gleichung beschreibt, daß der Schmelzpunkt von Lipiden gegenüber ihrer "bulk"-Ware stark herabgesetzt wird, wenn diese in sehr feinen Partikeln auskristallisieren (z. B. Nanopartikel oder Partikel in einem Größenbereich von wenigen Mikrometern) (Hunter, R.J., Foundations of colloid science, Vol. 1, Oxford University Press, Oxford, 1986).

Beispiele für bei Raumtemperatur feste Lipide sind, Karnaubawachs, Hydroxyoctacosanylhydroxystearat, Chinesisches Wachs, Cetylpalmitat, Bienenwachs und ähnliche Wachse. Weitere Beispiele für feste Lipide beinhalten C_{20-40} Di- und Triglyceride, mit gesättigten und ungesättigten Fettsäuren, C_{20-40} Fettalkohole, C_{20-40} Fettamine und ihre Verbindungen, sowie Sterole.

Als Lipide zur Herstellung von Mischungen aus flüssigen und festen Lipiden sind geeignet: Natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit z. B. Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben. Besonders geeignet sind synthetische Monoglyceride, Diglyceride und Triglyceride als

individuelle Substanzen oder als Mischung (z. B. Hartfett), Imwitor 900, Triglyceride (z. B. Glyceroltrilaurat, Glyceroltrimyristat, Glyceroltripalmitat, Glyceroltristearat und Glyceroltribehenat) und Wachse wie z. B. Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB). Außerdem Kohlenwasserstoffe, wie z. B. Hartparaffin.

Die Tropfengröße der Öltropfen (O/W-Typ) oder Wassertropfen (W/O-Typ) in der Dispersion ist größer als 100 nm (bestimmt mit Photonenkorrelationsspektroskopie – PCS). Das empfohlene obere Größenlimit für die Tropfen ist 10 µm, anderenfalls kommt es zum Aufrahmen aufgrund der Flotation der Tropfen, was zu physikalischer Instabilität führt (Tropfenkoaleszenz). Um Flotation zu minimieren, sollte die Größe kleiner als 5 µm sein, vorzugsweise unterhalb von 1 µm (PCS-Durchmesser), was zu den sogenannten physikalisch "autostabilen" Dispersionen führt. Die optimale Stabilität wurde gefunden im Größenbereich ähnlich zu parenteralen Fettemulsionen mit PCS-Durchmessern von 200 nm bis 500 nm.

Der Gehalt an Stabilisatoren in parenteralen Zubereitungen sollte so niedrig wie möglich gehalten werden, um Toxizität und Störungen des Metabolismus zu minimieren. Von Lecithin-haltigen Emulsionen zur parenteralen Ernährung ist es bekannt, daß eine zu hohe Zuführung von Lecithin metabolische Störungen bewirken kann, typische Tagesvolumina appliziert sind hier z. B. 500 ml Emulsion und mehr. Dies führte zu der Entwicklung der Lecithin-reduzierten Emulsionen, d. h. man reduzierte den Lecithingehalt von 1,2% weiter auf nur 0,6% Lecithin. Einige Systeme zur Applikation von schwerlöslichen Arzneistoffen verwenden einen relativ hohen Emulgatorgehalt (z. B. Solubilisierung mit Tensiden, SEDDS – self-emulsifying drug delivery systems basierend auf der Solubilisation von Öl mit hohen Tensidkonzentrationen). Eine spezielle Eigenschaft der vorliegenden Erfindung ist, daß sie die Tensidbelastung minimiert. Eine typische Zusammensetzung des O/W-Types der erfindungsgemäßen Dispersion ist: 20 g Öl, 1,2 g Lecithin, 0,1 g Arzneistoff und 78,3 g

Wasser. Dies bedeutet, daß die 21,2 g produzierter Öltropfen aus 20 g Ölphase (= 94,3%) und 1,2 g Stabilisator (= 5,7%) bestehen.

Weitere Beispiele für Emulgatoren sind neben Lecithinen die Polyethoxysorbitanester (Tween®-Typen), wie beispielsweise Laurate (Tween 20/21), Palmitate (Tween 40), Stearate (Tween 60/61), Tristearate (Tween 65), Oleate (Tween 80/81), oder Trioleate (Tween 85), Natriumglycocholat und Natriumlaurylsulfat (SDS) sowie die Sorbitanfettsäureester (Span® -Typen). Besonders bevorzugt ist Tween 80.

Bevorzugt werden weiterhin Tenside, Emulgatoren und Stabilisatoren eingesetzt, die für die Anwendung am und im Menschen zugelassen sind (z.B. Hilfsstoffe mit dem GRAS-Status).

Speziell für die Dispersionen vom Typ W/O werden die typischen Wasser-in-Öl-Tenside zur Stabilisierung benutzt, manchmal in Mischungen, auch in Mischungen mit O/W-Emulgatoren. Beispiele hierfür sind die Fettalkohole, Ethylenglykolmonostearat, Glycerolmonostearat, Sorbitanfettsäureester (Span®-Serie, z. B. Span 20-, Span 40-, Span 60- und Span 80-Serie, speziell Span 85), Ether von Fettalkoholen mit Polyethylenglykol (PEG) (z. B. Brij®-Serie), Ester von Fettsäuren mit PEG (z. B. Myrj®-Serie).

Im allgemeinen werden wieder Tenside und Stabilisatoren mit einem anerkannten Status bevorzugt, z. B. GRAS-Substanzen (Generally Regarded As Safe – Food Additives – GRAS substances, Food Drug Cosmetic Law Reports, Chicago (1994), Food Additive Database der FDA, Internet: www.fda.gov, 1999).

Im Fall daß die erfindungsgemäßen Dispersionen – zusätzlich zu den Öltropfen – noch Partikel von ungelöstem Wirkstoff enthalten, sollte die Partikelgröße so klein wie möglich sein, zum Beispiel zwecks Erhalt der physikalischen Stabilität und zur Vermeidung von Sedimentation. Zusätzlich, im Fall der intravenösen Applikation, sollten die Partikel klein genug sein, um Kapillar-

blockade zu vermeiden. Die kleinsten Blutkapillaren sind ungefähr 5-6 µm im Durchmesser. Daher sollte der Partikeldurchmesser 90% unterhalb von 5 µm sein, vorzugsweise auch der Durchmesser 95% und insbesondere der Durchmesser 100% sollte unterhalb 5 µm sein (gemessen mit Laserdiffraktometrie nach Abtrennung der Partikel von der Dispersion durch Zentrifugation, Volumenverteilungsdaten). Es ist noch günstiger, wenn diese Durchmesser alle unterhalb von 3 µm sind, da dann eine Sicherheitsdistanz zur Größe der kleinsten Kapillaren vorhanden ist.

Am vorteilhaftesten ist eine Partikelgröße des ungelösten Arzneistoffes unterhalb von 1000 nm (mittlere Partikelgröße gemessen mit Photonenkorrelationsspektroskopie). Diese Größe ist weit weg von den 5-6 µm der kleinsten Kapillardurchmesser und schließt gleichzeitig jegliche Sedimentationseffekte aus (diese Partikelgröße sedimentiert nicht relativ unabhängig von der Dichte des Arzneistoffes). Im Fall, daß eine schnellere Auflösung der Arzneistoffkristalle nach Applikation der Dispersion notwendig ist, sollte der mittlere PCS-Durchmesser im Bereich 100 nm bis ungefähr 400 nm, bevorzugt unter 100 nm sein.

Generell ist es günstig, den Wirkstoff zur Herstellung der Dispersion in der Form sehr feiner Kristalle zu verwenden, d.h., in mikronisierter Form mit einer mittleren Teilchengröße im Bereich von ca. 0,1 µm – 25 µm (Kolloidmühle, Gasstrahlmühle). Bevorzugt sind mittlere Teilchengrößen von 0,1µm – 5 µm, besonders bevorzugt von kleiner als 1 µm.

Der pH-Wert der erfindungsgemäßen Dispersionen liegt typischerweise zwischen 4 und 8, vorzugsweise zwischen 5 und 7,5, besonders bevorzugt zwischen 6 und 7,5 und wird in der Praxis bestimmt durch die Applikationsform.

Die Dispersion gemäß der Erfindung kann ferner eine wirksame Menge eines Antioxidanz, wie beispielsweise Vitamin E, insbesondere das Isomer alpha-Tocopherol enthalten. Alternativ

können auch beta- oder gamma-Tocopherol, oder Ascorbylpalmitat verwendet werden. Der Zusatz kann zwischen 10 mg und 2000 mg, vorzugsweise zwischen 25 mg und 1000 mg, bezogen auf 100 g Triglyceride betragen.

Eine typische Dispersion gemäß der Erfindung kann somit, bezogen auf die anwendungsfertige Gesamtzusammensetzung z.B. umfassen: 0,05 bis 1,0 Gew.-%, vorzugsweise 0,05 bis 0,5 Gew.-% des Wirkstoffes, 0,05 bis 2 Gew.-% eines Emulgators oder Emulgatorgemisches, beispielsweise Tween 80 und/oder Ei-Lecithin, dispergiert in einer O/W-Emulsion, die, bezogen auf die Emulsion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 20 Gew.-% Triglyceride enthält. Bei den Triglyceriden handelt es sich vorzugsweise um Sojabohnenöl, mittelkettige Triglyceride (wenigstens 90 % C8/C10) sowie Gemische aus Sojabohnenöl und mittelkettigen Triglyceriden (wenigstens 90 % C8/C10) im Gewichtsverhältnis 1:2 bis 2:1, vorzugsweise 1:1. Daneben können noch, bezogen auf die Gesamtzusammensetzung, 0,5 bis 5 Gew.-%, vorzugsweise 1 bis 3 Gew.-% übliche Isotonisierungsmittel, wie Glycerol, und 0,005 bis 0,05 Gew.-% Antioxidantien, wie beispielsweise alpha-Tocopherol enthalten sein. Ein besonders bevorzugter Wirkstoff ist insbesondere Amphotericin B. Zusätzlich können auch Konservierungsmittel zugesetzt werden. Die trifft insbesondere bei Abpackung der Dispersion in Gefäße zur Mehrfachentnahme zu.

Die Dispersion enthält den Wirkstoff gelöst in einer Menge, die größer ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit jeweils in der Wasser- und der Ölphase der Emulsion ergibt, wobei die "additive Menge" unter Normalbedingungen (20°C, Normaldruck) durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen) wird.

In der Dispersion sind typische Wirkstoffkonzentrationen 0,01 Gew.-% bis 30 Gew.-%, vorzugsweise 0,1 Gew.-% bis 10 Gew.-%,

besonders bevorzugt 1 Gew.-% bis 5 Gew.-%, bezogen auf die Gesamtmenge der Dispersion.

Arzneistoffe von besonderem Interesse – neben Amphotericin B – sind Vancomycin und Vecuronium. Des weiteren können schwerlösliche Arzneistoffe aus den Gruppen der Prostaglandine, z. B. Prostaglandin E_2, Prostaglandin $F_{2\alpha}$ und Prostaglandin E_1, Proteinase-Hemmstoffe, wie z. B. Indinavir, Nelfinavir, Ritonavir, Saquinavir, Zytostatika, z. B. Paclitaxel, Doxorubicin, Daunorubicin, Epirubicin, Idarubicin, Zorubicin, Mitoxantron, Amsacrin, Vinblastin, Vincristin, Vindesin, Dactiomycin, Bleomycin, Metallocene, z B. Titanmetallocendichlorid, und Lipid-Arzneistoff-Konjugate, wie z. B. Diminazenstearat und Diminazenoleat, und generell schwerlösliche Antiinfektiva wie Griseofulvin, Ketoconazol, Fluconazol, Itraconazol, Clindamycin, insbesondere antiparasitische Arzneistoffe, z. B. Chloroquin, Mefloquin, Primaquin, Pentamidin, Metronidazol, Nimorazol, Tinidazol, Atovaquon, Buparvaquon, Nifurtimox und antiinflammatorische Arzneistoffe, wie z. B.Ciclosporin, Methotrexat, Azathioprin, verwendet werden.

Dispersionen, die antiinflammatorische Arzneistoffe enthalten, können topisch, oral und parenteral angewendet werden. Im Falle einer topischen Anwendung auf der Haut, kann der Arzneistoff in das tiefere Gewebe penetrieren, wo entzündliche Prozesse stattfinden. Mit einer topischen Anwendung auf Schleimhäuten, wie z. B. am Auge, können Erkrankungen wie das "Trockene Auge"-Syndrom behandelt werden, dem ein entzündlicher Prozeß zugrunde liegt. Eine topische Anwendung auf den Schleimhäuten der Vagina ist ebenso vorteilhaft, z. B. ganz besonders für Antiinfektiva. Die Dispersion spreitet gut auf der Schleimhautoberfläche und gewährleistet so eine gleichmäßige Verteilung des Arzneistoffs. Insbesondere wenn diese Dispersion Öltröpfchen und zusätzlich sehr feine Arzneistoffkristalle enthält, da diese feinen Kristalle auf der vaginalen Schleimhaut haften und sich dort langsam auflösen und damit für eine verlängerte Arzneistoff-

wirkung sorgen (Depotwirkung). Für eine Anwendung am Auge ist es vorteilhaft, wenn man Dispersionen verwendet, die positiv geladen sind. Die Wechselwirkungen der positiv geladenen Partikel mit den negativ geladenen Zellmembranen verlängern die Verweilzeit des Arzneistoffs am Wirkort.

Die orale Anwendung der erfundenen Dispersion ist geeignet, die Bioverfügbarkeit von schwerlöslichen Arzneistoffen, die oral nicht ausreichend verfügbar sind, zu erhöhen. Beispiele hierfür sind Paclitaxel und Amphotericin B. Anstelle von wäßrigen Dispersionen können auch, durch Sprühtrocknung oder Gefriertrocknung überführte, trockene Formen verwendet werden.

Die parenterale, insbesondere die intravenöse Anwendung von arzneistoffhaltigen Dispersionen kann Nebenwirkungen reduzieren, z. B. bei Doxorubicin, Daunorubicin und Amphotericin B. Intravenös angewendete Dispersionen können durch Modifizierung der Oberfläche mit Apolipoproteinen gezielt zu gewünschten Zielorganen, wie Gehirn oder Knochenmark gelenkt werden. Dies ist bei Arzneistoffen, die keinen oder nur geringen Zugang zum Gehirn haben, von besonderem Interesse. Typische Beispiele hierfür sind zytotoxische Substanzen wie Doxrubicin. Eine gezielte Aufnahme zytotoxischer Dispersionen in das Gehirn ermöglicht die Behandlung von Hirntumoren, die bisher nur operativ oder lokal, z. B. mit implantierten therapeutischen Systemen und mit arzneistoffhaltigen Implantaten behandelt werden können. Dispersionen, die Antiinfektiva mit geringer Blut-Hirn-Schranken-Permeabilität enthalten, können nun genutzt werden, um diese Antiinfektiva zur Behandlung von persistierenden Parasiten durch die Blut-Hirn-Schranke zu transportieren.

Die Organverteilung von intravenös applizierten Arzneistoffträgern wird von deren physiko - chemischen Eigenschaften, wie z. B. Partikelgröße, Partikelladung und Oberflächenhydrophobie bestimmt. Negativ geladene Partikel werden zum Beispiel wesentlich schneller von den Makrophagen der Leber aufgenommen als

ungeladene Partikel (Wilkens, D, J. and Myers, P. A., Studies on the relationship between the electrophoretic properties of colloids and their blood clearance and organ distribution in the rat. Brit. J. Exp. Path. 47, 568-576, 1966). Um die In-vivo-Organverteilung zu modifizieren, kann die Ladung der erfindungsgemäßen Dispersion geändert werden, speziell positiv geladene Dispersionen sind vorteilhaft. Die positiv geladene Dispersion kann im Bereich der Einstichstelle an den negativ geladenen Zelloberflächen haften bleiben. Nach intravenöser Applikation der negativ geladenen Dispersion interagieren die Partikel mit negativ geladenen Proteinen, speziell mit Albumin, das mengenmäßig bedeutendste Protein im Blut. Aufgrund seiner Funktion als Dysopsonin kann es durch Adsorption an der Tropfenoberfläche und Bildung einer Albumin-Adsorptionsschicht die Verweilzeit der erfundenen Dispersion im Blut verlängern (z. B. verminderte Aufnahme durch Makrophagen der Leber).

Positiv geladene Dispersionen gemäß der Erfindung, können unter Verwendung positiv geladener Emulgatoren, Mischungen von positiv geladenen und ungeladenen Stabilisatoren (z. B. Poloxamere) und/oder negativ geladenen Emulgatoren (z. B. Lecithin) hergestellt werden. Positiv geladene Dispersionen, gemäß der Erfindung, haben ein positives Zetapotential. Das Zetapotential der Dispersionspartikel wird mit elektrophoretischer Messung in destilliertem Wasser (durch Zugabe von Natriumchlorid auf eine Leitfähigkeit von 50 µS/cm eingestellt) oder im Originaldispersionsmedium (äußere Phase der Dispersion) gemessen. Beispiele für positiv geladene Emulgatoren und Stabilisatoren sind Stearylamin, Cetypyridiniumchlorid (CPC), für positiv geladene Lipide N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniumchlorid (DOTMA), Didodecyldimethylammoniumbromid (DDAB), 2,3-Dioleyloxy-N-[2(spermidincarboxamid)ethyl]-N,N-dimethyl-1-propylammoniumtrifluoroacetat (DOSPA), 3β-[N-(N',N'-Dimethylaminoethan)carbamoyl]-cholesterol (DC-Chol).

Die Herstellung positiv geladener Dispersionen kann unter Verwendung positiv geladener Emulgatoren oder Emulgatormischungen im Produktionsprozeß durchgeführt werden (De novo-Herstellung). Der positiv geladene Emulgator kann alternativ auch zu einer negativ geladenen Dispersion zugefügt werden. Der Emulgator muß in ausreichender Menge zugefügt werden, damit eine Ladungsumkehr von negativ nach positiv eintritt.

Nähere Beschreibung des Produktionsprozesses: Die Mischung aus Lipid, Arzneistoff, Wasser und Emulgator oder andere Stabilisatoren muß einem hochenergetischem Dispergierprozeß unterzogen werden. Sollen Mischungen von Ölen und festen Fetten im Homogenisationsansatz verwendet werden, ist es vorteilhaft, das feste Fett bei erhöhter Temperatur im Öl zu lösen. Die bevorzugte Methode die erfindungsgemäße Dispersion herzustellen, ist die Hochdruckhomogenisation, z. B. mit Kolben-Spalt-Homogenisatoren oder Jet Stream-Homogenisatoren. Befindet sich Wasser in der äußeren Phase der Dispersion, wird die Homogenisation zwischen 0°C und 100°C durchgeführt. Die beste Dispergierung und schnellste Auflösung des schwerlöslichen Arzneistoffs wird erreicht, wenn die Homogenisation deutlich über Raumtemperatur durchgeführt wird, z. B. zwischen 35°C und 100°C. Die optimale Homogenisationstemperatur bei gleichzeitiger Berücksichtigung der chemischen Stabilität des Arzneistoffs wurde zwischen 45°C und 65°C ermittelt. Liegt ein extrem temperaturempfindlicher Arzneistoff vor, sollte die Homogenisation in der Nähe des Gefrierpunktes von Wasser durchgeführt werden (z. B. ungefähr 4°C).

Werden für die äußere Phase der Dispersion andere Flüssigkeiten als Wasser verwendet, die einen höheren Siedepunkt als Wasser besitzen, kann auch bei höheren Temperaturen oder unter 0°C (z. B. PEG 600) homogenisiert werden.

Im Fall von Mischungen aus Lipiden, Mischen von Öl und festem Lipid als "bulk"-Waren kann zu einer festen "bulk"-Mischung

führen – obwohl die daraus in der Dispersion produzierten Partikel flüssig sind (Thomson-Effekt). In diesem Falle sollte die Homogenisation bei einer Temperatur durchgeführt werden, die über dem Schmelzpunkt der "bulk"-Mischung liegt. Daßelbe gilt bei alleiniger Verwendung von festen Lipiden zur Herstellung der Dispersion gemäß der Erfindung. Der angelegte Homogenisationsdruck kann zwischen 10 und 11.000 bar liegen. Werden die Dispersionen mit 11.000 bar produziert, ist die resultierende Dispersion steril, da unter diesem hohen Druck Bakterien und Viren zerrissen werden. Ist eine Sterilisation durch Homogenisation nicht erwünscht, liegt der bevorzugte Produktionsdruck zwischen 200 bar und annähernd 4000 bar. Die in der Industrie in Produktionslinien verwendeten Hochdruckhomogenisatoren arbeiten gewöhnlich in einem Bereich von 200 bar bis 700 bar, daher wäre es nicht notwendig neue Maschinen anzuschaffen, wenn bei diesen Drücken gearbeitet wird. Die Produktion bei niedrigeren Drücken erfordert jedoch eine höhere Anzahl an Durchläufen (Zyklen). Muß eine höhere Anzahl an Durchläufen vermieden werden (z. B. begründet durch Aspekte der chemischen Stabilität des Arzneistoffes), sollte ein höherer Druck angewendet werden, der von 700 bar bis 4000 bar reicht. Für den Bereich 700-1500 bar können Homogenisatoren von APV Gaulin (Lübeck, Deutschland) verwendet werden, für den Bereich 700-2000 bar sind Maschinen der Firma Niro Soavi (Lübeck, Germany) geeignet, des weiteren ermöglichen spezielle Homogenisatoren der Firma Stansted (Stansted, UK) bei Drücken bis zu 4000 bar zu arbeiten.

Um die Dispersion herzustellen kann jede Homogenisatorausstattung verwendet werden, die eine genügend hohe Leistungsdichte erreicht, d. h. typischerweise über 10^4 W/m^3. Bei einigen Homogenisatoren kann die Leistungsdichte (dissipierte Energie pro Volumeneinheit der Dispergierzone) nicht errechnet werden, da die genaue Größe der Dispergierzone nicht bekannt ist (z. B. Microfluidizer). In diesem Fall muß die Eignung der Maschine für die Herstellung der erfundenen Dispersion auf empirischem Wege ermittelt werden. Beispiele für Homogenisatoren vom Kolben-Spalt-

Typ sind die Maschinen von den Firmen APV Gaulin, Niro Soavi, Stansted und French Press, ein Beispiel für Jet Stream-Homogenisatoren ist der Microfluidizer (Microfluidics, Inc., USA).

Die Erfindung wird durch die nachfolgenden Beispiele näher erläutert, ohne sie jedoch zu beschränken.

Beispiele

Beispiel 1

8 mg Amphotericin B wurden mit 40 g Lipofundin N 20 % angerieben (0,2 mg Amphotericin B/ml Emulsion) und die erhaltene Dispersion mit einem Ultra-Turrax-Rührer 5 Minuten bei 8000 Umdrehungen pro Minute gerührt. Anschließend wurde die Dispersion mit einem Micron LAB 40 bei 1.500 bar mit 20 Zyklen hochdruckhomogenisiert. Die Partikelgröße wurde mit einem Laserdiffraktometer bestimmt (Coulter LS 230, Coulter Electronics, USA). Der Durchmesser 50 % (D50%) der Volumenverteilung betrug 0,164 µm, D90% 0,340 µm, D95% 0,387 µm, D99% 0,466 µm und D100% 0,700 µm.

Beispiel 2

Es wurde ein Emulsionssystem mit Amphotericin B wie in Beispiel 1 hergestellt, die eingearbeitete Menge an Amphotericin B betrug jedoch 40 mg (d. h. 1 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50% 0,160 µm, D90% 0,362 µm, D95% 0,406 µm, D99% 0,485 µm und D100% 0,746 µm.

Beispiel 3

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die eingearbeitete Amphotericin B-Menge betrug jedoch 80 mg (d. h. 2 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50%

0,194 µm, D90% 0,381 µm, D95% 0,423 µm, D99% 0,494 µm und D100% 0,721 µm.

Beispiel 4

40 mg Amphotericin B-Pulver wurden mit 40 g Öl (Mischung 50 : 50 aus LCT und MCT) angerieben und die erhaltene Suspension wie in Beispiel 1 mit einem Ultra-Turrax für 5 Minuten gerührt. Anschließend wurde die Suspension mit einem Hochdruckhomogenisator Micron LAB 40 hochdruckhomogenisiert mit 2 Zyklen bei 150 bar, 2 Zyklen bei 500 bar und anschließend 20 Zyklen bei 1.500 bar. 8 g der erhaltenen öligen Suspension wurden dann in 32 g Wasser dispergiert, das 1,2 % Lecithin enthielt. Dispergierung erfolgte mit einem Ultra-Turrax für 5 Minuten bei 8000 Umdrehungen/Minute. Die erhaltene Dispersion wurde dann mit dem Micron LAB 40 hochdruckhomogenisiert bei 500 bar mit 10 Zyklen. Es wurden folgende Durchmesser gemessen: D50% 0,869 µm, D90% 2,151 µm, D95% 2,697 µm, D99% 3,361 µm.

Beispiel 5

Es wurde eine Emulsion analog Beispiel 4 hergestellt, allerdings erfolgte die Herstellung der Emulsion mit Hochdruckhomogenisation nicht bei Raumtemperatur, sondern in einem temperaturkontrolliertem LAB 40 bei 50°C. Es wurden folgende Durchmesser gemessen: D50% 0,647 µm, D90% 1,537 µm, D95% 1,768 µm, D99% 2,152 µm und D100% 3,310 µm.

Beispiel 6

Es wurde eine Amphotericin B-Emulsion durch Hochdruckhomogenisation analog Beispiel 1 hergestellt (0,2 mg Amphotericin B/ml Emulsion), die Hochdruckhomogenisation der Emulsion erfolgte bei Raumtemperatur. Der Arzneistoff wurde in 1,2%iger wäßriger Tween 80-Lösung angerieben, die Suspension vorhomogenisiert und 80 mg dieser Suspension mit 40g Lipofundin N 20% gemischt. Es wurden

folgende Durchmesser gemessen: D50% 0,142 µm, D90% 0,282 µm, D95% 0,331 µm, D99% 0,459 µm und D100% 0,843 µm.

Beispiel 7

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 1 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,245 µm, D90% 0,390 µm, D95% 0,426 µm, D99% 0,489 µm, D100% 0,700 µm.

Beispiel 8

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 2 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,237 µm, D90% 0,389 µm, D95% 0,426 µm, D99% 0,491 µm, D100% 0,701 µm.

Beispiel 9

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Hochdruckhomogenisation der Emulsion erfolgte bei 60°C. Es wurden folgende Durchmesser gemessen: D50% 0,197 µm, D90% 0,388 µm, D95% 0,436 µm, D99% 0,532 µm und D100% 0,953 µm.

Beispiel 10

Es wurde eine Emulsion analog Beispiel 7 hergestellt, der Homogenisationsdruck betrug jedoch 500 bar anstatt 1500 bar. Es wurden folgende Durchmesser gemessen: D50% 0,263 µm, D90% 0,401 µm, D95% 0,435 µm, D99% 0,493 µm und D100% 0,657 µm.

Beispiel 11

Die Partikelgrößenverteilung des Amphotericin B-Pulvers wurde mit Laserdiffraktometrie und Lichtmikroskopie analysiert. Abbildung 1 (oben) zeigt die Teilchengrößenverteilungskurve des Pulvers

nach Dispergierung in Wasser ermittelt mit Laserdiffraktometrie sowie die Partikelgrößenverteilung nach Einarbeitung in das erfindungsgemäße Emulsionssystem aus Beispiel 2 (Abbildung 1, unten). Im Emulsionssystem sind keine Amphotericin B-Kristalle mehr detektierbar, Amphotericin B wurde in das Emulsionssystem inkorporiert.

Beispiel 12

Die Amphotericin B-Emulsion wurde im Vergleich zu in Wasser dispergierten Amphotericin B-Kristallen mit Lichtmikroskopie untersucht. Abbildung 2 zeigt die lichtmikroskopische Aufnahme des Amphotericin B-Pulvers im polarisierten Licht, aufgrund der Anisotropie der Kristalle erscheinen sie hell. Abbildung 3 zeigt die lichtmikroskopische Aufnahme im polarisierten Licht nach Einarbeitung von Amphotericin B in das Emulsionssystem (Beispiel 1), anisotrope Strukturen sind nicht mehr detektierbar, das gesamte Bild ist nahezu schwarz. Für die Lichtmikroskopie wurde das Emulsionssystem unverdünnt auf den Objektträger aufgetragen.

Beispiel 13

Buparvaquon wurde analog zu Amphotericin B wie in Beispiel 6 in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,399 µm, D90% 0,527 µm, D95% 0,564 µm, D99% 0,635 µm und D100% 0,843 µm.

Beispiel 14

Atovaquon wurde analog zu Beispiel 1 anstelle von Amphotericin B in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,297 µm, D90% 0,437µm, D95% 0,475 µm, D99% 0,540 µm und D100% 0,744 µm.

Beispiel 15

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die Menge an eingearbeitetem Amphotericin betrug jedoch 5 mg/ml Emulsion. Die Löslichkeit in der Dispersion für Amphotericin war überschritten, neben Öltropfen lagen Arzneistoffkristalle vor (heterogene Dispersion).

Beispiel 16

Es wurde eine Amphotericin B-Emulsion durch Zumischung von 40 mg Amphotericin B zu 40 ml Lipofundin N 20 % hergestellt (d. h. Amphotericin B 1 mg/ml Emulsion). Die Mischung wurde mit 10 Zyklen bei 1500 bar und 45°C homogenisiert. Diese Emulsion wurde durch Autoklavieren bei 121°C für 15 Minuten (gemäß Deutschen Arzneibuches) sterilisiert. Der PCS-Durchmesser vor Autoklavierung betrug 203 nm, der Polydispersitätsindex 0,102, nach Autoklavierung lag der Durchmesser bei 208 nm, der Polydispersitätsindex bei 0,137.

Beispiel 17

100 mg Amphotericin B-Pulver wurden in 900 mg sterilen Wasser dispergiert, vorhomogenisiert und unter Verwendung von Pistill und Mörser in 20 g MCT-Öl mit 1,2% Lecithin eingearbeitet. Das Öl wurde in 80 g Wasser dispergiert und diese Mischung in einem Microfluidizer Typ Microfluidix M110y homogenisiert (d. h. Amphotericin B 1 mg/ml Emulsion). Die Homogenisation wurde bei 1000 bar für 10 Minuten durchgeführt. Der PCS-Durchmesser vor Autoklavierung betrug 192 nm, der Polydispersitätsindex 0,113, nach Autoklavierung lag der Durchmesser bei 196 nm, der Polydispersitätsindex bei 0,109.

Beispiel 18

Die unverdünnte Amphotericin B-Emulsion aus Beispiel 17 wurde auf größere Partikel und Amphotericin B-Kristalle mittels Lichtmikroskop untersucht. Abbildung 4 zeigt nur wenige größere Tröpfchen, Amphotericin B-Kristalle konnten nicht detektiert werden.

Beispiel 19

Es wurden Emulsionen, wie in Beispiel 16 beschrieben, hergestellt, wobei jedoch 15 Homogenisationszyklen durchgeführt wurden. Es wurden zwei Dispersionen hergestellt, die 1 mg/ml und 5 mg/ml Amphotericin B enthielten. Die Emulsionen wurden mit Lichtmikroskopie untersucht. Die lichtmikroskopische Aufnahme der Dispersion mit 1 mg/ml zeigt ein Emulsionssystem ohne detektierbare Amphotericin B-Partikel (Abb. 5), in der Dispersion mit 5 mg/ml Amphotericin B sind neben den Emulsionströpfchen kleine Amphotericin B-Kristalle detektierbar (Abb. 6)

Beispiel 20

Es wurde eine Amphotericin B-Emulsion, wie in Beispiel 16, hergestellt. Die Emulsion wurde 20 Zyklen bei einer Produktionstemperatur von 65°C homogenisiert. Der mittlere PCS-Durchmesser betrug 255 nm, der Polydispersitätsindex 0,098. Die Partikelgröße wurde mittels Laserdiffraktometrie mit einem Coulter LS 230 (Coulter Electronics, USA) durchgeführt. Der Durchmesser 50% war 0,247 µm, der Durchmesser 90% 0,410 µm, der Durchmesser 99% 0,566 µm und der Durchmesser 100% 0,938 µm. Die Amphotericin B-Konzentration lag bei 1 mg/ml, Sterilisation wurde mittels Autoklavieren bei 121°C für 15 Minuten durchgeführt. Die Arzneistoffkonzentration wurde mit HPLC analysiert, wobei in zwei Proben 93,8% und 91,0% wiedergefunden wurden.

Beispiel 21

100 mg Cyclosporin wurden mit 40 g Lipofundin N 20% angerieben. Die Homogenisation wurde mit 20 Zyklen bei 1500 bar und 25°C durchgeführt. Der mittlere PCS-Durchmesser betrug 234 nm, der Polydispersitätsindex 0,099. Der Laserdiffraktometerdurchmesser D50% lag bei 0,218 µm, der D90% bei 0,381 µm und der D100% bei 0,721 µm. Mit Lichtmikroskopie konnten keine Cyclosporin-Partikel detektiert werden (polarisiertes Licht, Dunkelfeld). Das Zetapotential der Emulsion wurde in destillierten Wasser mit einer eingestellten Leitfähigkeit von 50 µS/cm (durch Zugabe von Natriumchlorid) gemessen. Die Feldstärke lag bei 20 V/cm, die Umrechnung der elektrophoretischen Mobilität in das Zetapotential erfolgte mit der Helmholtz-Smoluchowski Gleichung. Das Zetapotential betrug -51 mV.

Beispiel 22

Es wurde eine Cyclosporin-Emulsion wie in Beispiel 21 beschrieben hergestellt. Während der Produktion wurden jedoch 0,5% Cetylpyridiniumchlorid (CPC) zugefügt. Die Emulsion war positiv geladen, das Zetapotential betrug +32 mV.

Beispiel 23

Es wurde eine Cyclosporin-Emulsion, wie in Beispiel 21 beschrieben, hergestellt. Während der Produktion wurden jedoch 1,0% Stearylamin zugefügt. Der PCS-Durchmesser betrug 247 nm, der Polydispersitätsindex 0.088. Der Laserdiffraktometerdurchmesser 50% lag bei 0,229 µm, der Durchmesser 90% bei 0,389 µm und der Durchmesser 100% bei 0,721 µm. das Zetapotential betrug +24 mV.

Beispiel 24

Eine Cyclosporin-Emulsion wurde de novo hergestellt. Die Zusammensetzung bestand aus 0,1% Cyclosporin, 0,5% Poloxamer 188, 0,5%

Eilecithin Lipoid E80, 0,15% Stearylamin, 10% Miglyol 812 und 2,25% Glycerol als Isotonisierungszusatz und Wasser ad 100%. Das Lecithin wurde in der Öl-Phase dispergiert, eine Prä-Emulsion wurde unter Zusatz der anderen Bestandteile durch Hochgeschwindigkeitsrühren hergestellt, das Cyclosporin-Pulver wurde im letzten Schritt zugefügt. Diese Mischung wurde bei 45°C mit 20 Zyklen und 1500 bar homogenisiert. Der PCS-Durchmesser betrug 226 nm, der Polydispersitätsindex 0,111. Der Laserdiffraktometerdurchmesser 50% lag bei 0,200 µm, der Durchmesser 90% bei 0,406 µm und der Durchmesser 100% bei 1,154 µm. Die Emulsion war positiv geladen, das Zetapotential betrug +31 mV.

Beispiel 25

Eine O/W-Dispersion wurde produziert mit der Zusammensetzung von 10 g Wasserphase, die 25 mg Amphotericin enthielt, 0,5 g Span 85, 0,25 Tween 80 und Miglyol 812 ad 50 g. 1,0 ml Amphotericin Suspension (2,5% Amphotericin/ml), stabilisiert mit 2,4% Lecithin Lipoid E 80 wurden gemischt mit destilliertem Wasser auf ein Gesamtgewicht von 10 g. Tween 80 wurde zur Wasserphase hinzugefügt, Span 85 zur Ölphase. Das Wasser wurde im Öl durch hochtouriges Rühren dispergiert. Die erhaltene Prä-Emulsion wurde bei 90°C homogenisiert unter Anwendung von 1500 bar und 20 Homogenisationszyklen. Größenanalytik wurde durchgeführt mit Laserdiffraktometrie (Mastersizer E, Malvern Instruments, United Kingdom). Der Durchmesser 50% war 2,25 µm, der Durchmesser 90% 4,21 µm.

Erklärungen zu Abbildungen:

Abb. 1: Partikelgrößenverteilung des Amphotericin-Pulvers vor Einarbeitung in die Dispersion (oben) und Partikelgrößenanalyse der erfindungsgemäßen Dispersion nach Einarbeitung des Amphotericin-Pulvers (unten, Beispiel

2), die Arzneistoffpartikel sind nicht mehr detektierbar (Laserdiffraktometrie)

Abb. 2: Lichtmikroskopische Aufnahme des Amphotericin-Pulvers vor Einarbeitung in die O/W-Emulsion (Beispiel 1) (Polarisations-Aufnahme im Dunkelfeld, anisotrope Kristalle erscheinen weiß, Balken wie in Abb. 3 (10 µm)).

Abb. 3: Lichtmikroskopische Aufnahme der O/W-Emulsion nach Einarbeitung des Amphotericin-Pulvers aus Abb. 2 (Beispiel 1) (Polarisations-Aufnahme, im Dunkelfeld nur schemenhafte Reflexe der isotropen Emulsionstropfen, Balken 10 µm).

Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/ml Amphotericin B aus Beispiel 19.

Abb. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/ml Amphotericin B aus Beispiel 19.

Patentansprüche

1. Dispersion, die eine ölige Phase und eine wäßrige Phase in Form einer O/W-Emulsion oder einer Wasser-in-Öl (W/O) Emulsion, mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfaßt, dadurch gekennzeichnet, daß die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wäßrigen Phase der Emulsion ergibt.

2. Dispersion nach Anspruch 1, dadurch gekennzeichnet, daß der Arzneistoff zusätzlich zum gelösten Zustand noch in hochdisperser fester kristalliner Form vorliegt, wodurch sich eine Dispersion mir einer heterogenen dispersen Phase aus Öltropfen und aus Arzneistoffkristallen ergibt.

3. Dispersion nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß mindestens 90%, bevorzugter 95% der vorhandenen Kristalle kleiner als 5 µm sind und insbesondere 100% kleiner als 5µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie), wobei besonders bevorzugt 90% kleiner als 3 µm, bevorzugter 95% kleiner als 3 µm und insbesondere 100% kleiner als 3µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

4. Dispersion nach Anspruch 3, dadurch gekennzeichnet, daß mindestens 90%, bevorzugt 95% und insbesondere 99% der Kristalle kleiner als 1 µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

5. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Öl-in-Wasser-Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 99,5 Gew.-%, vorzugsweise 10 bis 95 Gew.-% insbesondere 60 bis 95 Gew.-% und speziell 70-95% wäßrige Phase enthält.

6. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Wasser-in-Öl (W/O) Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 25 Gew.-% insbesondere 10 bis 20 Gew.-% wäßrige Phase enthält.

7. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Emulgator und/oder Stabilisator enthält.

8. Dispersion nach Anspruch 7, dadurch gekennzeichnet, daß sie, bezogen auf die Gesamtmenge Dispersion, weniger als 15%, bevorzugt weniger als 10% und insbesondere weniger als 2%, bevorzugt 0,6% bis 1,2% Emulgator und/oder Stabilisator enthält.

9. Dispersion nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß sie als Emulgatoren Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Sorbitanestern (insbesondere Span 85), Polyethylenglykolsorbitanester (insbesondere Tween 80), Natriumglycocholat, Natriumlaurylsulfat (SDS) oder Gemischen derselben und/oder als Stabilisatoren Block-Copolymere, insbesondere Poloxamere (bevorzugt Poloxamer 188 und 407) oder Poloxamine (bevorzugt Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide (bevorzugt Hyaluronsäure oder Chitosan und ihre Derivate), Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (bevorzugt Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester (bevorzugt Saccharosemonostearat) oder Natrium-

citrat einzeln oder in irgendeiner Mischung derselben enthält.

10. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie eine O/W-Emulsion umfaßt und die zur Herstellung der Dispersion verwendete ölige Phase (Lipidphase) nur bei Raumtemperatur feste Lipide oder nur bei Raumtemperatur flüssige Lipide umfaßt oder eine Mischung aus einem oder mehreren bei Raumtemperatur flüssigen Lipiden mit einem oder mehreren bei Raumtemperatur festen Lipiden umfaßt.

11. Dispersion nach Anspruch 10, dadurch gekennzeichnet, daß die Mischung aus flüssigem Lipid und festem Lipid von 99 + 1 bis zu 1 + 99 variiert (Gewichtsteile), insbesondere in der Mischung der Anteil von flüssigem Lipid mindestens 10 Teile beträgt, bevorzugt mindestens 30 Teile und insbesondere mindestens 50 Teile.

12. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Partikel aus folgenden einzelnen Lipiden oder deren Mischungen hergestellt werden: natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben insbesondere synthetische Monoglyceride, Diglyceride und Triglyceride als individuelle Substanzen oder als Mischung, vorzugsweise Hartfett, oder Imwitor 900, Triglyceride, insbesondere Glyceroltrilaurat, Glycerolmyristat, Glycerolpalmitat, Glycerolstearat und Glycerolbehenat, und Wachse, insbesondere Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB), sowie Kohlenwasserstoffe, insbesondere Hartparaffin.

13. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als Ölphase Sojaöl, Safloröl, langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT), insbesondere Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride (bevorzugt wie in Stesolid) einzeln oder in Mischungen enthält.

14. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als wäßrige Phase Wasser, Mischungen von Wasser mit wassermischbaren organische Flüssigkeiten, insbesondere flüssigen Polyethylenglykolen (PEG) (bevorzugt PEG 400 und 600) enthält.

15. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die wäßrige Phase Zusätze enthält, insbesondere Elektrolyte, Nichtelektrolyte (bevorzugt Glycerol, Glucose, Mannit, Xylit zur Isotonisierung) und/oder Gelbildner (bevorzugt Cellulosederivate zur Viskositätserhöhung).

16. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die eingesetzte Emulsion eine O/W-Emulsion ist und Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid oder Salvilipid ist.

17. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Arzneiwirkstoffen zur Behandlung des menschlichen und tierischen Körpers.

18. Dispersion nach Anspruch 17, dadurch gekennzeichnet, daß sie einen oder mehrere Arzneistoffe aus den Gruppen der Anaesthetika, Antibiotika, Antimykotika, Antiinfektiva, Kortikoide, Hormone, Antioestrogene, Antispetika, gefäßaktive Substanzen, Glaukomittel, Beta-Blocker, Cholinergi-

ka, Sympathomimetika, Carboanhydrase-Hemmer, Mydriatika, Virustatika, Mittel zur Tumortherapie, Antiallergika, Vitamine, antiinflammatorische Wirkstoffe sowie Immunsuppressiva enthalten, insbesondere Cyclosporin, oder irgendeine Kombination daraus enthält.

19. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladen ist.

20. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladene Stabilisatoren enthält, insbesondere Natriumlaurylsulfat (SDS), Stearylamin, und/oder positiv geladene Phospholipide und/oder positiv geladene Lipide.

21. Dispersion nach Anspruch 20, dadurch gekennzeichnet, daß sie die eingesetzte Emulsion eine O/W-Emulsion ist und intravenös appliziert werden kann, wobei neben positiven Stabilisatoren auch Mischungen mit Lecithin und/oder nichtionischen Stabilisatoren eingesetzt werden können, insbesondere Poloxamer Polymere.

22. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Cyclosporin als Wirkstoff enthält.

23. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß als Wirkstoff ein Antimykotikum (bevorzugt Amphotericin B), ein Antiinfektivum (bevorzugt Buparvaquon oder Atovaquon), ein Immunsuppressivum (bevorzugt Cyclosporin A oder eines seiner natürlichen und synthetischen Derivate), ein Mittel zur Tumortherapie (bevorzugt Paclitaxel oder Taxotere) enthält.

24. Verfahren zur Herstellung einer Zusammensetzung gemäß einem der Ansprüche 1 bis 23, dadurch gekennzeichnet, eine wäßrige Phase und eine ölige Phase, die nicht oder nur

teilweise miteinander mischbar sind, sowie gegebenenfalls ein oder mehrere Emulgator(en) und/oder Stabilisator(en) und eine feste Phase, die mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff umfaßt, miteinander gemischt werden und die erhaltene Mischung aus flüssigen und festen Phasen einem hochenergetischen Homogenisationsprozeß mit einem Homogenisator unterzogen werden, wobei keine toxikologisch bedenklichen organischen Lösungsmittel verwendet werden.

25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß der Wirkstoff ohne vorherige Auflösung als Feststoff in die flüssigen Phasen der Dispersion eingearbeitet wurde.

26. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff mit einer O/W-Emulsion oder einer W/O-Emulsion angerieben oder gemischt wird und diese Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

27. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff in einer Emulgatorlösung dispergiert wird, diese Dispersion homogenisiert wird, anschließend mit einer O/W-Emulsion oder einer W/O-Emulsion gemischt wird und die so erhaltene Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

28. Verfahren nach einem der Ansprüche 24 bis 27, dadurch gekennzeichnet, daß als Homogenisator ein Rotor-Stator-Homogenisator (vorzugsweise eine Kolloidmühle) oder ein Hochdruckhomogenisator (vorzugsweise ein Kolben-Spalt-Homogenisator (APV Gaulin, French Press, Niro, Stansted) oder ein Rohrhomogenisator (jet stream) (Microfluidizer oder Nanojet)) eingesetzt wird.

29. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß sich der Wirkstoff am Ende des Homogenisationsprozesses vollständig oder nahezu vollständig aufgelöst hat, so daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10 Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

30. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

31. Verfahren nach einem der Ansprüche 24 bis 30, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 5 µm, bevorzugt einen Durchmesser 95% kleiner als 5 µm und insbesondere einen Durchmesser 100% kleiner als 5µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

32. Verfahren nach Anspruch 31, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 3 µm, bevorzugt einen Durchmesser 95% kleiner als 3 µm und insbesondere einen Durchmesser 100% kleiner als 3µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

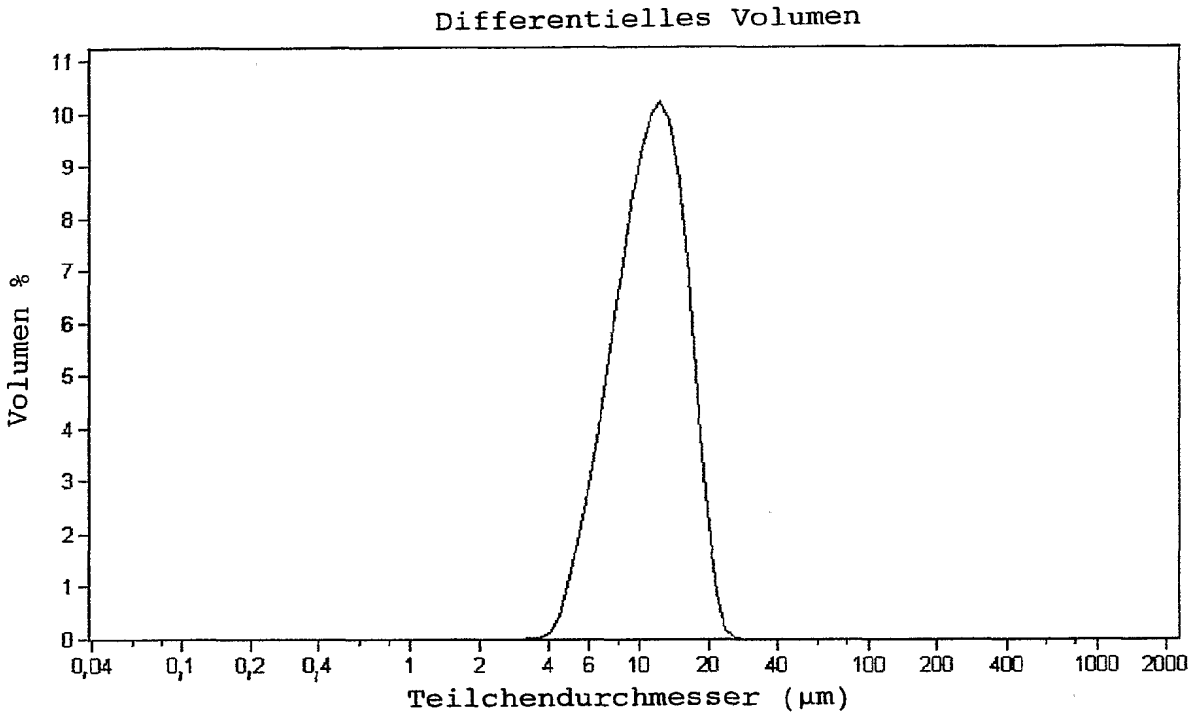
33. Verfahren nach Anspruch 32, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen mit Photonenkorrelationsspektroskopie (PCS) bestimmten Durchmesser kleiner als 1000 nm aufweisen.

34. Verwendung der Dispersion gemäß einem der Ansprüche 1 bis 23 oder hergestellt gemäß einem der Ansprüche 24 bis 33 zur Herstellung eines Arzneimittels.

35. Verwendung nach Anspruch 34, zur Herstellung eines Arzneimittels zur Behandlung von Mykosen, vorzugsweise systemischen Mykosen, Entzündungen, Allergien, Tumorerkrankungen, kardiovaskulären Erkrankungen, viralen und anderen Infektionen und zur Durchführung von Anästhesien.

36. Verwendung nach Anspruch 34 oder 35, dadurch gekennzeichnet, daß das Arzneimittel topisch, oral, peroral sowie parenteral, insbesondere intravenös, intra- und subkutan, intramuskulär, intraartikulär oder intraperitoneal wird, vorzugsweise am Auge angewendet wird und vorzugsweise Cyclosporin enthält.

37. Verwendung nach einem der Ansprüche 34 bis 36, dadurch gekennzeichnet, daß das Arzneimittel eine verlängerte Verweilzeit im Blut zeigt, verglichen mit negativ geladenen Dispersionen.


Differentielles Volumen
Volumen %
0
1
2
3
4
5
6
7
8
9
10
11
0,04
0,1
0,2
0,4
1
2
4
6
10
20
40
100
200
400
1000
2000
Teilchendurchmesser (µm)

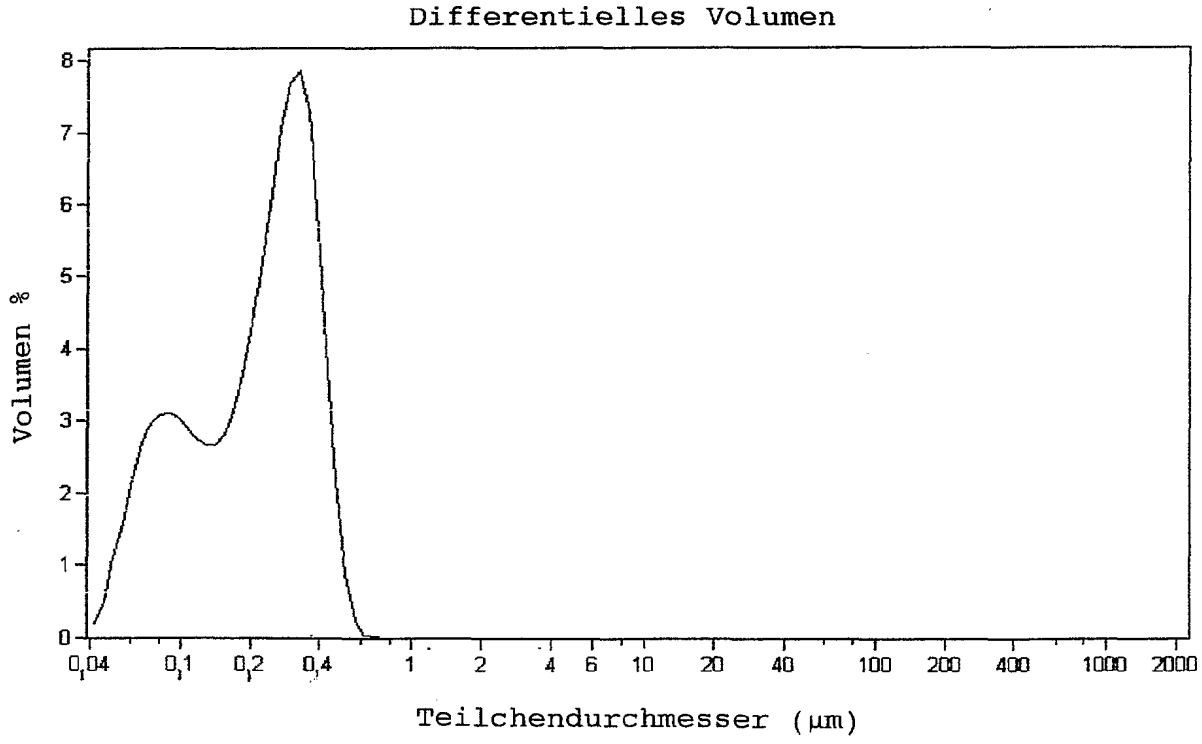

Differentielles Volumen
Volumen %
0
1
2
3
4
5
6
7
8
0,04
0,1
0,2
0,4
1
2
4
6
10
20
40
100
200
400
1000
2000
Teilchendurchmesser (µm)

Abbildung 1

Abbildung 2

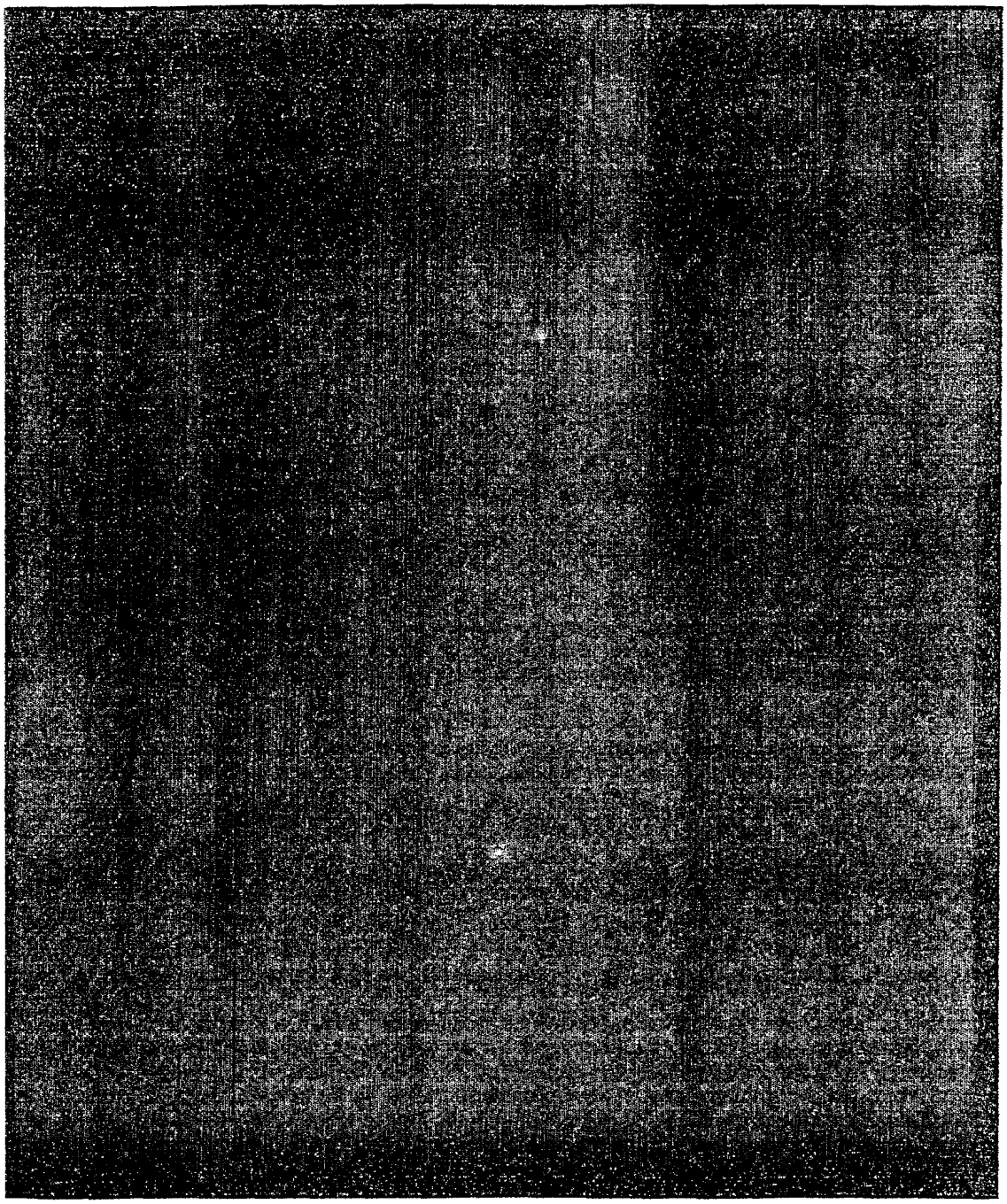


Abbildung 3

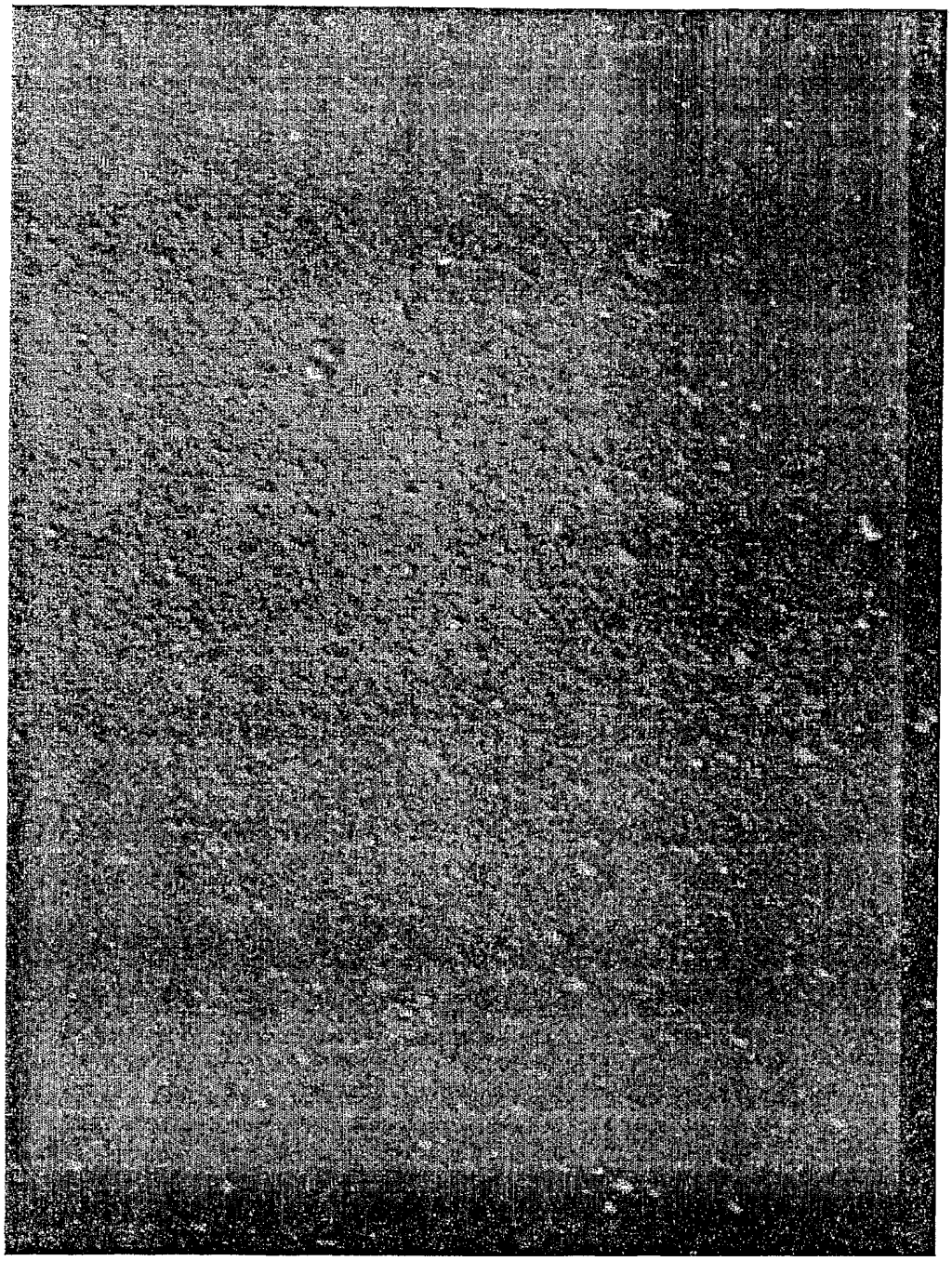


Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

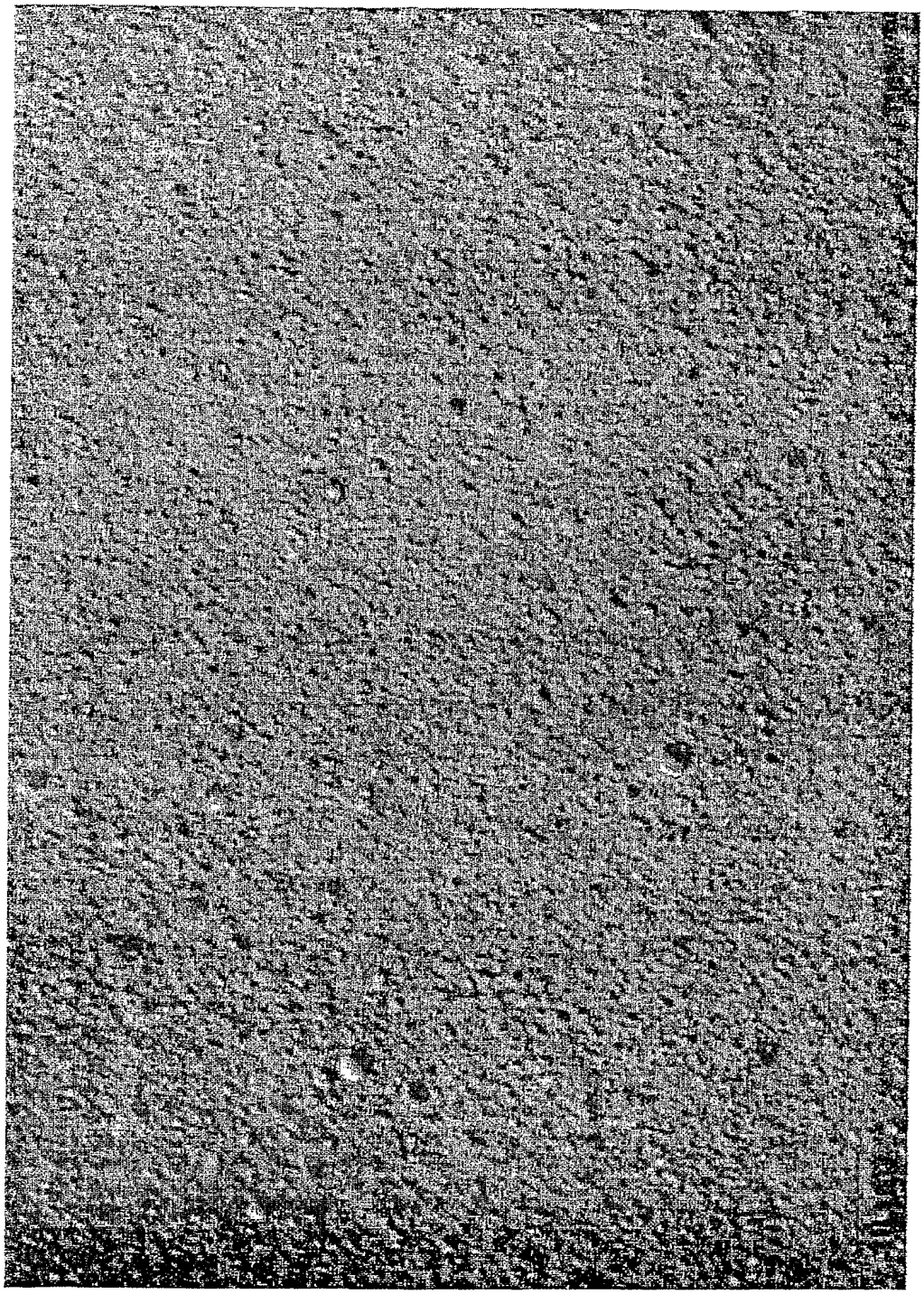


Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/mL Amphotericin B aus Beispiel 19.

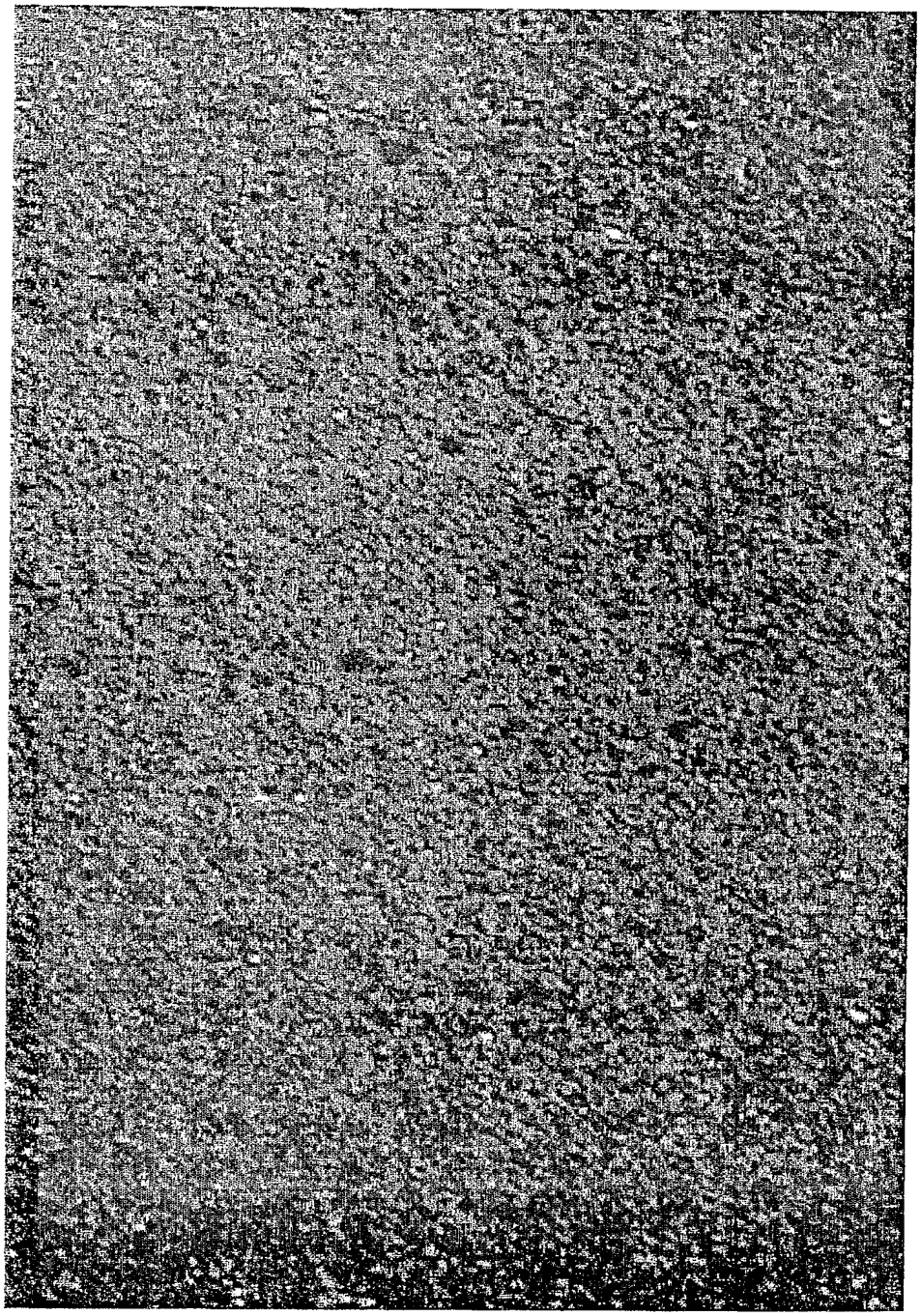


Fig. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

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(54) Title: COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

(57) Abstract: The present invention discloses a topical composition for prevention and alleviation of wrinkling which comprises one or two or more selected from the group consisting of Phenytoin, Valproic acid, Cyclosporin A, Nifedipine, Diltiazem, Verapamil HC1 and Amolldipine as an active ingredient having an effect of boosting collagen synthesis.

COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

Technical Field

The present invention relates to a topical composition for prevention and alleviation of skin wrinkles which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, in conjunction with conventional components of a formulation for transdermal absorption such as cream, ointment, lotion, skin tonic, gel, pack, patch or patch-type administering apparatus.

Background Art

Skin aging is developed by both endogenous causes, for example, aging, and environmental causes. The effects of aging are shown as wrinkles in the skin, which include neck wrinkles, worry lines, frown lines, crow's feet, the folds from the side of the nose to the corners of the mouth, and fine lines around the eyes, below the lips, and over the face. Skin wrinkles caused by aging, though there are individual differences, commonly occur in individuals in their early twenties and increase with age. With aging, the amount of dermal collagen of skin is decreased and alterations in elastic fibers occur, whereby the skin relaxes and fine wrinkles appear. Meanwhile, collagen is a major matrix protein produced by fibroblasts of the skin, being present in the extracellular matrix. It is a primary protein comprising 30 % by weight of proteins in the human body, and has a firm structure of a triple helix. It is known that collagen functions to provide structural stability to the skin, durability of connective tissues and cohesion of tissues while supporting cell coherence, cell proliferation, and induction of differentiation of unspecialized cells. Also, it is known that collagen is broken down by exposure to UV, an

environmental cause of skin aging, and the damage by UV is proportional to the accumulated time of exposure thereto. UV denatures collagenous fibers, causing wrinkles and decreasing elasticity of the skin. Other environmental causes known to promote skin aging include wind, heat and smoking.

As mentioned above, collagen is closely related with skin aging. The amount of collagen in the dermis is decreased with aging and by UV radiation. Collagen decreases by 65 % from age 20 to age 80. Such a decrease of collagen makes the skin thin and further, is closely associated with the formation of skin wrinkles.

Studies have been widely performed to find a method for the prevention and alleviation of skin wrinkles, elucidating important roles of collagen. The studies also elucidated that when collagen synthesis is activated in skin, dermal matrix components are increased, which has effects including alleviation of wrinkles, and increased elasticity and strength of skin. Therefore, using collagen having a moisture retention effect, some collagen-incorporated cosmetics have been developed. Such cosmetics, however, are poor in holding moisture, since the cosmetics are applied to the surface of skin and high molecular weight collagen is poor in transdermal absorption. As a result, their use fails to provide an intrinsic improvement in skin appearance. In the prior art, retinoic acid, TGF-β , protein derived from an animal placenta (JP8-231370), betulinic acid (JP8-208424) and *Chlorella* extract (JP9-40523, JP10-36283) are disclosed as substances for promoting collagen synthesis. As for retinoic acid, it is unstable and has a problem in its safety due to causing irritation and redness upon application the skin, limiting the available dosage thereof. As for other above substances including *Chlorella* extract, their effects of increasing collagen synthesis are weak, so they hardly improve skin appearance. Recently, several new procedures for treating wrinkles by promoting collagen synthesis have been introduced. Examples include ultrasonic treatment, skin scaling, laser peeling, botulinum toxin injection and Restilene injection. These procedures, however, have disadvantages in terms of cost effectiveness and duration of their effects. Thus, it is desirable to search for and develop a

highly effective agent for promoting collagen synthesis.

Disclosure of the Invention

Therefore, the present inventors have conducted studies to develop a compound having an effect of promoting collagen synthesis, and found that phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine which are already known as anticonvulsants, immunosuppressants or calcium channel inhibitors have very strong effects of promoting collagen synthesis in human fibroblast cell lines. Further, it was found that as applied to the skins of rats and mice, the compounds exhibited strong inhibition and alleviation effects of wrinkles, proving the effects of inhibiting and preventing signs of skin aging such as skin wrinkles. Accordingly, the present invention is directed to a composition comprising phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl or amoldipine as an active ingredient having an effect of promoting collagen synthesis.

Phenytoin and valproic acid have been widely used as anticonvulsants for treatment of epilepsy, and their effects on collagen synthesis are documented (USP5686489; Minerva Stomatol., 47(9): 387-398, Sep. 1998). Cyclosporine A has been widely used as an immunosuppressant for suppressing rejection of tissues after transplantation, and its effects on collagen synthesis are reported (J Periodontol., 72(7): 921-931, Jul. 2001). Nifedipine, diltiazem, verapamil HCl and amoldipine have already been used as calcium channel inhibitors, and their effects on collagen synthesis are also reported (J Periodontol., 72(8), Aug. 2001; Proc Natl Acad Sci USA, 93(11): 5478-5482, May 1996; J Urol., 156(6): 2067-2072, Dec. 1996). However, the above drugs are not disclosed for use as topical agents applied to the skin for preventing and alleviating skin wrinkles, as in the present invention.

Hereinafter, a topical composition for preventing and alleviating skin wrinkles will be described in detail, in conjunction with experimental

examples and examples.

Experimental example 1: Effect of active ingredients of the invention on promoting collagen production in fibroblasts

To investigate the effects of active ingredients of the invention on promoting collagen production in fibroblasts in cellular level, respective active ingredients were added to cultures of fibroblasts derived from a human. The synthesized collagen was measured using a modification of a method proposed by Martens (Gut, 33: 1664-1670, 1992) to evaluate the effects of the active ingredients. The experimental protocol in detail is as follows.

Human-derived fibroblasts were transferred to a 24 well plate and cultured in a medium containing 10 % fetal bovine serum (FBS) for 24 hours, followed by washing twice with phosphate buffered saline. The cells were then incubated in a medium containing 1 % FBS in the presence of phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl or amoldipine at final concentrations of 10^{-8} to 10^{-5} M. After 1 hr incubation, cultures were added with 10 μ Ci of ^{3}H-proline per well, followed by a final incubation for 24 hours. After the incubation was terminated, cells from each group were harvested and two fractions of each culture were prepared. One fraction from each culture was treated with collagenase. To all fractions was added trichloroacetic acid to precipitate proteins. The amount of radioactivity incorporated into collagenase-sensitive protein was measured and compared with that of the other fraction which was not treated with collagenase. The difference in radioactivity was attributed to the promoting effect of the compound. Samples without an active ingredient served as a control group, the amount of collagen synthesized being 100 %. The results are shown in Table 1.

Table 1: Effect of promoting collagen production in fibroblasts (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	215.28*	298.35	360.65	381.54
Valproic acid	100.00	201.13	283.24	332.11	370.21
Cyclosporine A	100.00	212.11	293.21	352.31	372.27
Nifedipine	100.00	204.31	292.21	330.30	358.16
Diltiazem	100.00	199.15	276.25	321.23	362.12
Verapamil HCl	100.00	183.25	280.23	331.09	355.12
Amoldipine	100.00	182.42	280.07	330.42	355.26

* Rate of collagen production = (collagen production of experimental group/ collagen production of control group) x 100

As shown in Table 1, the active ingredients in experimental groups have effects of promoting collagen production with increasing concentration of the compounds, ranging from the minimum of 182.42 % to the maximum of 381.54 % in a dose-dependent manner, compared to the control group which contains no active ingredient of the invention. This demonstrates that the active ingredients of the invention have excellent effects on promoting collagen synthesis.

Experimental example 2: Promotion of collagen production in rat skin

The effects of application of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on promoting collagen production in animal skin were investigated. The synthesized collagen was measured using a modification of a method proposed by Mard L DaCosta et al. (Surgery, 123: 287-293, 1998).

In brief, 5-week male SD rats were grouped with 5 rats per group. The rats were each incised 1 cm in the center of their abdomens and PVA sponges (Unipoint ind.) were inserted therein. After suturing, as for

experimental groups, respective active ingredients to be examined were applied to the PVA sponge-embeded regions in a volume of 200 ㎕ every day for 10 days. Upon autopsy, the PVA sponge was removed to quantify hydroxyproline. The PVA sponge was added with 4 ㎖ of 6 N HCl, hydrolized at 130℃ for 3 hours and was subjected to complete drying. 50 ㎕ of methanol was added and the solution was incubated at 110℃ until HCl was removed. 1.2 ㎖ of 50 % isopropanol was added to dissolve the remaining precipitate. 200 ㎕ of chloramine-T (sodium p-toluensulfochloramide trihydrate) solution was added while stirring, and let stand for 10 min. After adding 1.2 ㎖ of Ehrlich reagent and mixing, the solution was incubated at 50℃ for 90 min. The resulting solution was cooled to room temperature and absorbance at 558 ㎚ was measured. Hydroxyproline standard solutions were prepared by dissolving 1 ㎎ hydroxyproline in 1 ㎖ HCl and diluting it to concentrations of 0, 0.2, 0.4, 0.8, 1 ㎎ each relative to 25 ㎕ of 6 N HCl. The standard solutions were hydrolyzed at 130℃ for 3 hours. The quantified value of hydroxyproline, relative to hydroxyproline value (100 %) of the control group which was applied with solvent only, are shown in Table 2.

Table 2: Effect of promoting collagen production in animal skin (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	132.58*	143.51	167.41	182.47
Valproic acid	100.00	128.05	139.24	157.72	178.13
Cyclosporine A	100.00	131.02	143.07	164.82	179.26
Nifedipine	100.00	129.92	142.41	161.43	185.88
Diltiazem	100.00	122.44	136.76	157.45	175.23
Verapamil HCl	100.00	135.63	147.39	167.06	183.32
Amoldipine	100.00	132.50	149.65	163.84	181.12

* Rate of collagen production = (hydroxyproline value of experimental group/hydroxyproline value of control group) x 100

As shown in Table 2, the active ingredients increased collagen production in rat skin and the rates of increase ranged from the minimum of 122.44 % to the maximum of 185.88 %, compared to the control group to which no active ingredient of the invention was applied. This demonstrates that the active ingredients of the invention strongly promote dermal collagen synthesis.

Experimental example 3: Effect on inhibiting the generation of wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on inhibiting the generation of wrinkles in hairless mice were investigated.

6-week hairless mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective compounds at a concentration of 10^{-8} to 10^{-3} M. The control groups were applied with solvent only, without any active ingredient. The experimental protocol in detail is as follows. Hairless mice were radiated using simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 12 weeks, thereby generating wrinkles. Respective active ingredients or the solvent only were applied twice every day (specifically, on radiation days, the application was performed at 30 min before and after the radiation), at a volume of 100 $\mu\ell$ each for 10 weeks from the first radiation day. Degrees of inhibition of generated wrinkles were determined. The determination was performed by visual observation with naked eyes and photography. The degrees of inhibition of wrinkles in the compound-treatment groups (experimental groups) were compared with the control group (Score 0) and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 3a to 3c.

Table 3a: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-8} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	2	8
Exp. 2	Valproic acid	0	1	1	8
Exp. 3	Cyclosporine A	0	1	2	7
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	1	8
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	10	0	0	0

Table 3b: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-5} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	1	3	6
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	10	0	0	0

Table 3c: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-3} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	0	2	8
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	0	1	9
Exp. 7	Amoldipine	0	0	2	8
Control 1	-	10	0	0	0

As shown in Tables 3a to 3c, the active ingredients inhibited the generation of wrinkles by a high degree in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on inhibiting the generation of wrinkles.

Experimental example 4: Effect of alleviating wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on alleviating photo-induced wrinkles in 6-week hairless mice were investigated.

Mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective active ingredients at a concentration of 10^{-8} to 10^{-3} M. The control groups were the mice applied with solvent only without any active ingredient. The experimental protocol is as follows. Hairless mice were radiated using a simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 10 weeks, thereby generating wrinkles. Then, respective active ingredients or the solvent only were applied at a volume of 100 $\mu\ell$ each, twice a day for 6 weeks. Degrees of wrinkle

reduction were determined. The determination was performed by visually observing the compound-applied region with naked eyes, and the region was photographed. The degrees of alleviation of wrinkles in the compound-treatment groups (experimental groups) were compared with those of the control group and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 4a to 4c.

Table 4a: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-8} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	2	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	3	5
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	2	6
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	9	1	0	0

Table 4b: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-5} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	3	6
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	1	7
Exp. 7	Amoldipine	0	2	2	6
Control 1	-	9	1	0	0

Table 4c: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-3} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	0	2	8
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	8	2	0	0

As shown in Tables 4a to 4c, the active ingredients exhibited a high level of alleviation effects on the photo-induced wrinkles in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on alleviating wrinkles.

The results from the experiments employing the active ingredients of the invention for evaluating effects of promoting collagen synthesis in fibroblasts derived from human, rats and mice demonstrate that phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine with concentrations of 10^{-8} to 10^{-3} M each have excellent effects of promoting collagen synthesis.

A topical composition comprising an active ingredient of the invention may include any formulations applicable to skin, for example, cream, ointment, lotion, skin tonic, gel, pack, aerosol types thereof, patch and patch-type apparatus with micro needles. The compositions were especially prepared in formulations of cream, ointment and pack and applied to human skin for evaluating reduction of wrinkles. It was found that they significantly reduce wrinkle density.

Hereinafter, the present invention will be described in detail, in conjunction with examples and comparative examples. It is noted that these

examples are provided only for illustrative purposes, and the present invention is not to be construed as being limited to those examples.

Preparation of variable formulations comprising an active ingredient of the invention

Agents topically applicable to the skin were prepared with compositions given in Tables 5 to 7, employing each active ingredient and other supplementary components according to the invention. In the invention, ointment, cream, pack, essence, skin softner, nutrient emulsion, patch and patch-type apparatus with micro needles, each topically applicable to the skin, were prepared. It is noted that though only formulations employing phenytoin and cyclosporine A as active ingredients were prepared herein, the examples are not intended to limit the formulations and active ingredients.

Table 5: Formulation of ointment

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Diethyl Sebacate	8	8	8	8	8
Spermaceti	5	5	5	5	5
Polyoxyethylene*	6	6	6	6	6
Sodium benzoate	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.1	-	-	-
Cyclosporine A	-	-	0.00001	0.1	-
Total weight with Vaseline added	100	100	100	100	100

* Polyoxyethylene oleic ether phosphate

Table 6: Formulation of cream

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Stearic acid	15.0	15.0	15.0	15.0	15.0
Setanol	1.0	1.0	1.0	1.0	1.0
Potassium hydroxide	0.7	0.7	0.7	0.7	0.7
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	3.0	3.0	3.0	3.0	3.0
Preservative	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 7: Formulation of pack

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	4.0	4.0	4.0	4.0	4.0
Polyvinyl alcohol	15.0	15.0	15.0	15.0	15.0
Ethanol	8.0	8.0	8.0	8.0	8.0
Polyoxyethylene oleic ethyl	1.0	1.0	1.0	1.0	1.0
Paraoxy methyl benzoate	0.2	0.2	0.2	0.2	0.2
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.1	0.5	-	-	-
Cyclosporine A	-	-	0.1	0.5	-
Total weight with purified water added	100	100	100	100	100

Table 8: Formulation of essence

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Cyclometicon	15.0	15.0	15.0	15.0	15.0
Caprilic/capric triglyceride	3.0	3.0	3.0	3.0	3.0
Mineral oil	3.0	3.0	3.0	3.0	3.0
beeswax	1.0	1.0	1.0	1.0	1.0
Cetyl dimethicone copolyol	3.0	3.0	3.0	3.0	3.0
Glycerin	5.0	5.0	5.0	5.0	5.0
Magnesium sulfate	3.0	3.0	3.0	3.0	3.0
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Phenytoin	0.01	0.05	-	-	-
Cyclosporine A	-	-	0.01	0.05	-
Total weight with purified water added	100	100	100	100	100

Table 9: Formulation of skin softner

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	2.0	2.0	2.0	2.0	2.0
Hyaluronic acid	1.0	1.0	1.0	1.0	1.0
Polyoxyethylene oleic ether	0.1	0.1	0.1	0.1	0.1
Polyoxyethylene hydrogenated castor oil	0.1	0.1	0.1	0.1	0.1
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 10: Formulation of nutrient emulsion

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Setanol	1.0	1.0	1.0	1.0	1.0
Beeswax	0.5	0.5	0.5	0.5	0.5
Vaseline	2.0	2.0	2.0	2.0	2.0
Squalene	6.0	6.0	6.0	6.0	6.0
Ethanol	3.0	3.0	3.0	3.0	3.0
1,3-butyleneglycol	4.0	4.0	4.0	4.0	4.0
Polysolbait 60	1.0	1.0	1.0	1.0	1.0
Solbitan sesqi oleate	0.3	0.3	0.3	0.3	0.3
Carboxy-vinylpolymer	0.3	0.3	0.3	0.3	0.3
Triethanol amine	0.3	0.3	0.3	0.3	0.3
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 11: Formulation of patch

(unit: weight %)

Component	Compound	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Polymer	carboxymethylcellulose polyacrylic acid	1 2	1 2	1 2	1 2	1 2
Cross link agent	acetaldehyde	0.1	0.1	0.1	0.1	0.1
Humectant	glycerin	30	30	30	30	30
Inorganic filling agent	caolin	0.1	0.1	0.1	0.1	0.1
Preservative	paraoxy methyl benzoate paraoxy propyl benzoate	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05
Buffer	monosodium phosphate	0.1	0.1	0.1	0.1	0.1
	sodium tripoly phosphate	0.05	0.05	0.05	0.05	0.05
Active ingredient	phenytoin cyclosporine A	0.01 -	0.05 -	- 0.01	- 0.05	- -
Total weight with purified water added		100	100	100	100	100
Support		cotton	cotton	cotton	cotton	cotton
Protective film		silicon	silicon	silicon	silicon	silicon

With regard to a patch-type apparatus with micro needles, a main body of the patch apparatus, a reservoir which contains a solvent for a drug, is comprised of a polymer support for securing an entire patch type apparatus as well as preventing a drug such as phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine from being permeable thereto. The solvent for a drug may be water, polyethyleneglycol, transcutol or ethanol and is contained inside the reservoir. As for the polymer support, polyethylene, polypropylene, non-woven fabric or cotton fabric are available. The drug mentioned above is dispersed in powdered form in a lower part of the reservoir. The patch-type apparatus is an instrument for administering a drug transdermally, characterized by further comprising a support for micro needles and a number of micro needles. As for a support for micro needles, it is made of a polymer gel such as celluloses, polypropylene, fluorocarbon or polycarbonate and it has a swelling property

as the solvent is released after adhesion to the skin. As for micro needles, they are distributed and fixed perpendicular to the support for micro needles, and they come into contact with the skin. More particularly, 10 to 50 micro needles are attached per unit area (㎠) of the support for needles and each has a channel through which a drug can pass, the channel being 1 to 1000 ㎛ in diameter and the needles being fixed outward 0.01 to 1 ㎜ in length. The apparatus has an adhesive layer at its lower part which has a role in adhering the apparatus to the skin, the adhesive layer being made of a material such as polyacrylate or polybutene. It should be noted that the adhesive layer has no adverse effects on skin and is not dissolved by a solvent. Further, no decrease in adhesive property by a solvent is permitted. Finally, there is a protective film attached to the adhesive layer film, which is easily removable upon using the apparatus, to prevent a drug from being leaked, and protecting an adhesive. Hereinafter, the present invention will be described in detail in conjunction with examples, not to be construed as being limited to those examples.

Preparation of patch-type apparatus with micro needles comprising an active ingredient of the invention

Comparative example 1

1 g of 3 % gelatin solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A comparative matrix was thus obtained.

Example 1

0.001 % phenytoin was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A phenytoin-dispersed matrix was thus obtained.

Example 2

0.001 % cyclosporine A was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A cyclosporine A-dispersed matrix was thus obtained.

Evaluation of prevention and treatment effects on skin aging by a composition comprising an active ingredient of the invention

To evaluate the effect of the formulations prepared in above comparative example and examples including the examples as set forth in Tables 5 to 11 on alleviating skin wrinkles, female subjects aged 35 - 60 were employed. 760 females were placed into 38 groups, 20 subjects per group. Respective examples and comparative examples were applied to the face twice per day for 3 months (in case of the packs of Table 7, they were removed 30 min after application). The degrees of alleviating wrinkles were determined by a survey and an image analysis of wrinkles after 3 months. As for the survey, the degrees of alleviation of wrinkles and increase of elasticity were determined as one of 4 stages, that is, none, slight, moderate and high, as compared with the conditions before applying respective compositions, and the corresponding subjects were counted. The data are shown in Tables 12. For the evaluation by an image analysis of wrinkles, one replica of the region right below the eye of each subject was taken using Xantopren (Bayer) before beginning the experiment. Another replica was taken in the same region immediately after finishing the experiment. The replicas were subjected to an image analysis. Wrinkle density was measured by a two dimensional analysis. The measurements were represented as decrease rates, relative to wrinkle densities before the experiment. The results are shown in Table 13.

Table 12: Alleviation of wrinkles in human females

Degree of alleviation	Example	None	Slight	Moderate	High
Ointment	Ex. 1	1*	3	4	12
	Ex. 2	0	2	4	14
	Ex. 3	0	4	7	9
	Ex. 4	0	3	6	11
	Comp. Ex. 1	17	3	0	0
Cream	Ex. 1	0	1	8	11
	Ex. 2	0	1	6	13
	Ex. 3	0	2	7	11
	Ex. 4	0	3	7	10
	Comp. Ex. 1	13	7	0	0
Pack	Ex. 1	0	0	9	11
	Ex. 2	0	2	5	13
	Ex. 3	0	3	4	14
	Ex. 4	0	1	5	14
	Comp. Ex. 1	15	5	0	0
Essence	Ex. 1	0	2	5	13
	Ex. 2	0	0	5	15
	Ex. 3	0	3	6	11
	Ex. 4	0	1	7	12
	Comp. Ex. 1	12	7	1	0
Skin softner	Ex. 1	0	2	8	10
	Ex. 2	0	1	7	12
	Ex. 3	0	3	7	10
	Ex. 4	0	4	5	11
	Comp. Ex. 1	16	4	0	0
Nutrient emulsion	Ex. 1	0	1	5	14
	Ex. 2	0	0	5	15
	Ex. 3	0	1	7	12
	Ex. 4	0	2	7	11
	Comp. Ex. 1	13	7	0	0
Patch	Ex. 1	0	1	4	15
	Ex. 2	0	0	4	16
	Ex. 3	0	1	7	12
	Ex. 4	0	1	9	10
	Comp. Ex. 1	15	5	0	0
Micro-needle patch	Ex. 1	0	1	4	15
	Ex. 2	0	1	9	10
	Comp. Ex. 1	15	5	0	0

*: the number of subjects counted

Table 13: Effect of decreasing wrinkle density in human females

Example	Ointment	Cream	Pack	Essence	Skin softner	Nutrient emulsion	Patch
Ex. 1	45 %	43 %	40 %	39 %	44 %	45 %	46 %
Ex. 2	44 %	41 %	38 %	37 %	42 %	43 %	48 %
Ex. 3	50 %	40 %	41 %	40 %	48 %	46 %	45 %
Ex. 4	48 %	50 %	44 %	39 %	45 %	42 %	44 %
Comp. Ex. 1	98 %	98 %	94 %	97 %	99 %	98 %	96 %

As shown in Table 12, the examples according to the invention provide excellent effects of alleviating wrinkles and increasing skin elasticity. Specifically, more than 80 % showed high levels of improving effects. As shown in Table 13, when the examples comprising an active ingredient of the invention were applied to the subjects, wrinkle densities were considerably decreased to about 37 to 50 %, compared to that before the experiment. Also, when a patch-type administering apparatus with micro needles was applied, examples 1 and 2 exhibited significant decreases in wrinkle densities, 70 % and 60 % respectively, indicating that the examples are superior to the comparative example (98 %) (data not shown).

The above experimental results demonstrate that when the active ingredients of the invention are topically applied to the skin in the form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type apparatus with micro needle, skin wrinkles generated by intrinsic or extrinsic causes are effectively alleviated.

Industrial Applicability

As apparent from the above description, the present invention provides a topical composition which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, exhibiting the effects of inhibiting, alleviating and preventing skin aging, such as skin wrinkles.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

Claims:

1. A topical composition comprising one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis for prevention and alleviation of skin wrinkles.

2. The composition as set forth in claim 1, wherein the active ingredient is contained at an amount of 0.00001 to 30.00 % by weight, relative to the total weight of the composition.

3. The composition as set forth in claim 1 or claim 2, wherein the composition is formulated in a form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type administering apparatus with micro needles.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Korean patents and applications for inventions since 1975

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

CAPLUS(STN), SCISEARCH(STN), PASCAL(STN), BIOTECHNO(STN), INVESTEXT(STN), JICST-EPLUS(STN), KOSMET(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WOLF J. S. JR; SOBLE J. J.; RATFLIFF T. L.; CLAYMAN R. V."Ureteral cell cultures II : Collagen production and response to pharmacologic agents", Journal of urology, USA, 1996, Vol.156, No.6, p.2067-72	1-3
A	MOLONEY, STEPHEN J & LEARN DOUGLAS B, " The effect of systemic cyclosporin A on a hairless mouse model of photoaging", Photochemistry and Photobiology, UK, 1992, Vol.56, No.4, p 495-504	1-3
A	US 5686489 A (Tristrata Technology, Inc.) 11 NOV 1997 cited in the application	1-3

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

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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		EP 1053745 A1	22. 11. 2000
		FR 2793681 B1	22. 06. 2001
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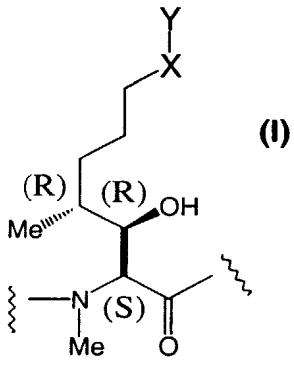
(54) Title: CYCLOSPORIN ANALOGS FOR THE TREATMENT OF LUNG DISEASES



(I)

(57) Abstract: The present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof. In formula (I), the formula for residue A is formula (II), where X is absent, -C1-C6 alkyl-, or -C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH_2-OC(O)R2; -C(S)-O-R1; and -C(S)- S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, 15 heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is -(D)Ala-, -(D)Ser- or -[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or -[O-(2-acyloxyethyl)(D)Ser]-. In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or pharmaceutically acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

WO 03/030834 A2

Cyclosporin Analogs for the Treatment of Lung Diseases

Technical Field

The present invention relates to novel cyclosporin analogs and methods for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

Background of the Invention

Respiratory diseases, such as asthma and other diseases characterized by airflow obstruction, are a global problem. Millions of people worldwide, both children and adults, suffer from these medical conditions. These diseases reduce quality of life by impairing the ability of sufferers to perform everyday tasks, and in some cases, cause death. One of the major respiratory diseases is asthma.

Asthma is a disease of unknown etiology in which the bronchi are inflamed and as a consequence obstructed. This narrowing results from a combination of bronchial smooth muscle contraction, mucosal oedema, inflammatory cell infiltrate and partial or total occlusion of the lumen with mucus, cells and cell debris. Bronchial obstruction is either partially or totally reversible, and this important feature distinguishes asthma from chronic bronchitis.

Asthma is an extremely common disease with a worldwide prevalence of 5% to 8%. In the developed world it is the most common chronic illness and, for reasons that are unclear, the disease is on the increase. It is now accepted that asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction. This type of airflow obstruction is often reversible either spontaneously or with treatment and causes associated increase in airway responsiveness to a variety of stimuli.

The illness has a wide clinical spectrum ranging from mild episodic bronchospasm (easily controlled by the occasional use of a bronchodilator) to a very severe intractable asthma sometimes resistant to treatment with high doses of

oral corticosteroids. Steroid resistance occurs in less than 5% of people with asthma. This translates to thousands of people. These patients with severe chronic disease may be dependent on corticosteroids and their disease is often so severe that full reversibility can be difficult or impossible to demonstrate.

Chronic obstructive airways disease, chronic obstruction lung disease and 'smoker's chest' have all been used to describe what is now known as COPD. COPD is characterized by progressive irreversible airway obstruction. It can lead to death from respiratory or cardio-respiratory failure. COPD consists of two subsets: chronic bronchitis and emphysema. In practice, it is very difficult to define the contribution of each of these two conditions to the obstruction of the airway and this has led to the displacement of these labels by the non-specific term COPD. The pathology of COPD is not fully elucidated, but features include hypertrophy of mucus-secreting glands, inflammation (including infiltration with lymphocytes) and goblet cell hyperplasia.

The treatment of COPD consists of bronchodilators, intermittent courses of antibiotics and, in some patients, inhaled and/or oral corticosteroids. The latter is claimed to reduce the decline in lung function in COPD.

Cystic fibrosis is an inherited condition. Excess viscid mucus is produced. This leads to recurrent chest infections and progressive bronchiectasis. Approximately 50% of cystic fibrosis sufferers have bronchial hyperresponsiveness and there is an increased incidence of atopy. There is widespread airway narrowing and wheeze. Most cystic fibrosis sufferers take bronchodilators, some take inhaled corticosteroids. And at least one study had reported benefit with oral corticosteroids.

Current drugs for treating asthma are corticosteroids (such as beclomethasone, triamcinolone), beta adrenergics (such as epinephrine, albuterol, bitolterol), NSAIDS, leukotriene antagonists, Xanthines (methyl xanthines such as theophylline, oxtriphylline) and anticholinergics (such as atropine, ipratropium bromide).

Corticosteroids are the mainstay of treatment of chronic asthma and they revolutionized the treatment of this disease when they were first introduced in the 1950's. Oral corticosteroids have today been largely replaced by inhaled corticosteroids, although severe asthmatics still require medication by mouth.

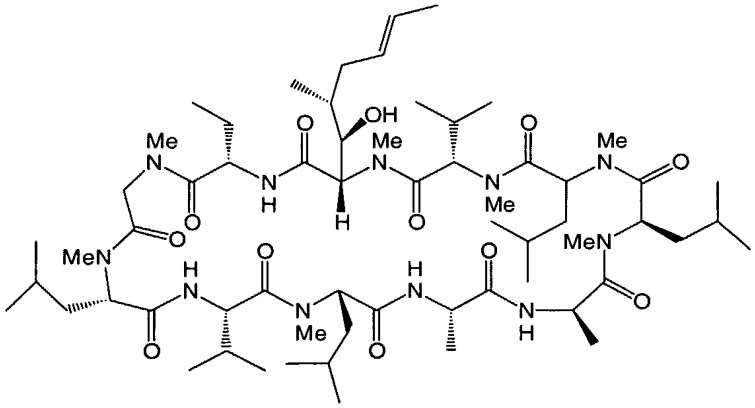
Inhaled cortisteroids are relatively safe and extremely effective in most patients, and improved the quality of life for millions of asthmatic sufferers. For those with severe asthma, however, oral therapy with corticosteroids is required. When taken for more than a few days oral corticosteroids have a number of serious side effects. These include growth retardation in children, severe osteoporosis (especially in old age), decreased responsiveness of the pituitary adrenal axis to stress, fluid retention, diabetes and precipitation of psychosis.

Furthermore, an appreciable number of patients have apparent corticosteroid resistance or unreponsiveness. Patients considered successfully treated with inhaled or oral steroids often have to be content with 60% of their predicted lung function. Further increasing the dose of oral corticosteroids runs the risk of concomitant side effects.

Although corticosteroids are effective for asthma, they are not ideal drugs. Over the years doctors have occasionally used immunosuppressive agents as adjuncts to corticosteroids in patients with extremely severe disease. Examples of immunosuppressive drugs are azathioprine, methotrexate, mycophenolic acid and prodrug, leflunomide, Cyclosporin A, ascomycin, FK-506 and rapamycin.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological activity, in particular immunosuppressive, anti-inflammatory or anti-parasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite

cyclosporin, *Cyclosporin A* represented as follows:



Since the original discovery of cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprising cyclosporins is thus now substantial and includes, for example, the naturally occurring Cyclosporins A through Z, for example, $[Thr]^2$, $[Val]^2$, $[Nva]^2$ and $[Nva]^{2-}$, $[Nva]^5$-Cyclosporin (also known as Cyclosporins C, D, G and M respectively), $[(D)MeVal]^{11}$-Cyclosporin (also known as Cyclosporin H), [cf., Traber et al.;1, Helv. Chim. Acta, 60, 1247-1255 (1977); Traber et al.; 2, Helv. Chim. Acta, 65, 1655-1667 (1982); Kobel et al.; Europ. J. Applied Microbiology and Biotechnology, 14, 273-240 1982); and Von Wartburg et al.; Progress in Allergy, 38, 28-45, 1986)]; as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the MeBmt- residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, for example, [3-O-acetyl-MeBmt]^1–Cyclosporin (also known as Dihydro-cyclosporin D), $[(D)Ser]^8$-Cyclosporin, $[MeIle]^{11}$-Cyclosporin, $[MeAla]^6$.Cyclosporin, $[(D)\ Pro]^3$-Cyclosporin etc., employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc., 15 Suppl. 1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products, 50, 123 (1986).

There is increasing evidence that chronic inflammation in asthma is mediated via a network of cytokines emanating from inflammatory and structural cells in the airways. The prominent eosinophilic inflammation that characterizes asthma appears to be orchestrated by cytokines derived from type 2 T-helper (Th2)-like lymphocytes, suggesting that immunosuppressants might be beneficial in the control of asthma (*see* for example, "Pharmacokinetics, pharmacodynamics, and safety of inhaled cyclosporin A after single and repeated administration in healthy male and female subjects and asthmatic patients," Rohatagi, S. et al., Aventis Pharmaceutical, Collegeville, PA, USA. J. Clin. Pharmacol. (2000), 40(11),

1211-1226). Cyclosporin A (hereinafter "CsA") is active against CD4+ lymphocytes and might, therefore, be useful for asthma. A trial of low-dose oral CsA in patients with steroid-resistant asthma indicated that it can improve control of symptoms in patients with severe asthma on oral steroids.

The mechanism of CsA action in asthma is of interest. CsA binds to the ubiquitous protein cyclophilin, in the cytosol, and the complex in turn binds to calcineurin, which is a calcium and calmodulin dependent serine threonine phosphatase. Calcineurin is necessary for the cytoplasmic portion of the transcription factor NF-AT, a nuclear factor of activated T-cells, to translocate to the nucleus and bind to its nuclear portion to become an active transcription factor. NF-AT forms a complex with AP-1 and regulates the transcription of the IL-2 gene, together with other genes, for example, IL-5. CsA prevents the cytoplasmic portion of NF-AT from translocating, resulting in reduced transcription of IL-2. CsA has a specific inhibitory effect in CD4+ cells through this transcription mechanism, but may also have inhibitory effects on other cells, including mast cells and eosinophils, through mechanisms that have not yet been defined.

Recently, three controlled trials of CsA in asthma have been reported. [Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **1992**; 339: 324-328; Niwanowska E, Dworski R, Domala B, Pinis G. Cyclosporin for steroid-dependent asthma. *Allergy*, **1991**; 46: 312-315; Lock SH, Kay AB, Barnes NC. Double-blinded, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* **1996**; 153: 509-14; Nizankowska E, Soja J, Pinis G, Bochenek G, Sladek K, Domagala B, et al. Treatment of steroid-dependent bronchial asthma with cyclosporin. *Eur Respir J* **1995**; 8: 1091-1099.]

CsA 5 mg/kg/day allowed a significant reduction in the use of corticosteroids by 60%. Side effects with systemic CsA were increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasis and paraesthesia. The systemic toxicity of CsA limits its use for the treatment of asthma, COPD and other related lung diseases. Therefore, it is desirable to synthesize analogs of CsA which retain CsA's potential utility as a primary or adjunct therapy for respiratory diseases, while reducing or eliminating CsA's systemic toxicity.

Summary of the Invention

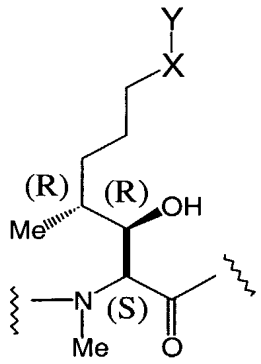
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 3 8 11

(I)

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH_2-OC(O)R2; -C(S)-O-R1; and -C(S)-S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or a pharmaceutically

acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject.

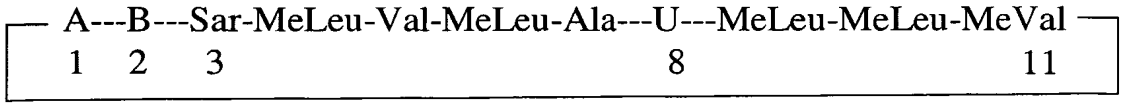
In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. In a preferred embodiment, the present invention relates to the processes for the production of cyclosporin analogs of formula I, with the structure of residue A as illustrated above.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

Detailed Description of the Invention

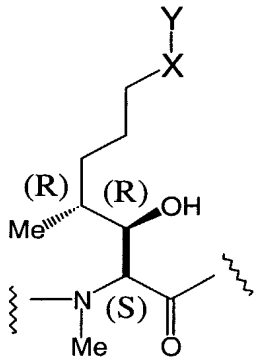
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production. The patents and publications identified in this specification indicate the knowledge in this field and are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure will prevail.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:



I

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; and -C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In formula I, abbreviation of amino acid residues, for example, -Ala-, -MeLeu-, -αAbu-, etc., are in accordance with conventional practice and are to be understood as having the L-configuration unless otherwise indicated (for example, -(D)Ala- represents a residue having the D-configuration). Abbreviation of residues preceded by "Me-" represents a α-N-methylated amino acid residue, for example, "Me-Leu" is a α-N-methylated-Leucine residue. Individual residues of a molecule of the cyclosporin analog of the present invention are numbered, as in the art, clockwise and starting with the residue -MeBmt-, corresponding to residue 1. The same numerical sequence is employed throughout the present specification and claims.

In a most preferred embodiment, a cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where residue B is -αAbu- and residue U is -(D)Ala-. In another preferred embodiment, the cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where X is absent in residue A, residue B is -αAbu- and residue U is - (D)Ala-.

Representative compounds of the invention include, but are not limited to, the following compounds as illustrated below:

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOH; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Cl$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_2CH_2O\ CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$C(=O)SCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is -$CH_2CH_2CH_2$- and Y = -$COOCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOFmoc; residue B = –αAbu-, and residue U = –(D)Ala-.

Cyclosporin analogs of the invention are accordingly useful for the treatment of diseases or conditions responsive to or requiring topical anti-inflammatory, immunosuppressive or related therapy, for example, topical administration for the treatment of such diseases or conditions of the eye, nasal passages, buccal cavity, skin, colon or, especially, airways or lung. In particular, cyclosporin analogs of the invention permit topical anti-inflammatory, immunosuppressive or related therapy with the concomitant avoidance or reduction of undesirable systemic side effects, for example general systemic immunosuppression.

Cyclosporin analogs of the invention useful for the treatment of diseases and conditions of the airways or lung, in particular, inflammatory or obstructive airway diseases. They are especially useful for the treatment of diseases or conditions of the airways or lungs associated with or characterized by inflammatory cell infiltration or other inflammatory events accompanied by inflammatory cell accumulation, for e.g., eosinophil and/or neutrophil. Most preferably, they are useful for the treatment of asthma.

Cyclosporin analogs of the invention are useful in the treatment of asthma of whatever type of genesis including both intrinsic and, especially, extrinsic asthma. They are useful for the treatment of atopic and non-atopic asthma, including allergic asthma, bronchitic asthma, exercise induced asthma, occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. Treatment of asthma is also to be understood as embracing treatment of "wheezy-infant syndrome," that is treatment of subjects, for example, of less than 4 to 5 years of age, exhibiting wheezing symptoms, in particular at night, and diagnosed or diagnosable as "wheezy infants," an established patient category of major medical concern and now more correctly indentified as incipient or early-phase asthmatics. Cyclosporin analogs of the invention are in particular useful for the treatment of asthma in subjects whose asthmatic status is either steroid dependent or steroid resistant.

Cyclosporin analogs of the invention are also useful for the treatment of bronchitis or for the treatment of chronic or acute airways obstruction associated therewith. Cyclosporin analogs of the invention may be used for the treatment of bronchitis of whatever type or genesis, including, for example, acute bronchitis,

arachidic bronchitis, catarrhal bronchitis, chronic bronchitis, croupous bronchitis, phthinoid bronchitis and so forth.

Cyclosporin analogs of the invention are in addition useful for the treatment of pneumoconiosis (an inflammatory, commonly occupational, disease of the lungs, frequently accompanied by airways obstruction, whether chronic or acute, and occasioned by repeated inhalation of dusts) of whatever type or genesis, including, for example, aluminosis, anthracosis, asbestosis, berylliosis, chalicosis, ptilosis, siderosis, silicosis, tabacosis and, in particular, byssinosis.

Cyclosporin analogs of the invention may also be used for the treatment of eosinophil-related disorders of the airways (e.g. involving morbid eosinophilic infiltration of pulmonary tissues) including hypereosinophilia as it effects the airways and/or lungs as well as, for example, eosinophil-related disorders of the airways consequential or concomitant to Loffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoan) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma and eosinophil-related disorders affecting the airways occasioned by drug-reaction.

The word "treatment" as used herein in relation to the treatment of diseases of the airways and lungs, in particular asthma, is to be understood as embracing both symptomatic and prophylactic modes, that is for immediate treatment, for e.g., of acute inflammation (symptomatic treatment) as well as for advance treatment to prevent, ameliorate or restrict long term symptomatology (prophylactic treatment). The term "treatment" as used in the present specification and claims in relation to such diseases is to be interpreted accordingly as including both symptomatic and prophylactic treatment, for e.g., in the case of asthma, symptomatic treatment to ameliorate acute inflammatory events and prophylactic treatment to restrict on-going inflammatory status and to ameliorate future bronchial exacerbation associated therewith.

Cyclosporin analogs of the invention may also be used to treat any disease or condition of the airways or lungs requiring immunosuppressive therapy, for e.g., the treatment of autoimmune diseases, or as they affect, the lungs (for example, for the treatment of sarcoidosis, alveolitis or chronic hypersensitivity pneumonitis) or for the maintainance of allogenic lung transplant, for e.g., following lung or heart lung transplantation.

As previously indicated, for the above purposes, cyclosporin analogs of the invention will be administered topically within the airways, for e.g., by the pulmonary route or by inhalation. As also previously noted, while having potent efficacy when administered topically, cyclosporin analogs of the invention exhibit reduced systemic toxicity. Cyclosporin analogs of the invention thus provide a means for the treatment of diseases and conditions of the airways or lung, for example, as hereinabove set forth, with the avoidance of unwanted systemic side effect, e.g. consequent to inadvertent swallowing of drug substance during inhalation therapy. It is estimated that during the course of manoeuvres required to effect administration by inhalation, up to 90% or more of total drug substance administered will normally be swallowed rather than inhaled.

By the provision of cyclosporin analogs which are topically active, e.g. effective when inhaled, but systemically inactive the present invention makes cyclosporin therapy available to subjects for whom such therapy might otherwise be excluded, e.g. due to the risk of systemic, in particular immunosuppressive, side effect.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' opthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as COPD asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly

following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs; by inhibiting P-glycoprotein, they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Accordingly, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a cyclosporin analog of the invention in combination with a pharmaceutically acceptable carrier or excipient. In particular, compositions pertaining to the present invention are useful for treating a subject for a reversible obstructive airway disease.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients, as described throughout the present specification. Such treatment of the disease may be done by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compound may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Dosages of the cyclosporin analogs of the present invention employed in practicing the method of the present invention will of course vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (for e.g., in terms of body weight, age and so forth) as well as the effect desired. In general, for treating diseases or conditions of the airways or lungs, for e.g., inflammatory or obstructive airway disease such as asthma, cyclosporins of the invention can be suitably administered topically to the airways or lungs, for e.g., but not limited to, inhalation, at dosages from about 20 to about 400 mg/day, preferably from about 50 to about 300 mg/day, most preferably from about 200 to about 300 mg/day. Dosages will appropriately be administered from a metered delivery system in a series of from 1 to 5 puffs at each administration, with administration performed once to four times daily. Dosages at each administration will thus conveniently be from about 5 to 100 mg/day, more preferably from about 12.5 to about 100 mg/day, e.g. administered with a metered delivery device capable of delivering, for e.g., 1 to 25 mg cyclosporin per actuation. For purposes of oral administration, more preferable doses may be in the range from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

Definitions

The terms "C_1-C_3-alkyl" or "C_1-C_6-alkyl" as used herein refer to saturated, straight- or branched-chain hydrocarbon radicals containing between one and

three or one and six carbon atoms, respectively. Examples of C_1-C_3 alkyl radicals include methyl, ethyl, propyl and isopropyl, and examples of C_1-C_6-alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl and n-hexyl.

The term "C_1-C_6-alkoxy" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom. Examples of C_1-C_6-alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *tert*-butoxy, neopentoxy and n-hexoxy.

The term "C_1-C_6-alkylthio" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through a sulfur atom. Examples of C_1-C_6-alkylthio include, but are not limited to, thiomethoxy, thioethoxy, thiopropoxy, thio-isopropoxy, *n*-thiobutoxy, *tert*-thiobutoxy, neothiopentoxy and n-thio-hexoxy.

The term "aryl" as used herein refers to a carbocyclic ring system having one or more aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including multi-cyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from lower alkyl, substituted loweralkyl, haloalkyl, alkoxy, thioalkoxy, lower alkylenedioxy, lower alkylidenedioxy, amino, alkylamino, dialkylamino, acyamino, cyano, hydroxy, acyl, halo and/or trifluoromethyl, mercapto, nitro, carboxylaldehyde, carboxy, alkoxycarbonyl, carbamoyl, sulfamoyl, lower alkoxycarbonylamino, lower alkanoyl, ureido, amidino and carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "C_3-C_6-cycloalkyl-" as used herein refers to carbocyclic groups of 3 to 6 carbons, respectively; for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "heterocyclics", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined

to the rest of the molecule via any of the ring atoms, such as, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

The term "subject" as used herein refers to a mammal or animal. Preferably the mammal is a human. A subject refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs and the like.

The term "pro-drug" as used herein refers to pharmacologically acceptable derivatives, for example, but not limited to, esters and amides, such that the resulting biotransformation product of the derivative is the active drug. Pro-drugs are known in the art and are described generally in, e.g., Goodman and Gilman's "Biotransformation of Drugs," in the Pharmacological Basis of Therapeutics, 8th Ed., McGraw Hill, Int. Ed. 1992, page 13-15, which is hereby incorporated by reference in its entirety.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate,

oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a compound of the present invention formulated together with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminun hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgement of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert

diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required.

Pharmaceutically acceptable diluents or carriers may be diluents or carriers acceptable for topical application at the intended site of therapy, e.g. diluents or carriers acceptable for topical administration pulmonary, dermally, nasally, ocularly or rectaly.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide,

calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

Forms in topically administrable form, e.g. enabling or facilitating topical administration, include, e.g. dry powder preparations of the active ingredient (i.e. cyclosporin analog of the invention) in substantially pure form, for example as employed in the art for delivery from a dry powder inhalation device. Means or devices enabling or facilitating topical administration include, in particular, inhalation devices as well as containers and the like from which the active ingredient may be delivered in a form capable of topical application. Preferred embodiments as defined under C will be such as permit topical administration within the airways or lungs, e.g. by inhalation.

It is clear that safety may be maximized by delivering the drugs by the inhaled route either in nebuliser form or as dry powder. Clearly the great advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or of chronic sinusititis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

Preparation of forms suitable for administration by inhalation may be carried out by methods known in the art. It should be noted that several antibiotics have recently developed for topical inhaled usage, particularly in cystic fibrosis, where they have been shown to be effective against pseudomonas infections. Various inhalants are described. For example, in DE 1491707, GB 1,392,945, GB 1,457,351, GB 1,457,352, NL 147939, DE 1491715, GB 1,598,053, EP 5585, EP 41783, EP 45419, EP 360463 and FR 2628638. DE 1491715, in particular, is said to be suitable for inhalation therapy intended for bronchial or lung diseases.

For this purpose cyclosporin analogs of the invention may be employed in any suitable finely dispersed or finely dispersible form, capable of administration

into the airways or lungs, for example in finely divided dry particulate form or in dispersion or solution in any appropriate (i.e. pulmonarily administerable) solid or liquid carrier medium. For administration in dry particulate form, cyclosporin analogs of the invention may, for example, be employed as such, i.e. in micronised form without any additive materials, in dilution with other appropriate finely divided inert solid carrier or diluent (e.g. glucose, lactose, mannitol, sorbitol, ribose, mannose or xylose), in coated particulate form or in any other appropriate form as know in the art for the pulmonary administration of finely divided solids.

Pulmonary administration may be effected using any appropriate system as known in the art for delivering drug substance in dry or liquid form by inhalation, e.g. an atomizer, nebulizer, dry-powder inhaler or like device. Preferably a metered delivery device, i.e. capable of delivering a pre-determined amount of cyclosporin analog at each actuation, will be employed. Such devices are known in the art.

For nasal administration, cyclosporin analogs of the invention will suitably be administered in liquid form from a nasal applicator. Suitable topical forms for the treatment of diseases or conditions of the skin will include, for example, creams, gles, ointments, pastes, cataplasms, plasters, transdermal patches and the like. Formulations for dermal application will appropriately contain a skin penetration enhancer, e.g. as know in the art, for example azone. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, again as known in the art. For rectal administration, i.e. for topical therapy of the colon, cyclosporin analogs of the invention may be administered in suppository or enema form, in particular in solution, e.g. in vegetable oil or like oily system for use as a retention enema.

According to the present invention, cyclosporin analogs may be used for the manufacture of a topical preparation for the treatment, with or without the concurrent use of other drugs. For the above purposes, cyclosporin analogs of the invention may be employed in any dosage form appropriate for topical administration to the desired site. For example, for the treatment of diseases of the airways or lungs, cyclosporin analogs of the invention may be administered via the pulmonary route, by inhalation from an appropriate dispenser device.

Dosage for the topical preparation will in general be one tenth to one hundredth, of the dose required for oral preparation.

Abbreviations

Sar:	Sarcosine
MeLeu:	N-Methyl-Leucine
Val:	Valine
Ala:	Alanine
MeVal:	N-Methyl Valine
Et:	Ethyl
Ph:	Phenyl
Fmoc:	9-Fluorenylmethoxycarbonyl-
MeBmt:	N-Methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine
α-Abu:	α-Aminobutyric acid

Synthetic Methods

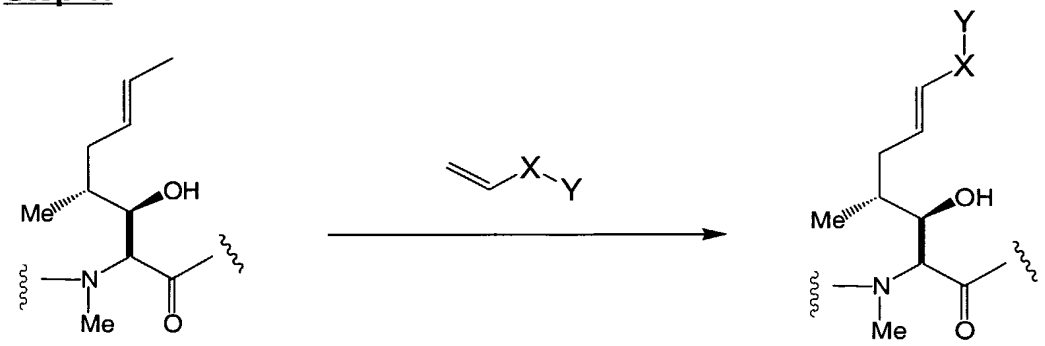
The compounds and processes of the present invention will be better understood, but are not limited to, the following synthetic scheme which illustrates the methods by which the compounds of the present invention (illustrated by formula I) may be prepared. The groups X and Y, and the amino acid residues B and U in formula I are as defined earlier in the specification. The starting material for Scheme I, illustrated by formula I where A'= -MeBmt-, may be, for example, but not limited to, a fermentation product or a synthetic product made by solution phase chemistry. Preferably, the starting material is commercially available. The starting material as a fermentation product may be made from highly productive strains, for example, but not limited to, *Sesquicillopsis rosariensis* G. ARNOLD F605; *Tolypocladium inflatum* wb6-5; Fusant, *Tolypocladium inflatum* KD461 etc. (in U.S. Patent Nos. 5,256,547; 5,856,141 etc.). Alternately, the starting material may be made by solution phase chemistry either by sequentially assembling amino acids or by linking suitable small peptide fragments, where the units are linked by, for example, but not limited to, amide, ester or hydroxylamine linkages (described in, *Müller, Methoden der organischen*, Chemie Vol. XV/2, pp 1 to 364, Thieme Verlag, Stuttgart, 1974; Stewart, Young, Solid Phase Peptide Synthesis, pp 31 to 34, 71 to 82, Pierce Chemical Company, Rockford, 1984; Bodanszky, Klausner, Ondetti, Peptide Synthesis, pp 85 to 128, John Wiley & Sons, New York, 1976 and other standard books on solution phase peptide chemistry). For amide linkages particular preference is given to the azide method, the symmetric and mixed anhydride method, *in situ* generated or preformed active esters and methods using

coupling reagents (e.g., dicyclohexylcarbodiimide, N,N-dimethyl-4-aminopyridine, N-hydroxy-benzotriazole, PyBrop® etc.). Classical solution phase chemistry using standard Z- and Boc- methodology may be used.

Residue A, which is -MeBmt- in the starting material is further modified, as illustrated in the following reaction scheme.

Scheme:

Step 1:

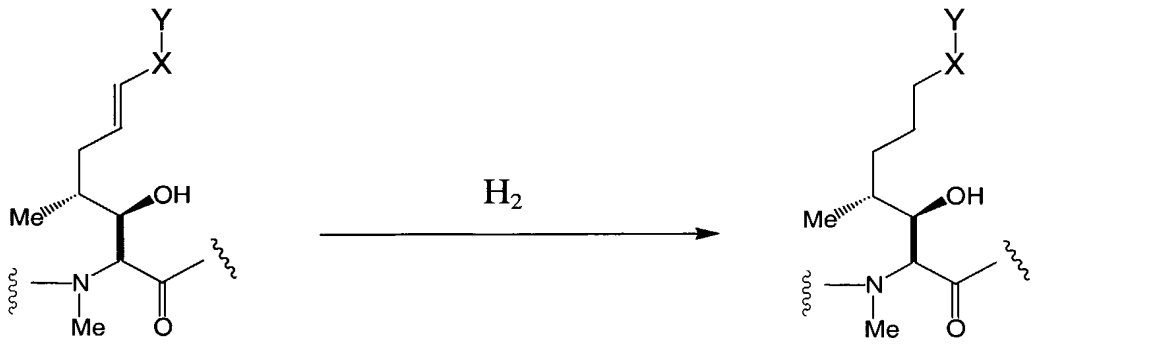


A' = -MeBmt- A", wherein X, Y are as defined (i)

The process for the preparation of the compounds of formula I comprises reacting a compound of formula I, where A' = -MeBmt- (for example, Cyclosporin A) with an olefin having a terminal double bond with catalysts such as Grubb's ruthenium alkylidene, Grubbs dihydroimidazole ruthenium, Shrock-Hoveyda molybdenum catalysts or benzylidene catalysts [*see* (a) US Patent 6,111,121; (b) Reviews: Synlett, **1999**, *2*, 267; (c) Reviews: Ivin, K J; Mol, J.C. *Olefin Metathesis and Metathesis Polymerization*, 2nd ed., Academic Press, New York, **1997**; (d) *J. Org. Chem.*, **1999**, *64*, 4798-4816; (e) *Angew. Chem.*, Int. Ed. English, **1997**, *36*, 2036-2056; (f) *Tetrahedron* **1998**, *54*, 4413-4450.] or Nolan's ruthenium catalyst [see (a) International Patent Application No. WO 00/15339; (b) *Org. Lett.*, **2000**, *2*, 1517-1519; (c) *J. Org. Chem.*, **2000**, *65*, 2204-2207] or Molybdenum catalysts [see (a) *J. Am. Chem. Soc.*, **1990**, *112*, 3875 (b), *J. Am. Chem. Soc.*, **1996**, *118*, 10926-10927] in the presence of a lithium salt such as lithium bromide, lithium chloride, lithium trifluoroacetate, lithium triflate of a lewis acid such as titanium isopropoxide in an organic solvent. The organic solvent used may be solvents such as, for example, dichloromethane, chloroform, toluene, benzene, tetrahydrofuran, dimethylformamide and the like or mixtures thereof. The reaction may be carried

out from room temperature to about 100 °C for 1-7 days to provide a compound of formula I, where residue A' is converted to residue A" having formula (i).

Step 2:



A", wherein X, Y are as defined

(i)

A, wherein X, Y are as defined

The compounds of formula I in an organic solvent, where residue A" has formula (i), are then subjected to standard hydrogenation conditions using a catalyst such as, but are not limited to, a catalytic amount of palladium on carbon in a hydrogen atmosphere to provide the saturated compounds of formula I, where in particular, residue A" having formula (i) is converted to residue A, as described throughout the specification.

The organic solvents used can be solvents such as methanol, ethanol, ethyl acetate or mixtures thereof. Other catalysts useful to assist hydrogenation may be, for example, but not limited to, platinum metal or its oxide [*see* standard books on catalytic hydrogenation, e.g., Rylander, P.N., *Hydrogenation Methods*, Academic Press: NY, 1985; *Catalytic Hydrogenation in Organic Synthesis*, Academic Press: NY, 1985; Červený, L., *Catalytic Hydrogenation*, Elsevier: NY, 1986 etc.]. The reaction may be carried out at room temperature or elevated temperature, for example, but not limited to, 50 °C or 100 °C.

Examples

The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intentended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications,

including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods fo the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Cyclosporin methyl ester (0.030 mg, 0.024 mmol) and palladium on carbon (0.0012 mg, 0.0012 mmol) were added to a flask and the flask was evacuated and backfilled with hydrogen gas three times. Anhydrous methanol (3 ml) was added and the reaction was stirred for 18 h at ambient temperature under an atmosphere of hydrogen. After filtration and concentration in vacuo, the product was isolated as a white solid (0.021 mg, 70 % yield). Electrospray mass spectrum (ESMS) M+H: 1248.91

Example 2: Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 2 was prepared from cyclosporin ethyl ester and palladium on carbon according to the procedures described in Example 1. ESMS M+H: 1262.3

Example 3: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 3 was prepared from cyclosporin propyl ester and palladium on carbon according to the procedures described in Example 1.

Example 4: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 4 was prepared from cyclosporin benzyl ester and palladium on carbon according to the procedures described in Example 1.

Example 5: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 5 was prepared from cyclosporin fluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 6: Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 6 was prepared from cyclosporin difluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 7: Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 7 was prepared from cyclosporin trifluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1.

Example 8: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 8 was prepared from cyclosporin trifluoroethyl ester ester and palladium on carbon according to the procedures described in Example 1.

The cyclosporin analogs of the present invention have potent immunosuppressive and anti-inflammatory activity. In particular, they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. In vivo this activity is apparent following topical administration, e.g., pulmonary route.

The immunosuppressive and anti-inflammatory properties of cyclosporin analogs of the invention may be demonstrated in standard test models *in vitro* and *in vivo* for example as follows.

Example 9: Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with Cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide

representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green (Biomol AK-111).

Compounds in DMSO (2.4µl) were added to a 96-well microplate and mixed with 50µl assay buffer (50mM Tris-HCl, pH 7.5; 100mM sodium chloride; 6mM magnesium chloride; 0.5mM dithiothreitol, 0.025% NP-40, 500µM calcium chloride, 0.27µM Calmodulin) containing 10µM Cyclophilin and 3nM Calcineurin. After warming to 37 °C for 60 mins, the enzymatic reaction was initiated by addition of phosphopeptide (7.5µl) to give a final concentration of 94µM. Phosphate release after 60 min at 37 °C was determined by addition of Biomol Green (100 µl) and measurement of the absorbance at 620nm after 15 mins at room temperature.

IC_{50} values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 0.1 to 0.0015 µM.

Example 10. NFAT reporter gene assay

NFAT activation follows precisely the activation of calcineurin by increased free calcium levels in the cytoplasm. Researchers from diverse fields are interested in the NFAT family of transcription factors, which are potential targets for newer and safer immunosuppressive drugs. In addition, the activation of NFAT proteins involves various cellular signal transduction pathways, including calcium mobilization and MAP kinase pathways linked to T-cell receptors and Ras1. To assist researchers probing the activity of NFAT proteins, Stratagene has developed a PathDetect cis-reporter plasmid, the pNFAT-Luc reporter plasmid (Stratagene, Inc. catalog # 219094), containing the NFAT binding site from the human IL-2 gene.2,7-9. The NFAT cis-reporting system includes the transfection-ready pNFAT-Luc reporter plasmid and the pCIS-CK negative control plasmid.

Construction of the pNFAT-Luc Plasmid:

The backbone of the 5749-base-pair pNFAT-Luc plasmid is the pFR-Luc reporter plasmid of the aforementioned PathDetect trans-reporting system. To this backbone, the GAL4 binding element was replaced with four direct repeats of the NFAT binding sequence (–286 to –257) from the IL-2 gene promoter, the most studied and widely used NFAT binding sequence. For all reporter plasmids of the PathDetect cis-reporting systems, activation of the luciferase gene indicated

interaction of uncharacterized gene products, extracellular stimuli, growth factors, or drug candidates with specific enhancer elements. Then a plasmid expressing the gene of interest was cotransfected into mammalian cells along with a cis-reporter plasmid to indicate transcription activation.

Testing the pNFAT-Luc Plasmid in Jurkat Cells:

Pharmacology studies have established that NFAT proteins can be activated by the protein kinase C activator phorbol ester (PMA) in combination with the calcium ionophore ionomycin, reagents that raise free intracellular calcium. When Jurkat cells, a mature human T-cell line, or CHO cells were transfected with the pNFAT-Luc plasmid and treated with 60 ng/ml of PMA and 1μg/ml of inomycin, luciferase activity increased by 13- and 16-fold, respectively. Therefore, the enhancer element in the pNFAT-Luc plasmid is responsive to calcium mobilization. Cells transfected with pNFAT-Luc and then treated with either PMA or ionomycin alone did not show a significant increase in luciferase activity.

Cyclosporin inhibits the activity of calcineurin, a protein phosphatase regulated by intracellular calcium mobilization. All the isoforms of NFAT protein contain a calcineurin-binding domain and are activated by calcineurin. The inhibition of luciferase expression from pNFAT-Luc in the present model, in both Jurkat and CHO cells induced by PMA and ionomycin, was monitored for cyclosporin (as a positive control) and the cyclosporin analogs of the present invention.

In another set of experiments, rat basophilic leukemia cells stably transfected with chemokine receptors were transfected with pNFAT-Luc and then treated with their respective ligands (data not shown). When both luciferase expression and calcium levels were monitored in these cells, luciferase expression correlated very well with calcium mobilization. Therefore, luciferase expression from pNFAT-Luc indeed reflects the activation of endogenous NFAT proteins by calcium immobilization.

Example 11. Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is

added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

Mishell-Dutton Test

Ca. 10^7 lymphocytes from the spleen of OFI, female mice are co-cultured with ca. $3x10^7$ sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

Delayed-type Hypersensitivity Resonse

On Day 0 groups of ten mice (having BALB/cByJ or any other acceptable strain) are dosed with test compound (1 to 10%), vehicle or the positive control, cyclophosphamide (Cyclosporin A), and monitored from Day-2 to 7. The mice are anesthetized and their abdomens shaved. 100 µl of a 3% solution of ovalbumin are applied to the abdomen and dried. Seven days later, the mice are challenged by applying 5 µl of ovalbumin to each side of the right ear. After 24 hours, both the right and left ear thickness are measured using a micrometer caliper.

Popliteal Lymph Node Assay

First, an inducer (phenytoin) is injected into the mice footpad (having BALB/cByJ or any other acceptable strain). Then the mice are challenged (subcutaneously or po) with ester and control agent using graded doses, for example, 2.5, 10, 20 mg/Kg (based on cyclosporine A data). On day 7 the popliteal lymph nodes are excised from the dosed mice and the lymph nodes are weighed. Then single cell suspensions of each lymph node are prepared and enumerated. The weight index for each animal is calculated (for example, a mean weight index <2 would indicate suppression of immune response).

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 µg/ml) with $Al(OH)_3$ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies, the procedure is repeated 1x after 2 weeks and the animals are used one

week later. For inhalation studies, the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

Challenge is effected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered (a) inhalation and/or (b) orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hrs. prior to and 6 hrs. after OA challenge. For inhalation studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 mins. prior to OA challenge.

Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliqots of Ca^2 + and Mg^2 + free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 μl of this cell suspension is added to 190 μl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per smear are counted and the total population of each cell type is calculated.

In untreated animals, OA challenge induces increase of all cell types in BAL fluid 24 hours after challenge. Prior administration of cyclosporin analogs in accordance with the present invention by inhalation at dosages of the order of from 1.0 to 15.0 mg/kg reduces eosinophil count in BAL in a dose dependent manner as compared with untreated controls. Cell counts for other leucocytes (macrophages, neutrophils etc.) are also reduced.

Claims

What is claimed is:

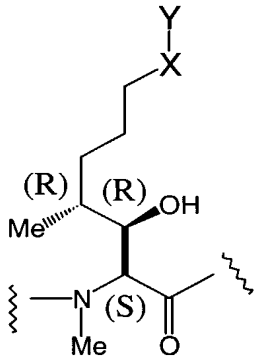
1. A cyclosporin analog of formula (I) or a pro-drug or a pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 8

(I)

wherein,

(a) A is of the formula:



wherein

X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-;

Y is selected from the group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

iii. -C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl, optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

iv. -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

v. C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio.

(b) B is –αAbu-, -Val-, -Thr- or –Nva-; and

(c) U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-; or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

2. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula (I), B is -αAbu-, and U is -(D)Ala-.

3. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula I:

(i) A is of the formula A1 or A2, wherein:

X is absent; and

Y is selected from a group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

iii. C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

(ii) B is -αAbu-; and

(iii) U is -(D)Ala-.

4. A cyclosporin analog according to claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, selected from the group consisting of:

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOH;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOEt;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CH_2CH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Ph$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2F$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCHF_2$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Cl$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_2CH_2O$ CH_3;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$C(=O)SCH_2Ph$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is -$CH_2CH_2CH_2$-, Y = -$COOCH_3$; and

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOFmoc.

5. A chemical process for preparing a cyclosporin analog of formula I as claimed in Claim 1, comprising:
   a. reacting a compound of formula I, wherein A= -MeBmt- with:
      i. an olefin of formula CH_2=CH-X-Y, wherein X and Y are as defined in Claim 1; and
      ii. a catalyst;
      in the presence of a lithium salt in an organic solvent; and
   b. hydrogenating the product of step a in an organic solvent under hydrogen with a catalyst;
      and optionally converting the product of said reaction into a pharmaceutically acceptable salt.

6. The chemical process as claimed in Claim 5, wherein the catalyst in step (a) (ii) is Grubb's ruthenium alkylidene, Nolan's catalyst, a benzylidene catalyst or a molybdenum catalyst.

7. The chemical process as claimed in Claim 5, wherein step (b) is performed at room temperature.

8. The chemical process as claimed in Claim 7, wherein the catalyst in step (b) is Palladium on carbon.

9. A pharmaceutical composition, said composition comprising at least one cyclosporin analog of formula 1 as claimed in Claim 1, said cyclosporin analog being present alone or in combination with a pharmaceutically acceptable carrier or excipient.

10. A method for treating diseases characterized by airflow obstruction in a subject in need of treatment which comprises the step of administering to said subject a therapeutically effective amount of at least one cyclosporin analog of formula I as claimed in Claim 1.

11. The method of Claim 10, wherein said disease is asthma.

12. The method of Claim 10, wherein the step of administering the cyclosporin analog of formula I is done by topical administration.

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(54) Title: METHOD AND COMPOSITION FOR DRY EYE TREATMENT

(57) Abstract: A method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil, a non-toxic emulsifying agent and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres thereto and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

METHOD AND COMPOSITION FOR DRY EYE TREATMENT

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the treatment of a dry eye condition, an in particular to a method and composition for this purpose which forms an artificia tear film on the surface of the eye acting to inhibit evaporation therefrom an delivering to the eye surface an efficacious medicament to treat an eye disease.

STATUS OF PRIOR ART

The main concern of the present invention is with the treatment of a dry ey condition by a method and composition that acts to lubricate the eye and to reduc evaporation of fluid from the cornea surface. The cornea normally functions t maintain this surface in a moist and lubricated state which is impaired when the ey suffers from a dry eye condition.

Dehydration of moisture from the eye gives rise to various discomforts suc an ocular dryness as well as burning and scratching sensations. But the mor serious consequence of a dry eye condition is a loss of visual acuity which if i persists and is not corrected, may result in permanent damage. Dry eye disease act to degrade the exposed ocular surface and may cause a complete breakdown c corneal tissues. In an extreme case, this may necessitate a corneal transplant.

Symptoms accompanying a dry eye condition are exacerbated when the ey is covered by a contact lens. The rate of evaporation of liquid from the eye i accelerated by the contact lens whose presence results in a meniscii formation tha promotes evaporation even when the eye has an adequate natural tear film.

The usual treatment prescribed for a dry eye condition is to alleviate it symptoms by the topical application of a tear film substitute that adds a substantia

volume of liquid to the anterior surface of the eye. A typical composition functioning as a tear film substitute includes soluble polymer solutions. Of prior art interest in this regard is the US patent to Trager 4,421,740 which discloses an artificial tear composition formed by an aqueous hypotonic solution of lecithin, a phospholipid, and a viscosity-adjusting agent.

Of particular prior art interest are the following US patents in each of which Korb is a co-inventor. Hence these patents will hereinafter be referred to as Korb patents:

I. 4,914,088 (1990)

II. 5,278,151 (1994)

III. 5,371,108 (1994)

IV. 5,294,607 (1994)

The Korb patents point out that a normal eye has an ocular surface coated with a tear film composed of:

(a) a mucous inner layer in contact with the ocular surface of the eye

(b) an aqueous middle layer which is the source of moisture, and

(c) a lipid outer layer which minimizes evaporation of the moisture from the film.

"Dry eye" is experienced when the outer layer (c) of the tear film is defective. The dry eye treatment disclosed and claimed in Patents I to IV involves the topical application to the eye of phospholipids which form an artificial film over the eye that replicates a normal outer lipid layer and maintains the eye in moist condition.

Patent I is directed to an artificial tear film formed by:

"a layer of a complex phospholipid having a net positive or negative charge".

According to this Korb patent, the significance of a net positive or negative net charge is that in either case, the charged molecules in the film coating the surface of the eye *"repel each other"* and in doing so, maintain *"the integrity of the phospholipid therein"* so that it acts *"as a barrier reducing*

evaporation." Hence it is a negative or positive repelling charge that the inventor regards to be the crux of his invention.

Patent I fails to take into account that the surface of the eye being treated is anionic and therefore will interact electrostatically with a charged coating in a way that depends on the polarity of the charge. According to Patent I, the polarity of the charge doesn't matter, for in either polarity the charged molecules in the film repel each other.

An important aspect of the present invention is not only that it has a positive net charge, but also that the strength and distribution of the charge is such as to cause the film to adhere electrostatically to the entire anionically-charged eye surface to provide an effective moisture barrier. A weak positive charge would not achieve this result. Inasmuch as in present invention, the positively-charged molecules in the film covering the eye surface electrostatically engage the negatively-charged molecules on this surface, the resultant electrostatic couple is neutral and the couples do not repel each other.

Korb patent II discloses an eye treatment composition comprising

"a layer of a complex phospholipid having a net charge" and

"a layer of an essentially non-polar oil over said phospholipid layer", the phospholipid and oil layers being in an amount *"below that amount that would result in significant prolonged blurring of vision"*.

According to Patent II, the preferred phospholipids are those "carrying a net negative charge because the negatively-charged molecules would be repelled by the negatively-charged ocular surface, thereby permitting the maintenance of a relatively thick aqueous layer".

In contradistinction, the present invention which resides in a positively-charged composition, exploits the fact that the eye surface is negatively charged (anionic) so that the composition is electrostatically attracted to this surface to create a coating which prevents the escape of moisture from the eye surface for a prolonged retention period.

Korb patent III also discloses a composition in which the phospholipid has a net negative or positive charge causing molecules in the tear film coating the eye surface to repel each other to maintain the integrity of the film. In Korb patent IV, the eye treated composition is a mixture of a charged phospholipid and a non-polar oil in a meta-stable water emulsion.

Essential to the present invention is that the emulsion coating the eye surface to form a film thereon carries a net positive charge which is distributed uniformly throughout the film so that it is electrostatically attracted to the entire anionic eye surface whereby the molecules on the film surface do not repel each other but are attracted to the eye surface.

Also of particular prior art interest is PCT patent publication WO 95/31211 (25 Nov. 1995) of Allergan, Inc. This publication discloses an emulsion for topical application to ocular tissue which includes cyclosporin admixed with castor oil. As noted in this publication, cyclosporin comprises a group of cyclic oligopeptides, the major component of which is cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$), Clyclosporin has been found to be effective in the treatment of a dry eye condition.

SUMMARY OF THE INVENTION

In view of the foregoing, the main object of this invention is to provide an improved method and composition for treating a dry eye condition by topically applying to the eye surface an emulsion forming a tear film that adheres electrostatically to the entire surface of the eye and acts to lubricate the eye and to inhibit evaporation of moisture therefrom.

Among the significant advantages of a method and composition in accordance with the invention are the following:

A. The tear film derived from the emulsion carries a strong net positive charge that is uniformly distributed throughout the film surface whereby the film is electrostatically attracted to the entire area of the negatively-charged eye surface and there is no uncoated zone.

B. The electrostatic attraction between the artificial tear film and the eye surface maintains adhesive contact therebetween for a prolonged retention period and the tear film cannot be readily washed away.

C. The tear film coating the eye surface has no adverse effects, for the film includes no toxic or other harmful agents.

Also an object of this invention is to provide a composition of the above type which incorporates therein a therapeutic agent for treating an eye disease, such as cyclosporin A which when the composition is topically applied then delivers the agent to the eye. The release of the agent from the coating film to the surface of the eye is maintained for a prolonged period in that the film is held electrostatically in contact therewith.

Briefly stated, these objects are attained in a method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres to the eye and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

DETAILED DESCRIPTION OF THE INVENTION

Cyclosporin A (CsA), a lipid-soluble cyclic endecapeptide, is a potent and well established immunomodulator drug mainly for oral use. With oral formulations, CsA bioavailability is limited because of the drug's insolubility in water and its tendency to separate immediately as a solid after coming into contact with water. Moreover, the bioavailability is highly dependent on complex

interactions occurring between the formulation and the physiological environment of the lumen.

CsA has been found to be effective in treating the immune-mediated keratoconjunctivitis sicca (KCS or dry eye disease) by the enhancement or restoration of lachrymal gland tearing in patient suffering from this syndrome. Dry eye disease is characterized by chronic drying of the conjunctiva and cornea, as well as by decreased tear production and changes in the composition of the tear film. In order to enhance the efficiency of CsA treatment, it becomes necessary to increase the absorption of the drug in the lachrymal gland as well as the conjunctiva and cornea target tissues, using for the purpose a suitable dosage of the drug to suppress ocular inflammation without significant systemic CsA exposure.

Since the aqueous solubility of CsA is between about 20 to 30 μg/ml, there is no adequate aqueous formulations available for ocular administration of the drug. Moreover, if cyclosporin is administered orally for the treatment of KCS, the accompanying side effects due to systemic circulation may cause adverse reactions such as hypertrichosis or renal dysfunction. In addition, the concentration of CsA present in oral formulations is limited due to the drug's hydrophobic nature.

Studies on ocular CsA penetration in animals were carried out using CsA formulations based on olive oil and corn oil. Local toxic effects on the cornea attributable to topical CsA formulations or the intrinsic solvent were observed. Upon using CsA in olive oil, in an *ex vivo* examination on bovine cornea, histological study revealed that the corneal epithelium was keratinized with some necrotic cells and rare pycnotic nuclei. Moreover, several researchers have confirmed that the probable toxic effect was due to topically administered CsA dissolved in olive oil. The conclusion reached is that olive oil, rather than CsA was responsible for the surface epithelial defects developing in the cornea. Hence, because of its high hydrophobicity, it is necessary to formulate CsA with compatible vehicles. These are not always biocompatible with ophthalmic administration, and may present some problems of stability such as the rancidity of olive oil. The drawback of corn-oil concentrated ointment formulations is that they

may exacerbate the symptoms (early-burning, redness and itching) of a dry eye condition.

It is possible to minimize problems related to unpleasant sensations and syndrome exacerbation by reducing the oil content and dispersing the oil phase in a water phase, resulting in an emulsion. We have found that when castor oil is used in developing the emulsion dosage, there are additional benefits to patients with dry eye disease arising from the long ocular retention time of the emulsion vehicle. The castor oil droplets in the emulsion form a lipid layer over the tear film, reducing the evaporation of the limited natural tears produced while the emulsion remains in the eye of a patient.

Our investigation of a positively-charged submicron emulsion containing a phospholipid having Zeta potential values ranging from 34 – 45 mV and a mean droplet size of around 150-250 nm supports the significant advantages which are gained when the emulsion vehicle carries a net-positive charge, rather than either a negative or neutral charge.

The resultant electrostatic attraction between the positively-charged submicron oil droplets in the emulsion and the corneal eye surface, which is negatively-charged results in a more prolonged residence or retention time conducive to topical drug flux enhancement.

Hence a positively-charged submicron emulsion of CsA enhances the local concentration of this medicament in conjunctiva and cornea which are the target ocular tissues. A positively-charged emulsion in accordance with the invention is therefore far more efficacious therapeutically than a negative charge emulsion having a similar composition.

The Composition The following represent formulations for a composition in accordance with the invention for treating a dry eye condition and other eye diseases.

Formulation (1) is a positive blank emulsion to be applied topically to an eye surface to create on the surface an artificial tear film. Formulation (2) which is for a CsA positive emulsion has the same ingredients as formulation (1), to which is

added cyclosporin. The resultant film serves as a vehicle to deliver the medicament to the eye surface.

Cyclosporin A	0.00	0.20
Castor oil	2.50	2.50
Lipoid E-80	0.50	0.50
Stearylamine	0.12	0.12
Vitamin E	0.01	0.01
Pluronic F-68	0.42	0.42
Glycerol	2.25	2.25
Benzalkonium chloride	0.01	0.01
Distilled water to	100.00	100.00

Lipid E-8 is a non-polar phospholipid, stearylamine is a cationic lipid and therefore imparts to the emulsion which also includes a non-polar castor oil a net positive charge. Pluronic F-68 is the trademark for poloxamer 188, a polyoxyalkyline derived from polypropylene glycol. Poloxamer 188 is an emulsifying agent and the glycerol in the formulation functions as an osmotic agent. Benzalkonium chloride is a cationic surfactant antiseptic agent acting as a preservative of the emulsion and strengthening the positive charge imparted to the emulsion by the cationic lipid. Vitamin E acts as a lipophillic antioxidant and as an eye lubricant.

In practice a composition may include instead of the cationic lipid stearylamine, cationic lipid oleylamine. The relative percentages of the ingredients included in the composition are not limited to those set forth above. Thus the relative percentage of castor oil may be in the range of 0.5 to 10%, that of the phospholipid (Lipoid E-80) in the range of 0.1 to 2.0%, that of the cationic lipid in the range of 0.1 to 0.5%, and that of the emulsifying agent, (Pluronic F-68), in the range of 0.5 to 2.0%.

It is vital however that whatever are the relative ranges of these ingredients, that the emulsion carry a net positive charge of sufficient strength to cause the emulsion when forming a film on the anionic surface of an eye, that it be electrostatically attracted to the surface so that it adheres thereto and cannot be readily washed away.

Lipoid E80, Pluronic F-68 and stearylamine coact to improve the stability of the emulsion droplets which are preferably in the submicron range, by enhancing the mechanical strength of the interfacial films formed around the droplets

It is important to bear in mind that in a composition in accordance with the invention which is to be administered topically to the anionic surface of an eye, that the phospholipid and castor oil included in the formulation carry no charge and that the aggregate net positive charge imparted to the submicron droplets is derived from the cationic surfactant plus the cationic antiseptic agent.

The advantage of this formulation over a dry eye treatment composition in which the charge imparted to the droplets is derived only from the phospholipid, as in the Korb patents, is that with the present formulation the positive charge of the emulsion is uniformly distributed over the entire area of the artificial tear film which is produced when the emulsion coats the anionic surface of the eye.

This results in electrostatic attraction throughout the entire area of the eye surface so that no portion thereof remains uncoated and untreated. Hence the present invention affords a treatment for a dry eye condition in which evaporation moisture is inhibited over the entire eye surface and no moisture is permitted to escape therefrom.

Preparation of Composition

Poloxamer 188 (Pluronic F-68) the osmotic agent (glycerol), and benzalkonium chloride were dissolved in the aqueous phase. The lipid E-80 is first dissolved in ethanol (1:5) and then dispersed in the aqueous phase. The ethanol is evaporated during the heating process of the aqueous phase. An antioxidant (α-tocopherol), the cationic lipid stearylamine (or oleylamine) and the CsA were dissolved in the castor oil phase. Both phases were heated separately to 70°C. The water phase was slowly incorporated into the oily phase and mixed with a magnetic stirrer. The resulting mixture was further heated to a temperature of 85°C.

The coarse emulsion obtained was emulsified for 5 minutes, using a high shear Polytron mixer and then rapidly cooled to below 20°C. After cooling in an ice bath, the emulsion was homogenized using a two stage homogenizer valve

assembly for 5 minutes. After further rapid cooling below 20°C, the pH was adjusted to 7.0 using 0.1 N hydrochloric acid. The emulsion was then filtered through a TE membrane filter (Schleicher & Schuell, Dassel, Germany) with a pore size of 0.45 μm. Finally, the emulsion was packed under nitrogen atmosphere in siliconized glass bottles and then sterilized by autoclaving at 121°C for 15 minutes. It is desirable that the droplets of the emulsion be in the submicron range and it is vital that the emulsion which is to be applied topically to the eye surface be sterile.

Medicaments: In an emulsion in accordance with the invention which is to be applied topically to the surface of an eye to treat a dry eye condition can also function as a vehicle to deliver a therapeutic agent to the eye to treat an eye disease.

The common practice in treating an eye infection is to deposit drops of an antibiotic agent in the eye, the number of drops to be applied on any one occasion being prescribed by a physician. Since this number defines the dosage of the drug applied to the eye, one must be careful that the drops are limited to the eye and that none of the applied liquid escapes therefrom. But in practice, it is difficult to deposit a drop of liquid into the eye so that none of the liquid flows beyond the eye borders, for there is little to hold the liquid to the eye surface.

The advantage of using an emulsion in accordance with the invention as a vehicle to deliver a therapeutic agent to the eye is that the emulsion which coats the entire surface of the eye and spreads the agent over its anionic surface, adheres electrostatically to this surface so that all of the therapeutic agent in a predetermined dosage is delivered to the eye. And because the coating electrostatically adheres to the eye surface and cannot be washed away, the residence time of treatment is prolonged and the therapeutic agent is therefore more effective.

The fact that the droplets in the charged emulsion in accordance with the invention are of submicron size is significant. This results in a much greater charge density per unit area of the emulsion film than would be produced had the droplet size been in the micron range and therefore produces a more powerful electrostatic force.

We have in the foregoing disclosed cyclosporin A as a preferred medicament to be incorporated in the emulsion. But other water-insoluble medicaments may be used provided that they possess properties for the treatment of eye disease similar to those of cyclosporin and are non-polar. Should the medicament carry a negative charge, then the amount of the cationic ingredient included in the emulsion must be such as to provide a net positive charge.

Thus among suitable medicaments that can be incorporated in an emulsion in accordance with the invention are those in the family of compounds including tacrolimus disclosed in US Patent 4,894,366. Also suitable is Sirolimus (Rapamycin) disclosed in US Patent 3,993,749.

While there has been disclosed preferred embodiments of the invention, it is to be understood that many changes may be made therein without departing from the spirit of the invention.

CLAIMS:

1. An emulsion to be topically applied to the anionic surface of an eye to form a tear film thereon which lubricates the eye to inhibit evaporation of fluid therefrom; said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

2. An emulsion as set forth in Claim 1, to treat a dry eye condition, the emulsion being defined by droplets in the submicron range.

3. An emulsion as set forth in Claim 1, in which the oil is castor oil.

4. An emulsion as set forth in Claim 1, in which the phospholipid is Lipoid E-80.

5. An emulsion as set forth in Claim 1, in which the cationic lipid is stearylamine.

6. An emulsion as set forth in Claim 1, in which the cationic lipid is oleylamine.

7. An emulsion as set forth in Claim 4, in which the relative percentage of the phospholipid in the emulsion lies in the range of 0.1 to 0.5 percent.

8. An emulsion as set forth in Claim 1, in which included in the mixture is vitamin E.

9. An emulsion as set forth in Claim 1, in which the mixture further includes an emulsifying agent.

10. An emulsion as set forth in Claim 9, in which the emulsifying agent is poloxamer.

11. An emulsion as set forth in Claim 10, in which the relative percentage of the emulsifying agent in the emulsion lies in the range of 0.5 to 2.0 percent.

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

AMENDED CLAIMS

[Received by the International Bureau on 23 December 2002 (23.12.02) ; new claims 22-24 ; remaining claims unchanged]

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

22. A method of treating immune-mediated keratoconjunctivitis sicca comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a

non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge, and further includes cyclosporin A; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

23. Use of an emulsion for the preparation of a pharmaceutical composition for the treatment of a dry eye condition, said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

24. The use according to Claim 23 for the preparation of a pharmaceutical composition for the treatment of immune-mediated keratoconjunctivitis sicca, wherein said emulsion further comprises cyclosporin A.

Statement under Article 19(1)

New Claim 23 is a reformulation of claim 19 in a form acceptable to the European Patent Office. New claims 22 and 24 claim a particular embodiment of the invention and find support in the specification on page 5, lines 3-11 and page 7, lines 20-24.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/01015

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/107 A61K38/13 A61K31/436 A61P27/02

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 18852 A (YISSAM RESEARCH DEV COMPANY OF) 30 September 1993 (1993-09-30) page 1, line 2 - line 6 examples 1-14,16 claims 1,3,18 --- -/--	1,2, 4-12,15, 19-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2002	23/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Epskamp, S

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

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ABDULRAZIK M ET AL: "Effect of emulsion droplet surface charge on cyclosporine ocular tissue distribution." IOVS, vol. 42, no. 4, 15 March 2001 (2001-03-15), page S925 XP008005391 Annual Meeting of the Association for Research in Vision and Ophthalmology;Fort Lauderdale, Florida, USA; April 29-May 04, 2001 abstract	1-21

X	KLANG SH ET AL: "Physiochemical characterization and acute toxicity evaluation of a positively-charged submicron emulsion vehicle" JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 46, no. 12, 1994, pages 986-993, XP008005426 ISSN: 0022-3573	1,2,4,5, 7-12,15, 19-21
Y	abstract page 986, left-hand column, line 1 - line 9 page 987, left-hand column, last line -right-hand column, paragraph 3 page 991, left-hand column, paragraph 3 -right-hand column, paragraph 1 page 992, left-hand column, paragraph 3	1-21

X	KLANG SH ET AL: "Evaluation of a positively charged submicron emulsion of piroxicam on the rabbit corneum healing process following alkali burn" JOURNAL OF CONTROLLED RELEASE, vol. 57, no. 1, 1999, pages 19-27, XP004155636 ISSN: 0168-3659	1,2,4,5, 7-12,15, 19-21
Y	abstract paragraph '02.2! paragraph '0004!	1-21

X	KLANG S ET AL: "Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution" PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY, vol. 5, no. 4, 2000, pages 521-532, XP008005503 ISSN: 1083-7450	1,2,4,5, 7-12,15, 19-21
Y	abstract page 522, right-hand column, last paragraph -page 523, left-hand column, paragraph 2 page 531, left-hand column, paragraph 2	1-21

	-/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 09667 A (PHARMASOL GMBH) 7 February 2002 (2002-02-07) page 19, line 1 - line 5; examples 22-24	1,2,4,5, 7-12,15, 16,19-21

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IL 01/01015

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ional Application No
PCT/IL 01/01015

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9318852 A	30-09-1993	IL	101241 A	20-11-1997
		AT	182485 T	15-08-1999
		AU	670443 B2	18-07-1996
		AU	4368393 A	21-10-1993
		CA	2132210 A1	30-09-1993
		DE	69325796 D1	02-09-1999
		DE	69325796 T2	09-03-2000
		DK	630286 T3	29-11-1999
		EP	0630286 A1	28-12-1994
		ES	2134850 T3	16-10-1999
		GR	3031623 T3	31-01-2000
		JP	7504848 T	01-06-1995
		WO	9318852 A1	30-09-1993
		US	6007826 A	28-12-1999
WO 0209667 A	07-02-2002	DE	10036871 A1	14-02-2002
		AU	8976801 A	13-02-2002
		BR	0107042 A	04-06-2002
		WO	0209667 A2	07-02-2002

Electronic Acknowledgement Receipt

EFS ID:	18616509
Application Number:	14222478
International Application Number:	
Confirmation Number:	9616
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine/Ken Dinh
Filer Authorized By:	Laura Lee Wine
Attorney Docket Number:	17618CON6CON1 (AP)
Receipt Date:	28-MAR-2014
Filing Date:	
Time Stamp:	16:53:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	17618C6C1-IDS_03_27_2014.pdf	100632 e3c732425d699939c0954dc4c59d408637b7c237	no	25

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This is not an USPTO supplied IDS fillable form					
2	Foreign Reference	DE19810655A1.pdf	642493 a134d524e4c7505c5732a8cab9f7ff85e484f7b9	no	6
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3	Foreign Reference	EP-0471293.pdf	1658633 f4204d9aae9add3360e71d625b87af99e5fcbb41	no	7
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4	Foreign Reference	EP-0547229.pdf	8161789 81ccdbb5d595b1c2a663b402f22b952405b3ade6	no	17
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5	Foreign Reference	EP-0760237.PDF	364223 11ca6edfbedb8a6c1f617c247c539021b6d7c292	no	11
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6	Foreign Reference	EP0448856.pdf	750356 8b79ca2026d78f8b534de30a1a3826eadd24e75c	no	4
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7	Foreign Reference	EP-0480690.pdf	3237017 c925e59e7d776b37bfe8702e003d8589bd6f1fc5	no	18
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8	Foreign Reference	WO-1995-031211.pdf	609318 785816ee787dd5564887887c172789c0434e5316	no	28
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9	Foreign Reference	WO2000-000179.pdf	1156948 9ccd79b7e6bf89086a66181561ecf369d4c6df5a	no	67
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10	Foreign Reference	WO-2001-032142.pdf	393715 2363e2b822d3076474c793023490c20efaf9c7fa	no	19

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11	Foreign Reference	WO2001-041671.pdf	477723 a384ff47dd523974818c54030a51b37a8918ffbf	no	21
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12	Foreign Reference	WO2002-009667.pdf	2610140 f18e7844afe73b52f294b3cde2c525461d8f6da8	no	47
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13	Foreign Reference	WO2002-049603.pdf	540840 206d73970083fdb8f2793db01373453fd9928caf	no	25
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14	Foreign Reference	WO2003-030834.pdf	884924 73f73deb9297abb6844121ece2cf99d1170cce48	no	36
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15	Foreign Reference	WO2003-053405.pdf	495859 0d8699e29563ce29b3373ac58605c6958ccbbfb1	no	22
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16	Non Patent Literature	Abdulrazik-2001.pdf	2020446 b968c117d8bcaecd650fd6654bae4ba3db2b0fef	no	6
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17	Non Patent Literature	Acheampong-1996.pdf	77280 fdcc9ecdd28fa37ec7a80c28fe04a96b186308e3	no	1
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18	Non Patent Literature	Acheampong-1998.pdf	143254 66c7df564002661f5af66d6c394f16f314f024f4	no	4
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19	Non Patent Literature	Acheampong_1999.pdf	3760656 56867109cd7e843043e532d47d09b7e2ef238663	no	13

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20	Non Patent Literature	AkpekOph111_3_476_482_2004.pdf	667675 749fbcc7440650cffe44ba25d09130c7ca7ffd2f	no	8
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21	Non Patent Literature	Angelov-1998.pdf	747092 3babe85b48f13e0b436934e2fb66a6f224c2cb44	no	5
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22	Non Patent Literature	AngelovSafetyAssessmentAN98071079_1997.pdf	214241 ae47dd3e3dccda539cebee3d5766073e8171482a	no	4
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23	Non Patent Literature	ArdizzoneGBPoroDrugs519_542_1998.pdf	3937988 ddc7128be23a94fb204d5a5f13047aa0133a9a11	no	26
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24	Non Patent Literature	BanicDigDisSci1362_1638_2002.pdf	848297 bd631465aef4a7f1f3f1660872a340421d06bd24	no	8
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25	Non Patent Literature	BFGoodrich-1987.pdf	2452032 677b03ff97ebd0a2f498dfc177a6227c36b0ee6e	no	25
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26	Non Patent Literature	BFGoodrich-1995.pdf	517715 4a32f10eb088de77f57e1117881d32c0c376ec79	no	2
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27	Non Patent Literature	BFGoodrich-1993.pdf	2194648 96133277635c2655043d559600e4043b2f165dd6	no	9
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28	Non Patent Literature	Bonini_2004.pdf	2094249 9295f1e169d3ca4f4a97007838344654f73a0ab4	no	9

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29	Non Patent Literature	Brewster_1994.pdf	2611645 f8dd875c527914d4d072c7d0b00c7c10c30e04b0	no	7
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30	Non Patent Literature	Brewster_1997.pdf	2034358 c8c310235391b73a2515b79e5b7e69614cc154a1	no	5
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31	Non Patent Literature	Brewster_1995.pdf	2435845 a87c613e9945ed2cb3e12ca36fc33523b6a62f2d	no	6
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32	Non Patent Literature	Brinkmeier_PyodermatitisActaDerm_4_2001.pdf	462833 060bc24a76f2e8db058262428845c9fb8b9f914b	no	4
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33	Non Patent Literature	Castillo_1995.pdf	2056885 f717067b8ac69bd61564f4dd29c6d1b10f5005d6	no	8
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34	Non Patent Literature	CheeksInfluenceofVehicle1992.pdf	2450001 42b02fe1b3a1aac46ca35d929021b4907a8823ca	no	9
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35	Non Patent Literature	Database_200044.pdf	76280 a1aa73b3e4580a5dbf752ca3d23b9aa23e0f0fb3	no	2
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36	Non Patent Literature	DingPharmacAn98040585_1997.pdf	48725 0b046fb716cb7285c9f07c53e6595d8370eaaa2e	no	1
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37	Non Patent Literature	Donnenfeld_2003.pdf	1514822 7a86cc202c471cce379f185113a1353747c98e63	no	3

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38	Non Patent Literature	Drosos_1998.pdf	1638443 0e40d7b9b2ae76200baa9068530b5f21eefeaefb	no	5
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39	Non Patent Literature	Drosos_1986.pdf	1032892 820c8e084c1b12333b1f635ec09c8afefd19579e	no	5
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40	Non Patent Literature	Durrani-1992.pdf	1598136 0fbf5592ddf9a659736568779 47fb6950f34dc83	no	7
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41	Non Patent Literature	EisenTopicalcyclosporine6_1990.pdf	752102 32ad87759ef79c77880006a594495cd6b992032d	no	6
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42	Non Patent Literature	Epstein_1996.pdf	1582082 f316d19218cefdc0cca0e9be34264fe6f7503daa	no	5
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43	Non Patent Literature	ErdmannMeetingattheDeptofDerm4_1997.pdf	462543 9f3fbb7197e803def6b46260fd835c5aaa5eef07	no	4
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44	Non Patent Literature	FDA_Concludes_Restasis_1999.pdf	93984 63cff26925570cb091fa44ee5b19b28a3ff6ab6b	no	1
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45	Non Patent Literature	Gaeta_1994.pdf	1817635 70f11e97d1851a46d0c18bb53f489f7f7ac5a37e	no	9
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46	Non Patent Literature	Gipson_vol2no2_18_2004.pdf	2242587 0dc513ea67454195d9aef8bd3a8703267cb04156	no	18

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47	Non Patent Literature	Gremse_UlcerativeColitis_in_Children10.pdf	1041954 091237aaaaa18ec3e0d7b0639e55086a00c290a2	no	10
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48	Non Patent Literature	Gunduz_TopicalcyclosporinActaOphth6_1994.pdf	618144 4e3e7164d3de6e5be5691d0d7c6e8bc52a76e871	no	6
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49	Non Patent Literature	Polyethylene_Glycol_Ester_2001.pdf	459916 86544da882fd05b2012425cb171c42eb144c32ab	no	6
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50	Non Patent Literature	Hunter_1981.pdf	3004241 774fdd0660b54d79c4de0be05f7a3f8afdd571da	no	5
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51	Non Patent Literature	Jumaa_1999.pdf	1946168 f50e943dc61513d9fed6651eee99968f6d36a553	no	9
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52	Non Patent Literature	KanaiEffectontheCornea1989.pdf	868139 f60019d56376bcecff6bb85e0eb3238f954590b4	no	3
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53	Non Patent Literature	KanpolatPenetrationofCyclosporin1994.pdf	359650 43be2c9cd6ba4e612554d7dd526b1e2b4f343b4e	no	4
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54	Non Patent Literature	Kaur_1979.pdf	906300 496c705d93f0d7baa2424db48fbbf424512f96e6	no	2
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Information:					
55	Non Patent Literature	KuwanoCyclosporineA_Pharma19_1_108_111_2002.pdf	1878659 4e9a19149a7164165e1106060b80c8485b150be7	no	4

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56	Non Patent Literature	Lambert_2003.pdf	460126 a4c43396f9847fb7308e6c0741e187941cd8aca8	no	3
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57	Non Patent Literature	Leibovitz_1983.pdf	1024735 2c50222cc641655f84228b313ee6df845bcb2761	no	5
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58	Non Patent Literature	Lixin_2002.pdf	1887719 6552422b21a2e5430452221f662f614d7560ede7	no	4
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59	Non Patent Literature	LopatinChemicalcompositions31pgs2001.pdf	13079081 55c1ea8f4cfcf4343e8d36c01dadadf7516e0c55	no	31
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60	Non Patent Literature	LyonsInfluenceofThreeEmulsion2000.pdf	59547 ea3c95c367170b2d95ca78b32f69875baf2a4c35	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	94266360		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

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

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/15 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	17618CON6CON1 (AP)
First Named Inventor	Andrew Acheampong
Title	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COM
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [✓] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [✓] **Specification** [*Total Pages* 34] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [ ] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* ____]
6. **Inventor's Oath or Declaration** [*Total Pages* 3] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [✓] A copy from a prior application (37 CFR 1.63(d))
7. [✓] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee _____
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [✓] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [✓] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [ ] **Other:** _____

***Note**: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[✓] The address associated with Customer Number: 051957 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Laura L. Wine/	Date	March 21, 2014
Name (Print/Type)	Laura L. Wine	Registration No. (Attorney/Agent)	68681

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- [x] The attached application, or
- [ ] United States application or PCT international application number _____ filed on _____.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Diane D. Tang-Liu Date (Optional) : _____

Signature: _____

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

PTO/AIA/01 (06-12)
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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 13/961,828 filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Andrew Acheampong Date (Optional):

Signature: Andrew Acheampong

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA/01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 13/961,828 filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: DAVID F. POWER Date (Optional): 8-14-2013

Signature: David F. Power

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA/01 form for each additional inventor.

Doc code: Oath
Document Description: Oath or declaration filed

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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Methods of Providing Therapeutic Effects Using Cyclosporin Components Docket No.: 17618CON6(AP)

This statement is directed to:

☐ The attached application,

OR

☒ United States application or PCT international application number 13/961,828 filed on 8-7-13.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

James N. Chang

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Newport Beach	CA	US

Mailing Address (except for a deceased or legally incapacitated inventor):

36 Cervantes

City	State	Zip	Country
Newport Beach	CA	92660	US

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

- ☐ Legal Representative (for deceased or legally incapacitated inventor only),
- ☒ Assignee,
- ☐ Person to whom the inventor is under an obligation to assign,
- ☐ Person who otherwise shows a sufficient proprietary interest in the matter (petition under 37 CFR 1.46 is required), or
- ☐ Joint Inventor.