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(54) Titre : COMPOSITIONS DE CYCLOSPORINE
(54) Title: CYCLOSPORIN COMPOSITIONS

(57) **Abrégé/Abstract:**

Disclosed herein are therapeutic methods, compositions, and medicaments related to cyclosporine.



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CYCLOSPORIN COMPOSITIONS**by Inventors**

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10

RELATED APPLICATION

This application claims the benefit of and priority
to U.S. Patent Application Serial No. 11/858,200, filed
15 September 20, 2007, a continuation-in-part, which claims
priority to U.S. Patent Application Serial No.
11/781,095, filed July 20, 2007, which claims priority to
U.S. Provisional Application Serial No. 60/820,239, filed
July 25, 2006; U.S. Provisional Application Serial No.
20 60/829,796, filed October 17, 2006; U.S. Provisional
Application Serial No. 60/829,808, filed October 17,
2006; U.S. Provisional Application Serial No. 60/883,525,
filed January 5, 2007; U.S. Provisional Application
Serial No. 60/916,352, filed May 7, 2007; and U.S.
25 Provisional Application Serial No. 60/869,459, filed
December 11, 2006; each of which is hereby incorporated
by reference in their entirety.

BACKGROUND OF THE INVENTION

30

Abnormalities associated with the function of the
lacrimal gland or with tearing often cause discomfort to
mammals who suffer from these abnormalities.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 Mean ($\pm$ SD) cornea cyclosporine A concentrations (semi-log) following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Fig. 2 Mean ($\pm$ SD) conjunctiva cyclosporine A concentrations (semi-log) following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Fig. 3 Mean ($\pm$ SD) sclera cyclosporine A concentrations (semi-log) following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Fig. 4 Mean ($\pm$ SD) eyelid margin cyclosporine A concentrations (semi-log) following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Fig. 5 Mean ($\pm$ SD) nasolacrimal duct cyclosporine A concentrations (semi-log) following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

5

DETAILED DESCRIPTION OF THE INVENTION

A composition comprising cyclosporin A at a concentration of from about 0.0001% (w/v) to less than about 0.05% (w/v) is disclosed herein.

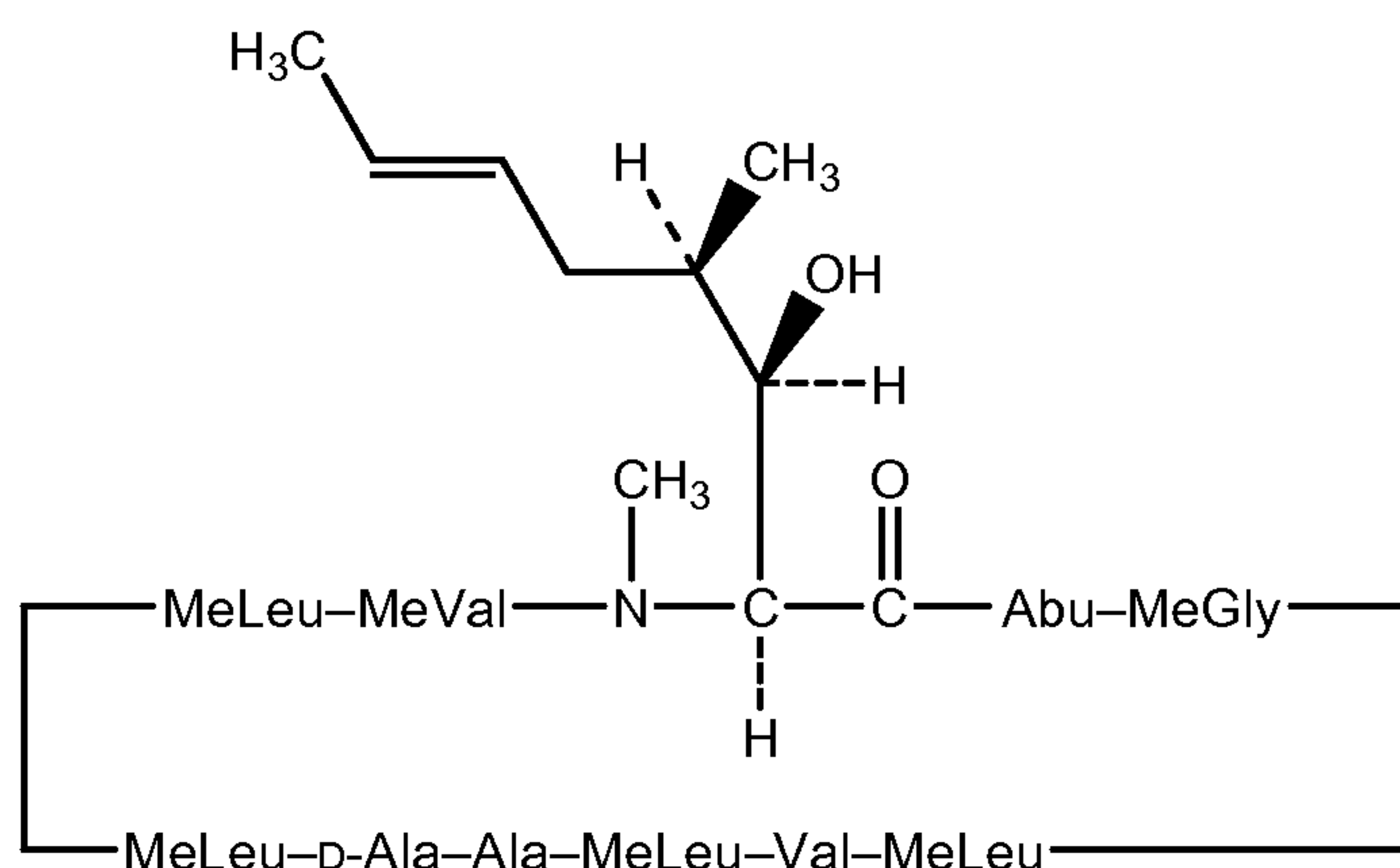
We have surprisingly discovered that compositions of cyclosporin A at a concentration of less than about 0.05% (w/v) can be prepared that will be therapeutically effective.

In one embodiment, the compositions disclosed herein are administered to an eye of a mammal in need thereof to treat loss of corneal sensitivity after surgery affecting the cornea.

In another embodiment, the compositions disclosed herein are administered to an eye of a mammal in need thereof to improve recovery of corneal sensitivity after surgery affecting the cornea.

In another embodiment, the compositions disclosed herein are administered to an eye of a mammal in need thereof to treat post herpetic loss of corneal sensitivity.

In another embodiment, the compositions disclosed herein are administered to an eye of a mammal in need thereof to treat dry eye disease.



Cyclosporin A

5

Cyclosporin A is a cyclic peptide with immunosuppressive properties having the structure shown above. It is also known by other names including cyclosporine, cyclosporine A, ciclosporin, and
 10 ciclosporin A.

Treatment Methods

One embodiment is a method of treating loss of corneal sensitivity comprising topically administering to
 15 a mammal in need thereof a composition comprising cyclosporin A at a concentration of from 0.0001% (w/v) to less than about 0.05% (w/v).

The treatment generally comprises administering 10-50 μ L drops of the compositions disclosed herein
 20 topically to the eye or eyes of the mammal or human. Determination of the number of drops administered per day to the person or mammal to provide effective relief is within the skill of the ordinary artisan.

Loss of corneal sensitivity may be related to a
 25 number of factors. For example, loss of corneal sensitivity is often caused by surgery affecting the cornea or by viral infection.

5 Examples of surgery that can cause loss of corneal sensitivity include keratorefractive surgery or penetrating keratoplasty, such as the following procedures:

 radial keratotomy,
10 photorefractive keratotomy,
 laser-assisted in situ keratomileusis (LASIK),
 laser assisted sub-epithelial keratomileusis (LASEK),
 SB-LASIK,
 EPI-LASIK,
15 and the like.

 Examples of viral infections that can cause loss of corneal sensitivity include:

 HSV-1,
 HSV-2,
20 VZV,
 and the like

 In one embodiment, the composition is administered from 1 to 4 times per day.

25 In another embodiment, the composition is administered twice a day.

 In another embodiment, the composition is administered only once a day.

 In another embodiment, less than 14% of patients
30 suffer ocular burning when the composition is administered only once a day for a period of three months.

 In another embodiment, less than 10% of patients suffer ocular burning when the composition is

5 administered only once a day for a period of three months.

In another embodiment, less than 8% of patients suffer ocular burning when the composition is administered only once a day for a period of three
10 months.

For the purposes of this disclosure, "treat," "treating," or "treatment" refer to the use of a compound, composition, therapeutically active agent, or drug in the diagnosis, cure, mitigation, treatment,
15 prevention of disease or other undesirable condition, or to affect the structure or any function of the body of man or other animals.

20

Compositions

The concentration of cyclosporin A is less than about 0.05%. This is intended to mean that the concentration is lower than the concentration in the
25 commercially available 0.05% cyclosporin A emulsion known as Restasis[®].

In another embodiment, the concentration of cyclosporin A is from about 0.005% (w/v) to about 0.04% (w/v).

30 In another embodiment, the concentration of cyclosporin A is from about 0.02% (w/v) to about 0.04% (w/v).

In another embodiment, the concentration of cyclosporine A is about 0.005% (w/v).

5 In another embodiment, the concentration of cyclosporine A is about 0.015% (w/v).

 In another embodiment, the concentration of cyclosporine A is about 0.0015% (w/v).

 In another embodiment, the concentration of
10 cyclosporine A is about 0.02% (w/v).

 In another embodiment, the concentration of cyclosporine A is about 0.03% (w/v).

 In another embodiment, the concentration of cyclosporine A is about 0.04% (w/v).

15 A liquid which is ophthalmically acceptable is formulated such that it can be administered topically to the eye. The comfort should be maximized as much as practicable, although sometimes formulation
20 considerations (e.g. drug stability, bioavailability, etc.) may necessitate less than optimal comfort. In the case that comfort cannot be maximized, the liquid should be formulated such that the liquid is tolerable to the patient for topical ophthalmic use. Additionally, an
25 ophthalmically acceptable liquid should either be packaged for single use, or contain a preservative to prevent contamination over multiple uses.

 For ophthalmic application, solutions or medicaments are often prepared using a physiological saline solution as a major vehicle. Ophthalmic solutions are often
30 maintained at a comfortable pH with an appropriate buffer system. The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.

 Various buffers and means for adjusting pH may be
35 used so long as the resulting preparation is

5 ophthalmically acceptable. Accordingly, buffers include, but are not limited to, acetate buffers, citrate buffers, phosphate buffers and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

10 In another embodiment, the composition contains a preservative.

Preservatives that may be used in the pharmaceutical compositions disclosed herein include, but are not limited to,

cationic preservatives such as

15 quaternary ammonium compounds including benzalkonium chloride, polyquad, and the like;
guanidine-based preservatives including PHMB, chlorhexidine, and the like;
chlorobutanol;

20 mercury preservatives such as thimerosal, phenylmercuric acetate and phenylmercuric nitrate; and
oxidizing preservatives such as stabilized oxychloro complexes (e.g. Purite[®]).

In another embodiment, the composition contains a
25 surfactant.

A surfactant may be used for assisting in dissolving an excipient or an active agent, dispersing a solid or liquid in a composition, enhancing wetting, modifying drop size, or a number of other purposes. Useful
30 surfactants include, but are not limited to surfactants of the following classes: alcohols; amine oxides; block polymers; carboxylated alcohol or alkylphenol ethoxylates; carboxylic acids/fatty acids; ethoxylated alcohols; ethoxylated alkylphenols; ethoxylated aryl
35 phenols; ethoxylated fatty acids; ethoxylated; fatty

5 esters or oils (animal & veg.); fatty esters; fatty acid
methyl ester ethoxylates; glycerol esters; glycol esters;
lanolin-based derivatives; lecithin and lecithin
derivatives; lignin and lignin derivatives; methyl
esters; monoglycerides and derivatives; polyethylene
10 glycols; polymeric surfactants; propoxylated &
ethoxylated fatty acids, alcohols, or alkyl phenols;
protein-based surfactants; sarcosine derivatives;
sorbitan derivatives; sucrose and glucose esters and
derivatives.

15 In particular, ethoxylate surfactants are useful.

An ethoxylate surfactants is one that comprises the
moiety $-O(CH_2CH_2O)_n-OH$, wherein n is at least about 1.

In one embodiment n is from about 1 to about 10,000.

In another embodiment, n is from 1 to about 1000.

20 In another embodiment, n is from about 1 to about
500.

Some ethoxylates contain one ethoxylate moiety. In
other words, there is a single ethoxylate chain on each
molecule.

25 Examples of surfactants with one ethoxylate moiety,
include, but are not limited to:

Ethoxylated alcohols wherein the alcohol has a
single hydroxyl unit; alkylphenol ethoxylates;
ethoxylated fatty acids; fatty acid methyl ester
30 ethoxylates; polyethylene glycols; and the like.

Ethoxylates may comprise more than one ethoxylate
moiety. In other words, there may be ethoxylate moieties
attached to several different parts of the molecule.
Examples include, but are not limited to: block polymers;

5 ethoxylated oils; sorbitan derivatives; sucrose and glucose ethoxylates; and the like.

Block Polymers: These are polymers with the structure A-B-A', wherein A and A' are polyethylene chains of 1 or more ethylene units, and B is a polypropylene chain of
10 one or more propylene units. Generally, but not necessarily, A and A' are approximately the same length. In one embodiment, A and A' contain from about 2 to about 200 ethylene units.

In another embodiment, A and A' contain from about 5 to
15 about 100 ethylene units.

In another embodiment, A and A' contain about 7 to about 15 ethylene units.

In another embodiment, A and A' contain about 7, about 8, or about 12 ethylene units.

20 In another embodiment, B contains from about 25 to about 100 propylene units.

In another embodiment, B contains from about 30 to about 55 propylene units.

In another embodiment, B contains about 30, about 34, or
25 about 54 propylene units.

In another embodiment, the molecular weight is from about 1000 to about 20000.

In another embodiment, the molecular weight is from about 2000 to about 10000.

30 In another embodiment, the molecular weight is about 2500, about 3000, about 3800, or about 8400.

These include but are not limited to:

Poloxalene: wherein A has about 12 ethylene oxide units, B has about 34 propylene oxide units, A' has about 12

5 ethylene oxide units, and the average molecular weight is about 3000.

Poloxamer 182: wherein A has about 8 ethylene oxide units, B has about 30 propylene oxide units, A' has about 8 ethylene oxide units, and the average molecular weight
10 is about 2500

Poloxamer 188: wherein A has about 75 ethylene oxide units, B has about 30 propylene oxide units, A' has about 75 ethylene oxide units, and the average molecular weight is about 8400.

15 Poloxamer 331: wherein A has about 7 ethylene oxide units, B has about 54 propylene oxide units, A' has about 7 ethylene oxide units, and the average molecular weight is about 3800;

Ethoxylated Alcohols

20 These include but are not limited to:

Ethoxylates of linear alcohols having from about 6 to about 20 carbon atoms.

In one embodiment, the linear alcohol has from about 10 to about 16 carbon atoms.

25 In another embodiment, n is from about 1 to about 100.

In another embodiment, n is from about 1 to about 50.

In another embodiment, n is from about 5 to about 50 ethylene oxide units.

In another embodiment, n is from about 1 to about 20
30 ethylene oxide units.

In another embodiment, n is from about 30 to about 50 ethylene oxide units.

Ethoxylated Alkylphenols

These are alkylphenols that are ethoxylated, i.e. the
35 phenolic OH is replaced with an ethoxylate moiety.

- 5 These include but are not limited to:
 octylphenol ethoxylate, i.e. $C_8H_{17}Ph(OCH_2CH_2O)_nH$.
 nonylphenol ethoxylate, i.e. $C_9H_{19}Ph(OCH_2CH_2O)_nH$.
 alkyphenols of the above formula wherein n is from about
 1 to about 100.
 10 alkyphenols of the above formula wherein n is from about
 1 to about 50.
 alkyphenols of the above formula wherein n is from about
 9 to about 15.

Octyl Phenol 1.5 Mole Ethoxylate (i.e. n is an
 15 average of about 1.5); Octyl Phenol 5 Mole Ethoxylate;;
 Octyl Phenol 7 Mole Ethoxylate; Octyl Phenol 9 Mole
 Ethoxylate; Octyl Phenol 12 Mole Ethoxylate; Octyl Phenol
 40 Mole Ethoxylate; Nonyl Phenol 1.5 Mole Ethoxylate;
 Nonyl Phenol 4 Mole Ethoxylate; Nonyl Phenol 6 Mole
 20 Ethoxylate; Nonyl Phenol 9 Mole Ethoxylate; Nonyl Phenol
 10 Mole Ethoxylate; Nonyl Phenol 10.5 Mole Ethoxylate;
 Nonyl Phenol 12 Mole Ethoxylate; Nonyl Phenol 15 Mole
 Ethoxylate; Nonyl Phenol 15 Mole Ethoxylate; Nonyl Phenol
 30 Mole Ethoxylate; and Nonyl Phenol 40 Mole Ethoxylate;

25 **Ethoxylated Fatty Acids,**

These include but are not limited to:
 ethoxylates which are esterified to form either:

monoesters, i.e. $RCO_2(CH_2CH_2O)_nOH$, where RCO_2H is a
 fatty acid; or

30 diesters, i.e. $RCO_2(CH_2CH_2O)_nC(=O)R$.

Fatty acids include, but are not limited to:

Saturated fatty acids, which have no C=C moieties and
 include myristic acid, palmitic acid, stearic acid,
 arachidic acid, behenic acid, lignoceric acid.

35 Unsaturated fatty acids, including the following:

5 monounsaturated fatty acids, which have one C=C
group such as palmitoleic acid, oleic acid, and
nervonic acid;
 diunsaturated fatty acids, which have two C=C
groups, such as linoleic acid;
10 triunsaturated fatty acids, which have three C=C
groups, such as α -linolenic acid and γ -linolenic
acid;
 tetraunsaturated fatty acids, which have four C=C
groups, such as arachidonic acid; and
15 pentaunsaturated fatty acids, which have five C=C
groups, such as eicosapentaenoic acid.

The following may also be used:

Lauric Acid; 14 carbon fatty acids such as myristic acid;
16 carbon fatty acids such as palmitic and palmitoleic
20 acid; 18 carbon fatty acids such as stearic acid, oleic
acid, linoleic acid, α -linolenic acid, and γ -linolenic
acid; 20 carbon fatty acids such as eicosapentaenoic
acid; 22 carbon fatty acids such as arachidic acid; and
24 carbon carbon fatty acids such as lignoceric acid and
25 nervonic acid.

In one embodiment, n is from about 2 to about 100.

In another embodiment, n is from about 5 to about 50.

In another embodiment, n is from about 30 to 50.

Ethoxylated Fatty Esters or Oils (Animal & Veg.).

30 These are the products which result from reacting
ethylene oxide with a fatty ester or an oil. When a
fatty oil is used, the products is a mixture of
ethoxylates of the fatty acids present in the oil,
ethoxylates of glycerine, ethoxylates of mono and
35 diglycerides, and the like.

5 Specific examples include, but are not limited to:
Ethoxylates of the following oils: Anise oil, Castor oil,
Clove oil, Cassia oil, Cinnamon oil; Almond oil, Corn
oil, Arachis oil, Cottonseed oil, Safflower oil, Maize
oil, Linseed oil, Rapeseed oil, Soybean oil, Olive oil,
10 Caraway oil, Rosemary oil, Peanut oil, Peppermint oil,
Sunflower oil, Eucalyptus oil and Sesame oil; Coriander
oil, Lavender oil, Citronella oil, Juniper oil, Lemon
oil, Orange oil, Clary sage oil, Nutmeg oil, Tea tree
oil, coconut oil, tallow oil, and lard;

15 In one embodiment, from 1 to about 50 moles of ethylene
oxide is used per mole of the oil triglyceride.

In another embodiment, from about 30 to about 40 moles of
ethylene oxide is used per mole of the oil triglyceride.

Ethylene oxide may also react with a fatty acid
20 ester with a formula $\text{RCO}_2\text{R}'$ to form $\text{RCO}_2(\text{CH}_2\text{CH}_2\text{O})_n\text{R}'$.
Thus, surfactants having the formula $\text{RCO}_2(\text{CH}_2\text{CH}_2\text{O})_n\text{R}'$,
where RCO_2H is a fatty acid and R' is alkyl having from 1
to 6 carbons are contemplated.

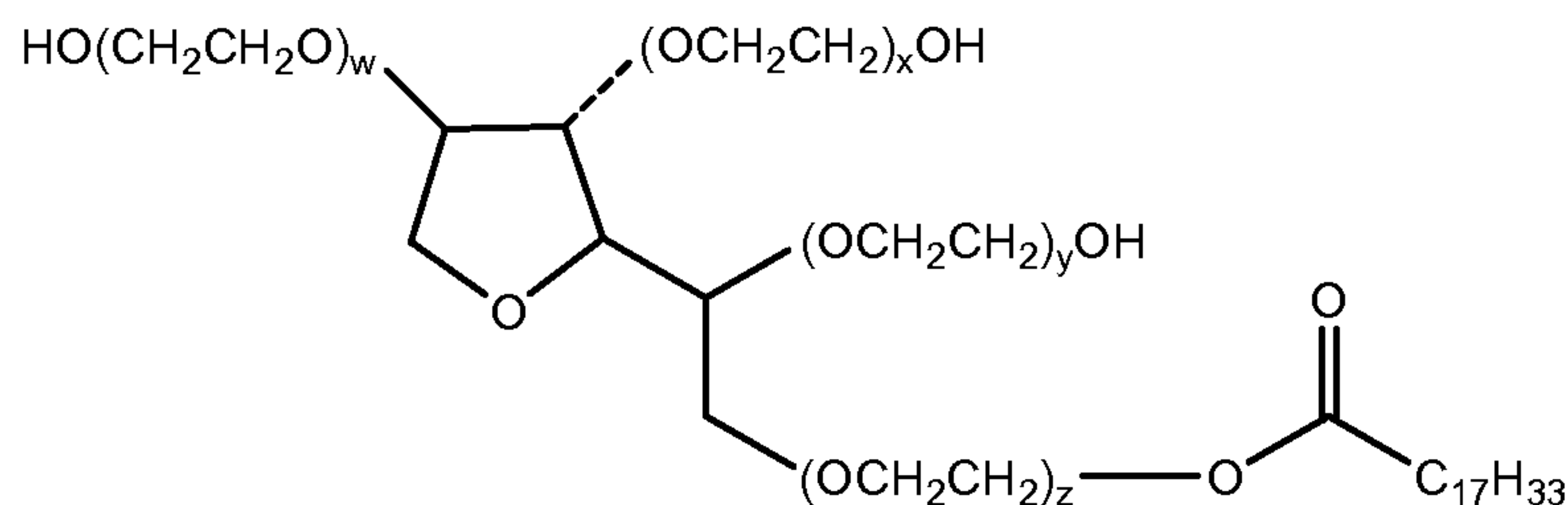
One embodiment is a fatty acid methyl ester
25 ethoxylate, wherein R' is methyl.

In another embodiment, RCO_2H is Lauric Acid; a 14
carbon fatty acid such as myristic acid; a 16 carbon
fatty acid such as palmitic and palmitoleic acid; an 18
carbon fatty acids such as stearic acid, oleic acid,
30 linoleic acid, α -linolenic acid, and γ -linolenic acid; a
20 carbon fatty acids such as eicosapentaenoic acid; a 22
carbon fatty acids such as arachidic acid; or a 24 carbon
carbon fatty acids such as lignoceric acid and nervonic
acid.

5 **Polyethylene Glycols** are ethoxylates that are unsubstituted, or terminated with oxygen on both ends, i.e. $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$,

Sorbitan Derivatives:

These are ethoxylated sorbates having a fatty acid capping one or more of the ethoxylated chains. For example, polysorbate 80 has an oleate cap as shown in the structure below.



Polysorbate 80
(Sum of w, x, y, and z is 20)

These compounds are named as POE (w+x+y+z) sorbitan mono (or di- or tri-) fatty acid.

For example, Polysorbate 80 is POE (20) sorbitan monooleate.

Thus, the number in parenthesis is the total number of ethylene oxide units on the molecule, and the ending is the number of acid caps and the capping acid.

These include but are not limited to:

Sorbitan derivatives wherein the total number of ethylene oxide units is from 3 to 30;

Sorbitan derivatives wherein the total number of ethylene oxide units is 4, 5, or 20;

Sorbitan derivatives wherein the capping acid is laurate, palmitate, stearate, or oleate;

The sorbitan derivative may be a POE sorbitan monolaurate;

a POE sorbitan dilaurate;

5 a POE sorbitan trilaurate;
a POE sorbitan monopalmitate;
a POE sorbitan dipalmitate;
a POE sorbitan tripalmitate;
a POE sorbitan monostearate;
10 a POE sorbitan distearate;
a POE sorbitan tristearate;
a POE sorbitan monooleate;
a POE sorbitan dioleate;
or a POE sorbitan trioleate;
15 Specific examples include:
POE (20) sorbitan monolaurate; POE (4) sorbitan
monolaurate; POE (20) sorbitan monopalmitate; POE (20)
monostearate; POE (20) sorbitan monostearate; POE (4)
sorbitan monostearate; POE (20) sorbitan tristearate; POE
20 (20) sorbitan monooleate; POE (20) sorbitan 15 monooleate;
POE (5) sorbitan 10 monooleate; POE (20) sorbitan
trioleate; and

Sucrose and Glucose Esters and Derivatives:

Although there are a number of sucrose and glucose based
25 surfactants, some sucrose and glucose esters and
derivatives are similar to the sorbate derivatives
described above. In other words, one, several, or all of
the hydroxyl moieties of the sugar are ethoxylated, and
one or more of the ethoxylate chains are capped with a
30 carboxylic acid. Other sucrose and glucose esters are
simply ethoxylated, but do not have a capping carboxylic
acid. Other sucrose and glucose esters may be ethoxylated
and capped with an alkyl group formed by reaction with an
alcohol. Other sucrose and glucose esters may be esters
35 or ethers of the sugars with hydrophobic chains and have

5 ethoxylates substituted in other positions on the sugar.

Various useful vehicles may be used in the ophthalmic preparations disclosed herein. These vehicles include, but are not limited to, polyvinyl alcohol, povidone,
10 hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose, and acrylates (e.g. Pemulen®).

Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts,
15 particularly sodium chloride, potassium chloride, mannitol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

In a similar vein, an ophthalmically acceptable antioxidant includes, but is not limited to, sodium
20 metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

Other excipient components which may be included in the ophthalmic preparations are chelating agents. A useful chelating agent is edetate disodium, although other
25 chelating agents may also be used in place or in conjunction with it.

Compositions may be aqueous solutions or emulsions, or some other acceptable liquid form. For an emulsion, one or more oils will be used to form the emulsion, and
30 in some instances one or more surfactants will be required. Suitable oils include, but are not limited to anise oil, castor oil, clove oil, cassia oil, cinnamon oil, almond oil, corn oil, arachis oil, cottonseed oil, safflower oil, maize oil, linseed oil, rapeseed oil,
35 soybean oil, olive oil, caraway oil, rosemary oil, peanut

5 oil, peppermint oil, sunflower oil, eucalyptus oil,
sesame oil, and the like.

In one embodiment, the composition is an aqueous solution.

10 In another embodiment, the composition contains no ethanol.

In another embodiment, the composition contains no hyaluronic acid.

In another embodiment, the composition contains no vitamin E TPMS.

15 In another embodiment, the composition contains no cyclodextrin A.

In another embodiment, the composition contains no cyclodextrin.

Example 1

20

Ingredients	Percent Ingredients (% w/v)	Amount needed (g) for a 1 liter batch
Cyclosporine	0% for Placebo (P)	0 grams for Placebo (P)
	0.03% (A)	0.30 (A)
	0.04% (B)	0.40 (B)
	0.05% (C)	0.5 (C)
Carboxymethylcellulose sodium	0.5	5.0
Polysorbate 80	1.0	10.0
Glycerin	1.0	10.0
Mannitol	0.5	5.0
Sodium Citrate Dihydrate	0.4	4.0
Boric Acid	0.25	2.5
Sodium Borate Decahydrate	0.41	4.1

Potassium Chloride	0.14	1.4
Purite	0.01	0.1
Purified Water	q.s. to 100%	q.s to 100%

5

Compositions P, A, B and C, are prepared according to the following procedure.

10 1. Measure **Purified Water** to about 90% of the batch size and place in an appropriate beaker or container.

2. Begin mixing the water with a strong mixer (Rotosolver) to obtain a strong vortex.

15

3. Add the pre-weighed **carboxymethylcellulose sodium** into the strong vortex. Continue strong mixing for at least 1 hour.

20 4. Slow mixer to a slow speed.

5. Add and dissolve the pre-weighed **polysorbate 80**.

6. Add and dissolve the pre-weighed **glycerin**.

25

7. Add and dissolve the pre-weighed **mannitol**.

8. Add and dissolve the pre-weighed **sodium citrate dehydrate**.

30

9. Add and dissolve the pre-weighed **boric acid**.

5 10. Add and dissolve the pre-weighed **sodium borate decahydrate**.

11. Add and dissolve the pre-weighed **potassium chloride**.

10 12. Check pH and adjust if necessary. **Target pH is 7.5 +/- 0.1**.

13. Add and dissolve the pre-weighed **Purite**.

15 14. Add sufficient quantity of **Purified Water** to attain the final batch volume. This will provide the finished placebo formulation (P).

Procedure for either 0.03% (A), 0.04% (B), 0.05% (C)

20

15. Measure the exact amount of Placebo (9815X) needed to satisfy the batch size requirements and place in a media bottle that contains a magnetic stir bar.

25 16. Add and dissolve the pre-weighed **cyclosporine**. Stir at a slow speed to avoid foaming. It will usually take overnight mixing to completely dissolve the cyclosporine.

17. After overnight mixing is completed, pump the
30 cyclosporine solution through a Millipore Milligard pre-filter and a Pall Suporlife sterilizing filter and collect the filtrate aseptically.

5 18. The sterile filtrate can then be aseptically
dispensed into multidose dropper bottles suitable for
ophthalmic purpose.

19. The finished product should be tested for
10 cyclosporine assay, pH, osmolality, viscosity, Purite,
sterility, and antimicrobial effectiveness.

20. The finished product should be store at room
temperature and protected from light.

15

Example 2

The following formulations were prepared. D and E
were prepared by standard methods known in the art. F
was prepared as described above for A-C except that
20 Pemulen TR-2 was substituted for carboxymethylcellulose
sodium, and the addition of the citrate and borate
buffers were omitted.

	D	E	F
	Emulsion	Emulsion	Solution
Cyclosporin A	0.05	0.05	0.05
Castor Oil	1.25	0.30	N/A
Polyoxyethylene 40 Stearate, NF	N/A	0.30	N/A
Polysorbate 80	1.00	0.30	1.00
Glycerin	2.20	1.00	1.00
Mannitol	N/A	2.00	2.00
Pemulen TR-2	0.05	0.10	0.10
Sodium Hydroxide (1N)	pH adjustment	pH adjustment	pH adjustment
Purified Water	QS	QS	QS
pH	pH=7.4	7.39	7.35

5 Bioavailability

The compositions disclosed and used herein provide a therapeutically effective amount of cyclosporin A to a mammal. However, while not intending to limit the scope of the invention in any way, concentrations of
10 cyclosporin A in the compositions may be significantly lower than those normally associated with a therapeutically effective concentration. For example, one commercial preparation, marketed as Restasis[®] by Allergan, Inc., is a 0.05% cyclosporin A castor oil
15 emulsion. Other compositions currently in development have concentrations of 0.1% or higher.

Reported herein are pharmacokinetic data for in vivo experiments on rabbits. However, the rabbit experiments are believed to be useful models for bioavailability in
20 other mammals including humans. Thus, although bioavailability parameters are disclosed and featured in the claims, they should not be construed as limiting the treatment to rabbits only, but the compositions characterized and defined by bioavailability in rabbits
25 are also contemplated for use in treatment in other mammals, particularly humans.

In one embodiment, the composition provides more cyclosporin A to the cornea of a person than Composition AA.

30 In another embodiment, the composition provides more cyclosporin A to the cornea of a person than Composition BB.

In another embodiment, the composition provides more cyclosporin A to the cornea of a person than Composition
35 CC.

5 In one embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
DD.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
10 EE.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
FF.

 In one embodiment, the composition provides more
15 cyclosporin A to the cornea of a person than Composition
GG.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
HH.

20 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
II.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
25 JJ.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
KK.

 In another embodiment, the composition provides more
30 cyclosporin A to the cornea of a person than Composition
LL.

 In another embodiment, the composition provides more
cyclosporin A to the cornea of a person than Composition
MM.

5 In one embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition AA.

 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
10 Composition BB.

 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition CC.

 In one embodiment, the composition provides more
15 cyclosporin A to the conjunctiva of a person than
Composition DD.

 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition EE.

20 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition FF.

 In one embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
25 Composition GG.

 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition HH.

 In another embodiment, the composition provides more
30 cyclosporin A to the conjunctiva of a person than
Composition II.

 In another embodiment, the composition provides more
cyclosporin A to the conjunctiva of a person than
Composition JJ.

5 In another embodiment, the composition provides more cyclosporin A to the conjunctiva of a person than Composition KK.

 In another embodiment, the composition provides more cyclosporin A to the conjunctiva of a person than
10 Composition LL.

 In another embodiment, the composition provides more cyclosporin A to the conjunctiva of a person than Composition MM.

 In another embodiment, topical administration of one
15 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 500 ng of cyclosporin A per gram of cornea of said rabbit at 30 minutes after said topical administration.

20 In another embodiment, wherein topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 1000 ng of cyclosporin A per gram of cornea of said rabbit at 30
25 minutes after said topical administration.

 In another embodiment, topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 1400 ng of cyclosporin A per gram
30 of cornea of said rabbit at 30 minutes after said topical administration.

 In another embodiment, wherein topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to
35 the corneas of said rabbit at least about 2000 ng of

5 cyclosporin A per gram of cornea of said rabbit at 30 minutes after said topical administration.

In another embodiment, topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 2400 ng of cyclosporin A per gram of cornea of said rabbit at 30 minutes after said topical administration.

In another embodiment, topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 17000 ng of cyclosporin A per gram of cornea of said rabbit over a period of 24 hours after said topical administration.

In another embodiment, said composition is an aqueous solution containing from 0.005% to about 0.04% cyclosporin A, wherein topical administration of one 35 μ L drop of said composition to each eye of a New Zealand rabbit provides at least about 17000 ng of cyclosporin A per gram of cornea to the corneas of said rabbit as determined by:

topically administering said composition to each eye of each of 15 female New Zealand white rabbit test subjects; and

determining the amount of cyclosporin A in the corneas of three subjects at times of about 0.5 hours, about 2 hours, about 6 hours, about 12 hours, and about 24 after administration to said subject;

wherein the amount of cyclosporin A in the cornea is determined only once for each subject.

5 In another embodiment said composition to each eye of a New Zealand rabbit provides at least about 30000 ng of cyclosporin A per gram of cornea to the corneas of said rabbit.

10 In another embodiment said composition to each eye of a New Zealand rabbit provides at least about 45000 ng of cyclosporin A per gram of cornea to the corneas of said rabbit.

15 In another embodiment said composition to each eye of a New Zealand rabbit provides at least about 95000 ng of cyclosporin A per gram of cornea to the corneas of said rabbit.

20 In another embodiment said composition to each eye of a New Zealand rabbit provides at least about 155000 ng of cyclosporin A per gram of cornea to the corneas of said rabbit.

25 In another embodiment, topical administration of one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the conjunctivas of said rabbit at least about 6000 ng of cyclosporin A per gram of conjunctiva of said rabbit over a period of 24 hours after said topical administration.

30 In another embodiment, said composition is an aqueous solution containing from 0.005% to about 0.04% cyclosporin A, wherein topical administration of one 35 μ L drop of said composition to each eye of a New Zealand rabbit provides at least about 6000 ng of cyclosporin A per gram of conjunctiva to the conjunctivas of said rabbit as determined by:

5 topically administering said composition to each eye of
each of 15 female New Zealand white rabbit test
subjects; and
determining the amount of cyclosporin A in the
conjunctivas of three subjects at times of about 0.5
10 hours, about 2 hours, about 6 hours, about 12 hours,
and about 24 after administration to said subject;
wherein the amount of cyclosporin A in the conjunctiva is
determined only a single time for each subject.

In another embodiment said composition to each eye
15 of a New Zealand rabbit provides at least about 5000 ng
of cyclosporin A per gram of conjunctiva to the
conjunctiva of said rabbit.

In another embodiment said composition to each eye
of a New Zealand rabbit provides at least about 7000 ng
20 of cyclosporin A per gram of conjunctiva to the
conjunctiva of said rabbit.

In another embodiment said composition to each eye
of a New Zealand rabbit provides at least about 10000 ng
of cyclosporin A per gram of conjunctiva to the
25 conjunctiva of said rabbit.

In another embodiment said composition to each eye
of a New Zealand rabbit provides at least about 17000 ng
of cyclosporin A per gram of conjunctiva to the
conjunctiva of said rabbit.

30 In another embodiment, the blood level of cyclosporin A
is less than 0.1 mg/mL for a person for whom the
composition has been administered twice a day topically
to both eyes in 35 microliter drops for twelve months.

35 **Pharmacokinetic Study 1**

5 A 35 μ L aliquot of one of three test formulations was topically administered to each eye of a female New Zealand White rabbit (n=3 rabbits/time point). At 0.5, 2, 6, 12, 24, 48 and 144 hours post-dose, cornea, conjunctiva, sclera, eyelid margin, nasolacrimal duct, and blood samples were collected. Samples collected from 10 naïve rabbits (n=2) served as pre-dose samples. The quantitation ranges were 0.2-40 ng/mL in blood, 0.1-200 ng in cornea and conjunctiva, 0.1-100 ng in eyelid margin and nasolacrimal duct, and 0.1-20 ng in sclera and 15 lacrimal gland.

The pharmacokinetic parameters of cyclosporine A in ocular tissues following a single ophthalmic instillation of one of three 0.05% cyclosporine A formulations are summarized in Table 1 below:

20 **Table 1**

Tissue/Matrix	Composition F			Composition E			Composition D		
	C_{max} (ng/g)	AUC_{0-t} (ng·hr/g)	$t_{1/2}$ (hr)	C_{max} (ng/g)	AUC_{0-t} (ng·hr/g)	$t_{1/2}$ (hr)	C_{max} (ng/g)	AUC_{0-t} (ng·hr/g)	$t_{1/2}$ (hr)
Cornea	4050	163000	41.3	1100	76200	41.7	536	29300	49.8
Conjunctiva	4460	18100	11.3	2560	11600	5.57	694	5290	4.55
Sclera	545	6110	29.7	136	2840	24.8	53.0	1040	18.7
Eyelid Margin	3120	38300	42.5	2020	42200	38.1	2450	27700	24.4
Nasolacrimal Duct	195	2190	NC	74.4	1190	NC	72.0	279	NC
Blood	221	NC	NC	0.441	NC	NC	BLQ	BLQ	NC

NC = Not calculable

BLQ = Below the limit of quantitation

Briefly summarizing, following a single ocular 25 instillation of a 0.05% cyclosporine A formulation, the

5 highest cyclosporine A ocular tissue exposure levels were observed from Composition F, followed by the Composition E, followed by Composition D.

Materials

10 **Test Articles**

Compositions D, E, and F, as described above, were used for these experiments.

Chemicals, Reagents and Supplies

All other chemicals were reagent grade or better.

15 **Animals**

Species, Strain, Sex, Age, Size, Source, and Identification

Female New Zealand White rabbits weighing 1.8 to 2.6 kg were purchased from Charles River (St. Constant, Quebec, Canada). A permanent ear tag was used to
20 identify animals.

Justification

Similarities between the ocular anatomies of rabbits
25 and humans make the rabbit an attractive animal model.

Animal Husbandry

All animals were housed in environmentally-controlled facility with a time-controlled fluorescent
30 lighting system providing a daily 12-hour light/12-hour dark period. Room temperature was maintained between 61 and 72°F, and relative humidity between 30 and 70%. Airflow ranged from 10 to 30 air changes per hour. Temperature, humidity, and airflow were monitored by the
35 Edstrom Watchdog system version 4.0.

5 The animals were provided Certified Hi-Fiber Rabbit
Diet. Diet certification and analysis were provided by
the vendor. No analysis outside those provided by the
manufacturer was performed.
Drinking water that was purified by a reverse osmosis
10 process was offered *ad libitum*. Water was periodically
analyzed for any contaminants that may interfere with the
conduct of this study. The manufacturer conducted
analysis of animal feed.

15 **Animal Acclimation**

 During the acclimatization period at Allergan,
animals were kept under daily observation for any change
in general health or behavior. Rabbits were quarantined
for at least five days prior to the start of the study.
20 All animals appeared healthy prior to and for the
duration of the study.

Animal Termination and Disposal

 Animals were euthanized via injection of at least 1
25 mL of sodium pentobarbital into a marginal ear vein.

Study Design and Experimental Procedures

Study Design

5 **Table 1 Study design**

Animal species and strain	Rabbit, New Zealand White
Gender	Female
Number	3 rabbits/time point 2 rabbits at pre-dose (bioanalytical controls)
Body Weights	1.8-2.8 kg
Dosing Regimen	Topical ocular, single dose, bilateral
Dose Volume	35 µL
Test Article	Formulations containing 0.05% AGN 192371 (cyclosporine A)
Time Points	0.5, 2, 6, 12, 24, 48, and 144 hours post-dose
Tissues/Matrices	Cornea, conjunctiva, sclera, nasolacrimal duct, eyelid margin and blood
Assay Method	LC-MS/MS
Analyte	AGN 192371 (Cyclosporine A)
Quantitation Range	Blood: 0.5-40 ng/mL Cornea: 0.1-200 ng Conjunctiva: 0.1-200 ng Eyelid Margin: 0.1-100 ng Nasolacrimal Duct: 0.1-100 ng Sclera:: 0.1-20 ng

Single bilateral dose, 3 rabbits (6 eyes and 3 blood samples) per time point. Two animals in group 4 were not
10 dosed and were used as bioanalytical controls. Prior to dosing, 65 animals were weighed and assigned to 4 study groups. The study design is presented in Table 1. The four study groups are presented in the Table 2 below:

15 **Table 2**

Group	Treatment	Dose (µL)	Frequency	n
1	Composition F	35	Single Bilateral Dose	3F per time point (total of 21F)
2	Composition E	35	Single Bilateral Dose	3F per time point (total of 21F)
3	Composition D	35	Single Bilateral Dose	3F per time point (total of 21F)
4	No Dose	--	--	2F (total of 2F)

5 n = Number of animals per group
F = Female

Pretreatment Examinations

10 Prior to placement on study, a physical examination was performed on each animal. Gross observations were recorded prior to drug administration and immediately after ocular dose using a standardized data collection sheet.

15

Randomization

Prior to dosing, 65 animals were weighed and randomly assigned to four study groups.

20 Dosing Procedure:

Animals were dosed once by ocular instillation bilaterally at Hour 0 of the study. Immediately prior to dosing, the eye was inspected for any abnormalities, such as infection, red eye, or visible damage. Only animals without visible abnormalities were used. The lower eyelid was gently pulled out and away from the eye. Using a Gilson precision pipette, 35 µL of dosing solution was instilled into the lower cul-de-sac of each eye. The time of dose administration was recorded. The

5 eye was gently held closed for approximately 5 seconds to
ensure even dose distribution around the eye. Gross
ocular observations were performed following dosing. The
animal, including the dosed eyes, were subjectively
evaluated for signs of irritation. Observations were
10 recorded.

Mortality/Morbidity

Animals were observed for mortality/morbidity during
the study.

15

Body Weights

Animals were weighed the day before dose
administration and subsequently randomized.

20 Pre-necropsy Blood Collection

Blood was collected from each rabbit prior to
euthanasia/necropsy. Animals were anesthetized with an
intravenous injection of a ketamine/xylazine cocktail
(87 mg/mL ketamine, 13 mg/mL xylazine) at a volume of
25 0.1 mL/kg. Blood was collected via cardiac puncture.
Approximately 5 mL of blood was collected into 10 mL
lavender top (K₃ EDTA) tubes. Blood samples were stored
at or below approximately -15°C until bioanalysis.

30

Euthanasia

Animals were euthanized with an intravenous
injection of commercial euthanasia solution following
blood collection.

35

5 **Necropsy and Collection of Ocular Tissues**

Ocular samples were collected from both eyes, blotted dry where applicable, weighed and placed in separate, appropriately labeled, silanized vials, at the time of necropsy. Both eyes were rinsed with LENS PLUS[®] in order to clear residual surface formulation remaining on the ocular surface.

Conjunctiva

The upper and lower conjunctiva from each eye were removed and pooled, weight recorded, placed into separate screw-cap glass 13x100 silanized test tubes and immediately placed on ice. Samples were stored at or below -15°C until bioanalysis.

Cornea

The entire cornea was removed from each eye; weight recorded, placed into separate screw-cap glass 13x100 silanized test tubes and immediately placed on ice. Samples were stored at or below -15°C until bioanalysis.

25 **Sclera**

The sclera was removed from each eye; weight recorded, placed into separate screw-cap glass 13x100 silanized test tubes and immediately placed on ice. Samples were stored at or below -15°C until bioanalysis.

30

Nasolacrimal Duct

Tissue containing the nasolacrimal duct associated with each eye was removed; weight recorded, placed into screw-cap glass 13x100 silanized test tubes and

5 immediately placed on ice. Samples were stored at or below -15°C until bioanalysis.

Eyelid Margin

The eyelid margins were removed from each eye;
10 weight recorded, placed into separate screw-cap glass 13x100 silanized test tubes and immediately placed on ice. Samples were stored at or below -15°C until bioanalysis.

Sample Storage

Blood and ocular tissue samples were stored at or below -15°C until bioanalysis.

Bioanalysis

20 Ocular tissue and blood concentrations were quantified using the following method.

Ocular tissue samples were extracted by soaking over night with 2.0 mL methanol at 4°C. This was followed by a second soak with 2.0 mL methanol and shaking for
25 approximately one hour at room temperature. An aliquot of 1 mL from a total of 4 mL organic extract was removed (all 4 mL were analyzed for lacrimal gland samples), and internal standard added (20 µL of 500 ng/mL of CsG). The methanolic extract was evaporated to dryness and
30 reconstituted with 200 µL of 2 mM ammonium acetate/0.4% formic acid in 50:50 acetonitrile:water for LC MS/MS analysis. The bioanalytical procedure for analysis of blood samples involved addition of internal standard, CsG (10 µL of 500 ng/mL) to 0.5 mL aliquots of K3 EDTA-
35 treated rabbit blood.

5 Following incubation of blood sample for 30 minutes
at 37°C, the samples were acidified with 0.1 N HCL (2
mL). Methyl t-butyl ether (4 mL) was added to each sample
and mixed for 15 minutes. The organic layer was removed
and made basic by addition of 0.1 N NaOH (2 mL). The
10 organic extract was separated from the aqueous layer,
evaporated to dryness and reconstituted with 200 µL of 2
mM ammonium acetate/0.4% formic acid in 50:50
acetonitrile:water for LC MS/MS analysis. Aliquots (50
µL) of the reconstituted samples were analyzed by LC-
15 MS/MS using a PE Sciex API 3000 mass spectrometer
(Applied Biosystems, Foster City, CA), Leap autosampler
(Carrboro, NC), and HPLC pumps (Shimadzu Scientific
Instruments, Columbia, MD). Reverse-phase HPLC was
performed on a Keystone BDS C8 column (3 µm, 2.1 x 50 mm,
20 65 °C) with solvent gradient elution (A=2mM ammonium
acetate/0.4% formic acid in water and B=2mM ammonium
acetate/0.4% formic acid in acetonitrile) at a flow rate
of 0.3 mL/min. The precursor-product ion pairs used in
MRM analysis were: 1203 (MH)⁺→425.5 for CsA and m/z 1217
25 (MH)⁺→ 425.5 for IS(Cyclosporin G). The total analysis
time was 5 min, with retention times of CsA and CsG at
approximately 1.82 and 1.86 minutes, respectively.

Data Treatment

30 **Data Collection**

- Pre and post treatment gross ocular examinations
- Body Weights: Randomization at Day -1
- Dosing Notes
- Mortality/Morbidity

- 5 • Blood Samples: Pre-necropsy
 • Ocular Tissue Samples: Post-necropsy

Data Calculation and Outlier Analysis

 All data was used in calculations unless omitted for
10 reasons justified in the raw data.

Pharmacokinetic Analysis

 Thermo Electron WatsonTM (Philadelphia, PA) and
 Microsoft[®] Excel (Redmond, Washington) were used for
15 pharmacokinetic calculations. The pharmacokinetic
 parameters listed below were calculated using a known
 non-compartmental approach (see Tang-Lui, *et. al.*
 Pharmaceutical Research, Vol 5, No. 4, 1988, 238-241).
 The pharmacokinetic data was described using descriptive
20 statistics such as mean and standard deviation whenever
 possible. Area under the concentration-time profile
 (AUC) values were reported as a composite AUC and
 whenever possible, $\pm$ standard error of the mean (SEM).

PK Parameter	Description
$C_{\max}$ (ng/mL) or (ng/g)	Maximum observed concentration
$T_{\max}$ (hr)	Time corresponding to maximum observed concentration
AUC _{0-t} (ng·hr/g)	Area under concentration time curve from time zero to the last quantifiable time point using the random method for non-sequential sampling
$t_{1/2}$ (hr)	Half-life
MRT (hr)	Mean residence time

25

Values below the Limit of Quantitation and Number Rounding

5 If more than half of the concentration values
contributing to a calculation of the mean were below
limit of quantitation (BLQ), then the statistics were
reported as non-calculable (NC). If half or more of the
values were quantifiable, then any BLQ values were
10 replaced with a value of "0", and the mean and its
standard deviation (SD) were calculated with these
replaced values. The mean and standard deviation of the
mean were calculated at each sampling time point within
each treatment group. Whenever the sample size was less
15 than or equal to 2, only mean values were listed. All
mean values were reported to 3 significant figures and
standard deviations were reported to the same decimal
place as their respective mean values.

20 **Protocol Deviations**

- Prior to collection of ocular tissue samples at the 6 hour time point, the eyes were not rinsed with Lens Plus[®] to clear any residual surface formulation remaining on the ocular surface. It is believed that this deviation will have minimal impact on the results derived from this study since in general this drug is rapidly absorbed from the ocular surface. In addition, blinking by the rabbits over 6
25 hours should also act to clear any residual surface formulation.

- **Abbreviations**

ACN	Acetonitrile	LLOQ	Lower Limit of Quantitation
ALQ	Above Limits of Quantitation	M	Male
AUC	Area Under the Plasma or Blood Drug Concentration - Time Curve	N, n, No., no.	Number
AUC _{Extrap}	Extrapolated Area Under the Plasma or Blood Drug Concentration Time Curve from Time 0 to the Last Quantifiable Timepoint	N/A, N.A., or n/a	Not Applicable
BID	Two Times Daily	N/C, N.C., NC, or n/c	Not Calculable
BLQ	Below Limit of Quantitation	NR	No Result / Not Reported
BMS	Bioanalytical Mass Spectrometry	NS	No Sample
CFR	Code of Federal Regulations	NZW	New Zealand White
C0 or C ₀	Extrapolated Plasma or Blood Drug Concentrations at the Time 0	OD	Right Eye
C _{max} or C _{max}	Maximal Drug Concentration	OU	Both Eyes
CONC	Concentration	PKDM	Pharmacokinetics and Drug Metabolism
DG	Day of Gestation	PO	By Mouth
DSE	Drug Safety Evaluation	QID	Four Times Daily
EDTA (K ₃)	Potassium Ethylenediaminetetraacetic Acid	QNS	Quantity Not Sufficient
F	Female	SD, S.D., or sd	Standard Deviation
GD	Gestation Day	SE	Standard Error
FDA	United States Food and Drug Administration	Sec	Seconds
GLP	Good Laboratory Practice	SMP	Sample
IC	Intracardiac	T _{1/2} or T _{1/2}	Drug Half Life
IS	Insufficient Sample Received	TA	Triamcinolone Acetonide
IM	Intramuscular	TID	Three Times Daily
IU	International Units	TK	Toxicokinetic
IV	Intravenous	T _{max} or T _{max}	Time at which C _{max} is Observed
IVT	Intravitreal	U	Units
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry	ULOQ	Upper Limit of Quantitation

5 Note: Not all abbreviations listed may appear in this report.

5 Results and Discussion

Cornea

The mean concentrations and pharmacokinetic parameters are summarized in Tables 3 and 4. The concentration-time profiles of cyclosporine A in cornea following a single bilateral ocular administration of one of three 0.05% cyclosporine A formulations to rabbits are presented in Figure 1.

Table 3 Mean cornea concentrations of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

	Cyclosporine A concentration (ng/g)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	4050	1220	1020	330	295	201
2	2740	620	1100	190	432	142
6	3030	750	1010	170	536	138
12	2530	430	858	267	417	127
24	1570 ^a	390	891 ^a	115	256 ^a	28.2
48	1240 ^a	230	622 ^a	118	238 ^a	76.6
144	222 ^a	61	125 ^a	47	52.5 ^a	13.2

Mean values represent an average of n=6

^a Concentration time points used to calculate $t_{1/2}$

5 **Table 4** **Pharmacokinetic parameters in cornea of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

Parameter	Composition F	Composition E	Composition D
C _{max} (ng/g)	4050 ± 1220	1100 ± 190	536 ± 138
T _{max} (hr)	0.500	2.00	6.00
AUC _{0-t} (ng·hr/g) ^a	163000 ± 7000	76200 ± 3300	29300 ± 2000
AUC ₀₋₂₄ (ng·hr/g)	59000	22100	9450
t _½ (hr)	41.3	42.2	49.8
MRT (hr)	50.3	56.5	61.6

10 ^a An AUC interval of 0-144 hours was used for calculations for the three formulations

Composition F

Following a single bilateral ocular instillation of
 15 Composition F, cyclosporine A was rapidly absorbed into the cornea with a peak corneal concentration (C_{max}) of 4050 ± 1220 ng/g, occurring 0.500 hours post-dose. The area under the concentration-time curve (AUC_{0-t}) value through the last quantifiable time point was 163000 ±
 20 7000 ng·hr/g and the AUC₀₋₂₄ value was 59000 ng·hr/g. The terminal half-life (t_{1/2}) was 41.3 hours and the mean residence time (MRT) was 50.3 hours.

Composition E

25 Following a single bilateral ocular instillation of Composition E, cyclosporine A was absorbed into the cornea with C_{max} value of 1100 ± 190 ng/g, occurring 2.00 hours post-dose. The AUC_{0-t} value was 76200 ± 3300 ng·hr/g and the AUC₀₋₂₄ value was 22100 ng·hr/g. The
 30 terminal t_{1/2} was 41.7 hours and the MRT was 56.5 hours.

5

Composition D

Following a single bilateral ocular instillation of Composition D, cyclosporine A was absorbed into the cornea with a $C_{\max}$ value of 536 ± 138 ng/g, occurring 6.00
10 hours post-dose. The AUC_{0-t} value was 29300 ± 2000 ng·hr/g and the AUC_{0-24} value was 9450 ng·hr/g. The terminal $t_{1/2}$ was 49.8 hours and the MRT was 61.6 hours.

Conjunctiva

15 The mean concentrations and pharmacokinetic parameters are summarized in Tables 5 and 6. The concentration-time profiles of cyclosporine A in conjunctiva following a single bilateral ocular administration of one of three 0.05% cyclosporine A
20 formulations to rabbits are presented in Figure 2.

5 **Table 5** Mean conjunctiva concentrations of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

	Cyclosporine A concentration (ng/g)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	4460	650	2560	1070	694	410
2	2170	530	1410	330	665	266
6	739	208	630 ^a	197	330 ^a	143
12	292 ^a	97	178 ^a	34	110 ^a	52.3
24	58.2 ^a	12.5	60.5 ^a	32.5	20.5 ^a	13.2
48	26.9 ^a	19.1	BLQ	-	BLQ	-
144	BLQ	-	BLQ	-	BLQ	-

10 Mean values represent an average of n=6

BLQ=Below the limit of quantitation

^a Concentration time points used to calculate $t_{1/2}$

15 **Table 6** Pharmacokinetic parameters in conjunctiva of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Parameter	Composition F	Composition E	Composition D
C _{max} (ng/g)	4460 ± 650	2560 ± 1070	694 ± 410
T _{max} (hr)	0.500	0.500	0.500
AUC _{0-t} (ng·hr/g)	18100 ± 800 ^a	11600 ± 700 ^b	5290 ± 480 ^b
AUC ₀₋₂₄ (ng·hr/g)	17100	11600	5290
$t_{1/2}$ (hr)	11.3	5.57	4.55
MRT (hr)	7.37	5.93	6.07

^a An AUC interval of 0-48 hours was used for calculations

20 ^b An AUC interval of 0-24 hours was used for calculations

Composition F

5 Following a single bilateral ocular instillation of
Composition F, cyclosporine A was rapidly absorbed into
the conjunctiva with a $C_{\max}$ value of 4460 ± 650 ng/g,
occurring 0.500 hours post-dose. The AUC_{0-t} value was
18100 $\pm$ 800 ng·hr/g and the AUC_{0-24} value was 17100
10 ng·hr/g. The terminal $t_{1/2}$ was 11.3 hours and the MRT was
7.37 hours.

Composition E

 Following a single bilateral ocular instillation of
15 Composition E, cyclosporine A was rapidly absorbed into
the conjunctiva with a $C_{\max}$ value of 2560 ± 1070 ng/g,
occurring 0.500 hours post-dose. The AUC_{0-t} value was
11600 $\pm$ 700 ng·hr/g. The terminal $t_{1/2}$ was 5.57 hours and
the MRT was 5.93 hours.

20

Composition D

 Following a single bilateral ocular instillation of
Composition D, cyclosporine A was rapidly absorbed into
the conjunctiva with a $C_{\max}$ value of 694 ± 410 ng/g,
25 occurring 0.500 hours post-dose. The AUC_{0-t} value was
5290 $\pm$ 480 ng·hr/g. The terminal $t_{1/2}$ was 4.55 hours and
the MRT was 6.07 hours.

Sclera

30 The mean concentrations and pharmacokinetic
parameters are summarized in Tables 7 and 8. The
concentration-time profiles of cyclosporine A in sclera
following a single bilateral ocular administration of one
of three 0.05% cyclosporine A formulations to rabbits are
35 presented in Figure 3.

5

Table 7 **Mean sclera concentrations of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

10

15

20

	Cyclosporine A concentration (ng/g)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	545	98	136	44	52.5	29.3
2	294	74	120	34	49.4	24.5
6	210	58	83.7	14.0	53.0	10.9
12	133	25	51.0	19.1	28.6 ^a	3.7
24	51.4 ^a	9.4	36.5 ^a	9.9	13.5 ^a	2.3
48	24.2 ^a	7.1	13.0 ^a	3.61	7.10 ^a	3.09
144	2.92 ^a	0.40	1.14 ^a	1.27	BLQ	-

Mean values represent an average of n=6

BLQ=Below the limit of quantitation

^a Concentration time points used to calculate $t_{1/2}$

5 **Table 8** **Pharmacokinetic parameters in sclera of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

Parameter	Composition F	Composition E	Composition D
C _{max} (ng/g)	545 ± 98	136 ± 43	53.0 ± 10.9
T _{max} (hr)	0.500	0.500	6.00
AUC _{0-t} (ng·hr/g)	6110 ± 260 ^a	2840 ± 150 ^a	1040 ± 50 ^b
AUC ₀₋₂₄ (ng·hr/g)	3900	1560	792
t _½ (hr)	29.7	24.8	18.7
MRT (hr)	25.3	26.9	23.8

10 ^a An AUC interval of 0-144 hours was used for calculations

^b An AUC interval of 0-48 hours was used for calculations

15

Composition F

 Following a single bilateral ocular instillation of Composition F, cyclosporine A was rapidly absorbed into the sclera with a C_{max} value of 545 ± 98 ng/g, occurring
 20 0.500 hours post-dose. The AUC_{0-t} value was 6110 ± 260 ng·hr/g and the AUC₀₋₂₄ value was 3900 ng·hr/g. The terminal t_{1/2} was 29.7 hours and the MRT was 25.3 hours.

Composition E

25 Following a single bilateral ocular instillation of Composition E, cyclosporine A was rapidly absorbed into the sclera with a C_{max} value of 136 ± 43 ng/g, occurring 0.500 hours post-dose. The AUC_{0-t} value was 2840 ± 150 ng·hr/g and the AUC₀₋₂₄ value was 1560 ng·hr/g. The
 30 terminal t_{1/2} was 24.8 hours and the MRT was 26.7 hours.

5

Composition D

Following a single bilateral ocular instillation of Composition D, cyclosporine A was absorbed into the sclera with a $C_{\max}$ value of 53.0 ± 10.9 ng/g, occurring
10 6.00 hours post-dose. The AUC_{0-t} value was 1040 ± 50 ng·hr/g and the AUC_{0-24} value was 792 ng·hr/g. The terminal $t_{1/2}$ was 18.7 hours and the MRT was 23.8 hours.

Eyelid Margin

15 The mean concentrations and pharmacokinetic parameters are summarized in Tables 9 and 10. The concentration-time profiles of cyclosporine A in the eyelid margin following a single bilateral ocular administration of one of three 0.05% cyclosporine A
20 formulations to rabbits are presented in Figure 4.

- 5 **Table 9 Mean eyelid margin concentrations of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

	Cyclosporine A concentration (ng/g)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	3120	1040	2020	980	1800	900
2	1710	300	1380	630	2450	970
6	679	135	547	300	430	214
12	787	280	910	199	662	506
24	263 ^a	158	138 ^a	87	222 ^a	172
48	223 ^a	207	362 ^a	437	112 ^a	82
144	40.0 ^a	22.5	24.9 ^a	23.4	7.30 ^a	12.64

Mean values represent an average of n=6

- 10 ^a Concentration time points used to calculate $t_{1/2}$

- 15 **Table 10 Pharmacokinetic parameters in eyelid margin of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

Parameter	Composition F	Composition E	Composition D
C _{max} (ng/g)	3120 ± 1040	2020 ± 980	2450 ± 970
T _{max} (hr)	0.500	0.500	2.00
AUC _{0-t} (ng·hr/g) ^a	38300 ± 5300	42200 ± 10800	27700 ± 3300
AUC ₀₋₂₄ (ng·hr/g)	19900	17600	18000
t _½ (hr)	42.5	38.2	24.4
MRT (hr)	40.5	38.4	21.9

^a An AUC interval of 0-144 hours was used for calculations for the three formulations

20

Composition F

5 Following a single bilateral ocular instillation of
Composition F, cyclosporine A was rapidly absorbed into
the eyelid margin with a $C_{\max}$ value of 3120 ± 1040 ng/g,
occurring 0.500 hours post-dose. The AUC_{0-t} value was
38300 $\pm$ 5300 ng·hr/g and the AUC_{0-24} value was 19900
10 ng·hr/g. The terminal $t_{1/2}$ was 42.5 hours and the MRT was
40.5 hours.

Composition E

 Following a single bilateral ocular instillation of
15 Composition E, cyclosporine A was rapidly absorbed into
the eyelid margin with a $C_{\max}$ value of 2020 ± 980 ng/g,
occurring 0.500 hours post-dose. The AUC_{0-t} value was
42200 $\pm$ 10800 ng·hr/g and the AUC_{0-24} value was 17600
ng·hr/g. The terminal $t_{1/2}$ was 38.1 hours and the MRT was
20 38.4 hours.

Composition D

 Following a single bilateral ocular instillation of
Composition D, cyclosporine A was absorbed into the
25 eyelid margin with a $C_{\max}$ value of 2450 ± 970 ng/g,
occurring 2.00 hours post-dose. The AUC_{0-t} value was
27700 $\pm$ 3300 ng·hr/g and the AUC_{0-24} value was
18000 ng·hr/g. The terminal $t_{1/2}$ was 24.4 hours and the
MRT was 21.9 hours.

30

Nasolacrimal Duct

 The mean concentrations and pharmacokinetic
parameters are summarized in Tables 11 and 12. The
concentration-time profiles of cyclosporine A in
35 nasolacrimal duct tissue following a single bilateral

- 5 ocular administration of one of three 0.05% cyclosporine
A formulations to rabbits are presented in Figure 5.

5 **Table 11** Mean nasolacrimal duct concentrations of cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

	Cyclosporine A concentration (ng/g)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	194	201	74.4	20.9	72.0	91.7
2	43.7	44.1	37.2	43.6	37.4	13.8
6	18.2	15.2	BLQ	-	11.8	10.0
12	24.2	12.0	35.5	21.5	14.9	8.4
24	BLQ	-	BLQ	-	BLQ	-
48	BLQ	-	4.68	5.15	BLQ	-
144	1.71	1.93	BLQ	-	BLQ	-

10 Mean values represent an average of n=6
 BLQ=Below the limit of quantitation

15 **Table 12** Pharmacokinetic parameters in nasolacrimal duct of Cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.

Parameter	Composition F	Composition E	Composition D
C _{max} (ng/g)	195 ± 201	74.4 ± 20.9	72.0 ± 91.7
T _{max} (hr)	0.500	0.500	0.500
AUC _{0-t} (ng·hr/g)	2190 ± 350 ^a	1190 ± 212 ^b	279 ± 39 ^c
AUC ₀₋₁₂ (ng·hr/g)	478 ± 86	465 ± 106	279 ± 39
t _½ (hr)	NC	NC	NC
MRT (hr) ^d	17.6	24.7	12.1

NC=Not calculable

^a An AUC interval of 0-144 hours was used for calculations

20 ^b An AUC interval of 0-48 hours was used for calculations

^c An AUC interval of 0-12 hours was used for calculations

^d A time interval of 0-12 hours was used for calculations

5 Composition F

Following a single bilateral ocular instillation of Composition F, cyclosporine A rapidly drained into and was then absorbed into the nasolacrimal duct tissue with a $C_{\max}$ value of 195 ± 201 ng/g, occurring 0.500 hours
10 post-dose. The AUC_{0-t} value was 2190 ± 350 ng·hr/g and the AUC_{0-12} value was 478 ± 86 ng·hr/g. The MRT was 17.6 hours.

Composition E

15 Following a single bilateral ocular instillation of Composition E, cyclosporine A rapidly drained into and was then absorbed into the nasolacrimal duct tissue with a $C_{\max}$ value of 74.4 ± 20.9 ng/g, occurring 0.500 hours post-dose. The AUC_{0-t} value was 1190 ± 210 ng·hr/g and
20 the AUC_{0-12} value was 465 ± 106 ng·hr/g. The MRT was 24.7 hours.

Composition D

Following a single bilateral ocular instillation of
25 Composition D, cyclosporine A rapidly drained into and was then absorbed into the nasolacrimal duct tissue with a $C_{\max}$ value of 72.0 ± 91.7 ng/g, occurring 0.500 hours post-dose. The AUC_{0-t} value was 279 ± 39 ng·hr/g. The MRT was 12.1 hours.

30

Blood

The mean concentrations of cyclosporine A in blood are summarized in Table 13.

5 **Table 13** **Mean blood concentrations of Cyclosporine A following a single bilateral topical ocular instillation of one of three 0.05% cyclosporine A formulations to New Zealand White rabbits.**

	Cyclosporine A concentration (ng/mL)					
	Composition F		Composition E		Composition D	
Time (hr)	Mean	SD	Mean	SD	Mean	SD
0.5	2.21	0.33	0.441	0.126	BLQ	-
2	0.463	0.021	BLQ	-	BLQ	-
6	BLQ	-	BLQ	-	BLQ	-
12	BLQ	-	BLQ	-	BLQ	-
24	BLQ	-	BLQ	-	BLQ	-
48	BLQ	-	BLQ	-	BLQ	-
144	BLQ	-	BLQ	-	BLQ	-

10 Mean values represent an average of n=3

BLQ=Below the limit of quantitation

Composition F

15 Following a single bilateral ocular instillation of Composition F, cyclosporine A was detected at 0.5 and 2 hours post-dose in the blood at concentrations of 2.21 ± 0.33 ng/mL and 0.463 ± 0.021 ng/mL, respectively. Cyclosporine A levels were below the limit of quantitation at all subsequent time points.

20

Composition E

25 Following a single bilateral ocular instillation of Composition E, cyclosporine A was detected at 0.5 hours post-dose in the blood at a concentration of 0.441 ± 0.126 ng/mL. Cyclosporine A levels were below the limit of quantitation at all subsequent time points.

5

Composition D

Following a single bilateral ocular instillation of Composition D, cyclosporine A levels were below the limit of quantitation at all time points.

10

Administration of Composition F to rabbits generally delivered the highest levels of cyclosporine A to ocular tissues, on average a 5-fold increase in area under the concentration-time profile (AUC) was observed when compared to Composition D. Administration of Composition E to rabbits resulted on average in a 2-fold increase in AUC when compared to Composition D. The pharmacokinetic profile observed following Composition D administration to New Zealand White rabbits in this study was in good agreement with previously reported data.

20

In general, the terminal half-life and mean residence time observed were greatest for Composition F, followed by the Composition E, followed by Composition D. Thus, AUC values were reported to the last quantifiable time point, in addition to AUC through 24 hours for cornea, conjunctiva, sclera and eyelid margin and AUC through 12 hours for nasolacrimal duct to make an assessment over the same interval as to the drug levels achieved following once a day dosing. Overall, the trends observed when comparing AUC_{0-t} values were consistent with the trends observed when comparing AUC_{0-24} or AUC_{0-12} .

25

30

In conclusion, following a single ocular instillation of a 0.05% cyclosporine A formulation, the

5 highest cyclosporine A ocular tissue exposure levels were observed when drug was formulated as an aqueous Composition F, followed by the Composition E followed by Composition D. A concomitant trend was observed in blood drug exposure.

10 While not intending to limit the scope of the invention, it is believed that these pharmacokinetic results suggest that significantly lower concentrations of cyclosporin A may be used in topical ophthalmic compositions than previously known and still achieve a
15 therapeutically effective amount cyclosporin A.

Pharmacokinetic Study 2

The compositions below were prepared in an analogous manner to compositions D, E, and F.

Formulations	Composition G	Composition H	Composition D
Ingredients	Aqueous Solution	Aqueous Solution	Emulsion
Cyclosporine A	0.020	0.030	0.050
Purite	0.01% (100 ppm)	0.01% (100 ppm)	0.0% (0 ppm)
Polysorbate 80	1.0	1.0	1.0
Glycerin	1.0	1.0	2.2
Mannitol	0.5	0.5	N/A
Sodium Carboxymethylcellulose (CMC) - 7LFPH	0.5	0.5	N/A
Sodium Citrate Dihydrate	0.4	0.4	N/A
Boric Acid	0.25	0.25	N/A
Sodium Borate Decahydrate	0.41	0.41	N/A
Potassium Chloride	0.14	0.14	N/A
Castor Oil	N/A	N/A	1.25
Pemulen TR-2	N/A	N/A	0.05
Sodium Hydroxide	N/A	N/A	pH 7.4

Purified Water	QS	QS	N/A
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5

A pharmacokinetic study was carried out using similar analytical methods to those already described. The parameters are shown below.

- **Test Formulations:** G,H, and D
- **Animal species/strain:** Rabbit NZW
- **Gender:** Female
- **Number:** 2 rabbits/timepoint (2 rabbits blanks)
- **Dosing Route:** Topical ocular
- **Dosing Regimen:** Bilateral, QD (Aqueous) / BID (Composition D) -5days
- **Dose Volume:** 35 μ L
- **Time points:** Day 1 and Day 5-0.5, 2, 6, 12, 24 hr post dose
- **Assay Method:** LC-MS/MS
- **Analyte:** Cyclosporine A
- **Data Analysis:** C_{max} , AUC_{0-24} , AUC dose normalized

The results in cornea, tear, and blood are shown in the tables below.

Table 14. Cyclosporin bioavailability in the cornea.

	Composition G		Composition H		Composition D Emulsion, BID	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
C_{max} (ng/g)	810 $\pm$ 530	2570 $\pm$ 650	1420 $\pm$ 930	3020 $\pm$ 440	583 $\pm$ 209	1670 $\pm$ 170

AUC ₀₋₂₄ (ng·hr/g)	14700 ±2500	33900 ±2200	22100 ±2800	48800 ±3900	12100 ±700	27900 ±1000
AUC/Dose (ng·hr/g/ng)	2.12	4.93	2.12	4.71	0.349	0.807
Total Dose/24hr (ng)	7000	7000	10500	10500	35000	35000

5

Table 15. Cyclosporin bioavailability in the blood.

	0.02% CsA Aqueous, QD		0.03% CsA Aqueous, QD		Restasis [®] (0.05%) Emulsion, BID	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
C _{0.5hr} (ng/mL)	0.741	0.883	0.727	0.604	BLQ	BLQ

n=2 rabbits/timepoint

BLQ-Below the limit of detection (0.2 ng/mL)

10 Table 16. Cyclosporin bioavailability in the tears.

	0.02% CsA Aqueous, QD		0.03% CsA Aqueous, QD		Restasis [®] (0.05%) Emulsion, BID	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
C _{max} (ng/mL)	18.2 ±6.3	50.1 ±29.2	31.4 ±45.2	39.4 ±9.7	44.2 ±18.4	83.5 ±33.2
AUC ₀₋₂₄ (ng·hr/mL)	109 ±15	371 ±62	327 ±121	397 ±127	368 ±51	663 ±110

Standard Compositions

These compositions (AA-MM) are particularly contemplated for use as standards for comparison for characterization of the compositions disclosed herein.

The following compositions are intended to mean those identical to those disclosed in Kanai et. al.,

5 *Transplantation Proceedings*, Vol 21, No 1 (February),
1989: 3150-3152, which is incorporated by reference
herein:

Composition AA: a solution consisting of 0.025%
cyclosporin A, 40 mg/mL alpha cyclodextrin, and water;

10 Composition BB: a solution consisting of 0.009%
cyclosporin A, 20 mg/mL alpha cyclodextrin, and water;
and

Composition CC: a solution consisting of 0.003%
cyclosporin A, 10 mg/mL alpha cyclodextrin, and water.

15 The following composition is intended to mean those
identical to that disclosed in Cheeks et. al., *Current
Eye Research*, Vol 11, No 7 (1992), 641-649, which is
incorporated by reference herein:

Composition DD: an alpha cyclodextrin solution at 40
20 mg/mL containing 0.025% cyclosporin A.

The following composition is intended to mean that
identical that disclosed in Tamilvanan, *Stp Pharma Sci*
Nov-Dec; 11(6):421-426, which is incorporated by
reference herein, except that the concentration of
25 cyclosporin A is different.

Composition EE: an emulsion consisting of cyclosporin A
(0.05 w/w%), castor oil (2.5 w/w%), stearylamine (0.12
w/w%), α -tocopherol (0.01 w/w%), benzalkonium chloride
(0.01 w/w%) and water up to 100 w/w%.

30 The following compositions are intended to mean
those identical to Samples C-E disclosed in United States
Patent No. 5,051,402 (column 7). The entire disclosure
is incorporated herein by reference.

Composition FF: 0.25 mL/mL of cyclosporin A, 40 mg/mL
35 of α -cyclodextrin, and 7.79 mg/mL of sodium chloride;

5 Composition GG: 0.10 mL/mL of cyclosporin A, 20 mg/mL of α -cyclodextrin, and 8.40 mg/mL of sodium chloride; and

Composition HH: 0.05 mL/mL of cyclosporin A, 10 mg/mL of α -cyclodextrin, and 8.70 mg/mL of sodium chloride.

10 The following composition is intended to mean that identical that disclosed in Abdulrizak, *Stp Pharma Sci* Nov-Dec; 11(6):427-432, which is incorporated by reference herein, except that the concentration of cyclosporin A is different.

15 Composition II: an emulsion consisting of cyclosporin A (0.05 w/w%), castor oil (2.5 w/w%), Poloxamer 188, (0.425 w/w%), glycerol (2.25 w/w%), Lipoid E-80 (0.5 w/w%), stearylamine (0.12 w/w%), tocopherol (0.01 w/w%), benzalkonium chloride (0.01 w/w%), and water.

20 The following composition is intended to mean that identical to that disclosed in Kuwano Mitsuaki *et al.* *Pharm Res* 2002 Aug;19(1):108-111.

Composition JJ: a solution consisting of cyclosporine A (0.0865%), ethanol (0.1%), MYS-40 (2%), HPMC (0.3
25 w/v%), sodium dihydrogen phosphate (0.2 w/v%), and disodium EDTA (0.01% w/v%), sodium chloride to adjust the tonicity to 287 mOsm, and water.

Composition KK is intended to mean that disclosed in US20010041671, incorporated by reference herein, as
30 Formulation 1, on Table 1. Composition LL is that disclosed in US20010041671 as Formulation 3, except that the concentration of cyclosporine is reduced.

Composition KK: cyclosporine A (0.02%), sodium hyaluronate (0.05%), Tween 80 (0.05%), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

5 (0.08%), sorbitol (5.46%), purified water added to 100 mL, pH 7.0-7.4, and mosm/L = 295-305.

Composition LL: cyclosporine A (0.2%), sodium
hyaluronate (0.10%), Tween 80 (5.00%), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$
(0.08%), sorbitol (5.16%), purified water added to 100
10 mL, pH 7.0-7.4, and mosm/L = 295-305.

The following composition is intended to mean that disclosed in Example 2 of US 5,951,971, incorporated herein by reference.

Composition MM: cyclosporine A (0.025 g), polyoxyl 40
15 stearate (0.5g), hydroxypropyl methylcellulose (0.2g), butylated hydroxytoluene (0.0005 g), ethanol (0.1 g), sodium chloride (0.73 g), sodium dihydrogen phosphate (0.2 g), sodium edethate (0.1 g), sodium hydroxide to adjust pH to 6.0, and water to make 100 mL.

20 In another embodiment the composition provides more cyclosporin A than Composition AA provides to the cornea of a female New Zealand white rabbit 30 minutes after topical ocular administration of one drop of said composition or Composition AA, wherein the drop of said
25 composition and the drop of Composition AA are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition BB provides to the cornea of a female New Zealand white rabbit 30 minutes after
30 topical ocular administration of one drop of said composition or Composition BB, wherein the drop of said composition and the drop of Composition BB are the same volume.

In another embodiment the composition provides more
35 cyclosporin A than Composition CC provides to the cornea

5 of a female New Zealand white rabbit 30 minutes after
topical ocular administration of one drop of said
composition or Composition CC, wherein the drop of said
composition and the drop of Composition CC are the same
volume.

10 In another embodiment the composition provides more
cyclosporin A than Composition DD provides to the cornea
of a female New Zealand white rabbit 30 minutes after
topical ocular administration of one drop of said
composition or Composition DD, wherein the drop of said
15 composition and the drop of Composition DD are the same
volume.

In another embodiment the composition provides more
cyclosporin A than Composition EE provides to the cornea
of a female New Zealand white rabbit 30 minutes after
20 topical ocular administration of one drop of said
composition or Composition EE, wherein the drop of said
composition and the drop of Composition EE are the same
volume.

In another embodiment the composition provides more
25 cyclosporin A than Composition FF provides to the cornea
of a female New Zealand white rabbit 30 minutes after
topical ocular administration of one drop of said
composition or Composition FF, wherein the drop of said
composition and the drop of composition FF are the same
30 volume.

In another embodiment the composition provides more
cyclosporin A than Composition GG provides to the cornea
of a female New Zealand white rabbit 30 minutes after
topical ocular administration of one drop of said
35 composition or Composition GG, wherein the drop of said

5 composition and the drop of composition GG are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition HH provides to the cornea of a female New Zealand white rabbit 30 minutes after
10 topical ocular administration of one drop of said composition or Composition HH, wherein the drop of said composition and the drop of composition HH are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition II provides to the cornea of a female New Zealand white rabbit 30 minutes after
15 topical ocular administration of one drop of said composition or Composition II, wherein the drop of said composition and the drop of composition II are the same
20 volume.

In another embodiment the composition provides more cyclosporin A than Composition JJ provides to the cornea of a female New Zealand white rabbit 30 minutes after
25 topical ocular administration of one drop of said composition or Composition JJ, wherein the drop of said composition and the drop of composition JJ are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition KK provides to the cornea of a female New Zealand white rabbit 30 minutes after
30 topical ocular administration of one drop of said composition or Composition KK, wherein the drop of said composition and the drop of composition KK are the same volume.

5 In another embodiment the composition provides more
cyclosporin A than Composition LL provides to the cornea
of a female New Zealand white rabbit 30 minutes after
topical ocular administration of one drop of said
composition or Composition LL, wherein the drop of said
10 composition and the drop of composition LL are the same
volume.

 In another embodiment the composition provides more
cyclosporin A than Composition MM provides to the cornea
of a female New Zealand white rabbit 30 minutes after
15 topical ocular administration of one drop of said
composition or Composition MM, wherein the drop of said
composition and the drop of composition MM are the same
volume.

 In another embodiment the composition provides more
20 cyclosporin A than Composition AA provides to the
conjunctiva of a female New Zealand white rabbit 30
minutes after topical ocular administration of one drop
of said composition or Composition AA, wherein the drop
of said composition and the drop of Composition AA are
25 the same volume.

 In another embodiment the composition provides more
cyclosporin A than Composition BB provides to the
conjunctiva of a female New Zealand white rabbit 30
minutes after topical ocular administration of one drop
30 of said composition or Composition BB, wherein the drop
of said composition and the drop of Composition BB are
the same volume.

 In another embodiment the composition provides more
cyclosporin A than Composition CC provides to the
35 conjunctiva of a female New Zealand white rabbit 30

5 minutes after topical ocular administration of one drop of said composition or Composition CC, wherein the drop of said composition and the drop of Composition CC are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition DD provides to the conjunctiva of a female New Zealand white rabbit 30 minutes after topical ocular administration of one drop of said composition or Composition DD, wherein the drop of said composition and the drop of Composition DD are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition EE provides to the conjunctiva of a female New Zealand white rabbit 30 minutes after topical ocular administration of one drop of said composition or Composition EE, wherein the drop of said composition and the drop of Composition EE are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition FF provides to the conjunctiva of a female New Zealand white rabbit 30 minutes after topical ocular administration of one drop of said composition or Composition FF, wherein the drop of said composition and the drop of composition FF are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition GG provides to the conjunctiva of a female New Zealand white rabbit 30 minutes after topical ocular administration of one drop of said composition or Composition GG, wherein the drop

5 of said composition and the drop of composition GG are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition HH provides to the conjunctiva of a female New Zealand white rabbit 30
10 minutes after topical ocular administration of one drop of said composition or Composition HH, wherein the drop of said composition and the drop of composition HH are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition II provides to the conjunctiva of a female New Zealand white rabbit 30
15 minutes after topical ocular administration of one drop of said composition or Composition II, wherein the drop of said composition and the drop of composition II are
20 the same volume.

In another embodiment the composition provides more cyclosporin A than Composition JJ provides to the conjunctiva of a female New Zealand white rabbit 30
minutes after topical ocular administration of one drop
25 of said composition or Composition JJ, wherein the drop of said composition and the drop of composition JJ are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition KK provides to the conjunctiva of a female New Zealand white rabbit 30
30 minutes after topical ocular administration of one drop of said composition or Composition KK, wherein the drop of said composition and the drop of composition KK are the same volume.

5 In another embodiment the composition provides more
cyclosporin A than Composition LL provides to the
conjunctiva of a female New Zealand white rabbit 30
minutes after topical ocular administration of one drop
of said composition or Composition LL, wherein the drop
10 of said composition and the drop of composition LL are
the same volume.

 In another embodiment the composition provides more
cyclosporin A than Composition MM provides to the
conjunctiva of a female New Zealand white rabbit 30
15 minutes after topical ocular administration of one drop
of said composition or Composition MM, wherein the drop
of said composition and the drop of composition MM are
the same volume.

 Comparison of two compositions in a person or animal
20 can be carried out by, among other means, administering
the claimed composition to one eye and the second
composition to the second eye.

 In another embodiment the composition provides more
cyclosporin A than Composition AA provides to the cornea
25 of a female New Zealand white rabbit over a period of 24
hours after topical ocular administration of one drop of
said composition or Composition AA, wherein the drop of
said composition and the drop of Composition AA are the
same volume.

30 In another embodiment the composition provides more
cyclosporin A than Composition BB provides to the cornea
of a female New Zealand white rabbit over a period of 24
hours after topical ocular administration of one drop of
said composition or Composition BB, wherein the drop of

5 said composition and the drop of Composition BB are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition CC provides to the cornea of a female New Zealand white rabbit over a period of 24
10 hours after topical ocular administration of one drop of said composition or Composition CC, wherein the drop of said composition and the drop of Composition CC are the same volume.

In another embodiment the composition provides more
15 cyclosporin A than Composition DD provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition DD, wherein the drop of said composition and the drop of Composition DD are the
20 same volume.

In another embodiment the composition provides more cyclosporin A than Composition EE provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of
25 said composition or Composition EE, wherein the drop of said composition and the drop of Composition EE are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition FF provides to the cornea
30 of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition FF, wherein the drop of said composition and the drop of composition FF are the same volume.

5 In another embodiment the composition provides more cyclosporin A than Composition GG provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition GG, wherein the drop of
10 said composition and the drop of composition GG are the same volume.

 In another embodiment the composition provides more cyclosporin A than Composition HH provides to the cornea of a female New Zealand white rabbit over a period of 24
15 hours after topical ocular administration of one drop of said composition or Composition HH, wherein the drop of said composition and the drop of composition HH are the same volume.

 In another embodiment the composition provides more
20 cyclosporin A than Composition II provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition II, wherein the drop of said composition and the drop of composition II are the
25 same volume.

 In another embodiment the composition provides more cyclosporin A than Composition JJ provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of
30 said composition or Composition JJ, wherein the drop of said composition and the drop of composition JJ are the same volume.

 In another embodiment the composition provides more cyclosporin A than Composition KK provides to the cornea
35 of a female New Zealand white rabbit over a period of 24

5 hours after topical ocular administration of one drop of said composition or Composition KK, wherein the drop of said composition and the drop of composition KK are the same volume.

10 In another embodiment the composition provides more cyclosporin A than Composition LL provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition LL, wherein the drop of said composition and the drop of composition LL are the
15 same volume.

In another embodiment the composition provides more cyclosporin A than Composition MM provides to the cornea of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of
20 said composition or Composition MM, wherein the drop of said composition and the drop of composition MM are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition AA provides to the
25 conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition AA, wherein the drop of said composition and the drop of Composition AA are the same volume.

30 In another embodiment the composition provides more cyclosporin A than Composition BB provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition BB, wherein

5 the drop of said composition and the drop of Composition BB are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition CC provides to the conjunctiva of a female New Zealand white rabbit over a
10 period of 24 hours after topical ocular administration of one drop of said composition or Composition CC, wherein the drop of said composition and the drop of Composition CC are the same volume.

In another embodiment the composition provides more
15 cyclosporin A than Composition DD provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition DD, wherein the drop of said composition and the drop of Composition
20 DD are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition EE provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of
25 one drop of said composition or Composition EE, wherein the drop of said composition and the drop of Composition EE are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition FF provides to the
30 conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition FF, wherein the drop of said composition and the drop of composition FF are the same volume.

5 In another embodiment the composition provides more cyclosporin A than Composition GG provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition GG, wherein
10 the drop of said composition and the drop of composition GG are the same volume.

 In another embodiment the composition provides more cyclosporin A than Composition HH provides to the conjunctiva of a female New Zealand white rabbit over a
15 period of 24 hours after topical ocular administration of one drop of said composition or Composition HH, wherein the drop of said composition and the drop of composition HH are the same volume.

 In another embodiment the composition provides more
20 cyclosporin A than Composition II provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition II, wherein the drop of said composition and the drop of composition
25 II are the same volume.

 In another embodiment the composition provides more cyclosporin A than Composition JJ provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration
30 of one drop of said composition or Composition JJ, wherein the drop of said composition and the drop of composition JJ are the same volume.

 In another embodiment the composition provides more cyclosporin A than Composition KK provides to the
35 conjunctiva of a female New Zealand white rabbit over a

5 period of 24 hours after topical ocular administration of one drop of said composition or Composition KK, wherein the drop of said composition and the drop of composition KK are the same volume.

10 In another embodiment the composition provides more cyclosporin A than Composition LL provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of one drop of said composition or Composition LL, wherein the drop of said composition and the drop of composition
15 LL are the same volume.

In another embodiment the composition provides more cyclosporin A than Composition MM provides to the conjunctiva of a female New Zealand white rabbit over a period of 24 hours after topical ocular administration of
20 one drop of said composition or Composition MM, wherein the drop of said composition and the drop of composition MM are the same volume.

In one embodiment, wherein topical administration of one 35 μ L drop of said composition to each eye of a
25 female New Zealand white rabbit provides to the corneas of said rabbit at least about 500 ng of cyclosporin A per gram of cornea of said rabbit at 30 minutes after said topical administration.

In another embodiment, topical administration of
30 one 35 μ L drop of said composition to each eye of a female New Zealand white rabbit provides to the corneas of said rabbit at least about 2000 ng of cyclosporin A per gram of cornea of said rabbit at 30 minutes after said topical administration.

5 In another embodiment, topical administration of one
35 μ L drop of said composition to each eye of a female
New Zealand white rabbit provides to the corneas of said
rabbit at least about 2400 ng of cyclosporin A per gram
of cornea of said rabbit at 30 minutes after said topical
10 administration.

 In another embodiment, topical administration of one
35 μ L drop of said composition to each eye of a female
New Zealand white rabbit provides to the corneas of said
rabbit at least about 17000 ng of cyclosporin A per gram
15 of cornea of said rabbit over a period of 24 hours after
said topical administration.

 In another embodiment, topical administration of one
35 μ L drop of said composition to each eye of a female
New Zealand white rabbit provides to the conjunctivas of
20 said rabbit at least about 3300 ng of cyclosporin A per
gram of conjunctiva of said rabbit over a period of 24
hours after said topical administration.

 In another embodiment, said composition is an
aqueous solution containing from 0.005% to about 0.04%
25 cyclosporin A, wherein topical administration of one 35
 μ L drop of said composition to each eye of a New Zealand
rabbit provides at least about 17000 ng of cyclosporin A
per gram of cornea to the corneas of said rabbit as
determined by:

30 topically administering said composition to each eye of
each of 15 female New Zealand white rabbit test
subjects, and
determining the amount of cyclosporin A in the corneas
of three subjects at times of about 0.5 hours, about 2

5 hours, about 6 hours, about 12 hours, and about 24
after administration to said subject,
wherein the amount of cyclosporin A in the cornea is
determined only once for each subject.

 In another embodiment, said composition is an
10 aqueous solution containing from 0.005% to about 0.04%
cyclosporin A, wherein topical administration of one 35
μL drop of said composition to each eye of a New Zealand
rabbit provides at least about 17000 ng of cyclosporin A
per gram of conjunctiva to the conjunctivas of said
15 rabbit as determined by:

 topically administering said composition to each eye of
each of 15 female New Zealand white rabbit test
subjects, and
determining the amount of cyclosporin A in the
20 conjunctivas of three subjects at times of about 0.5
hours, about 2 hours, about 6 hours, about 12 hours,
and about 24 after administration to said subject,
wherein the amount of cyclosporin A in the conjunctiva is
determined only a single time for each subject.

25 As mentioned above, these compositions are suitable
for use in other mammals other than rabbits, including
humans. Thus, any composition in the claims or
elsewhere which is characterized by in vivo rabbit
bioavailability testing is contemplated for use in a
30 person or in another mammal. Defining a composition in
terms of bioavailability in rabbits should not be
construed to limit a method of treatment using the
composition to use on rabbits, but treatment with the
composition should be construed to include treatment on
35 humans and other mammals.

5 The foregoing description details specific methods
and compositions that can be employed to practice the
present invention, and represents the best mode
contemplated. However, it is apparent for one of ordinary
skill in the art that further compositions with the
10 desired pharmacological properties can be prepared in an
analogous manner. Thus, however detailed the foregoing
may appear in text, it should not be construed as limiting
the overall scope hereof; rather, the ambit of the present
invention is to be governed only by the lawful
15 construction of the claims.

5 **What is claimed is:**

1. A method of treating loss of corneal sensitivity comprising administering a composition comprising cyclosporin A at a concentration of from about 0.0001%
10 (w/v) to less than about 0.05% (w/v) to a person in need thereof.

2. The method of claim 1 wherein the composition comprises cyclosporin A at a concentration of from 0.01% (w/v) to 0.02% (w/v) and a preservative.

15 3. The method of claim 2 wherein the concentration of cyclosporin A in the composition is about 0.015% (w/v).

4. The method of claim 1 wherein the concentration of cyclosporin A in the composition about 0.04% (w/v).

20 5. The method of claim 1 wherein the concentration of cyclosporin A in the composition is about 0.005% (w/v).

6. The method of claim 1 wherein the loss of corneal sensitivity is related to surgery affecting the cornea or viral infection.

25 7. The method of claim 6 wherein the loss of corneal sensitivity is associated with keratorefractive surgery or penetrating keratoplasty.

8. The method of claim 7 wherein the loss of corneal sensitivity is caused by the person having radial keratotomy.

30 9. The method of claim 7 wherein the loss of corneal sensitivity is caused by photorefractive keratotomy.

10. The method of claim 7 wherein the loss of corneal sensitivity is caused by laser-assisted in situ keratomileusis.

5 **11.** The method of claim 7 wherein the loss of corneal sensitivity is caused by laser assisted sub-epithelial keratomileusis.

12. The method of claim 7 wherein the loss of corneal sensitivity is caused by SB-LASIK.

10 **13.** The method of claim 7 wherein the loss of corneal sensitivity is caused by EPI-LASIK.

14. The method of claim 6 wherein the loss of corneal sensitivity is caused by viral infection.

15. The method of claim 14 wherein the viral infection
15 is HSV-1.

16. The method of claim 14 wherein the viral infection is HSV-2.

17. The method of claim 14 wherein the viral infection is VZV.

20 **18.** The method of claim 2 wherein the concentration of cyclosporin A in the composition is about 0.0015% (w/v).

19. A liquid composition comprising cyclosporin A at a concentration of about 0.0015% (w/v).

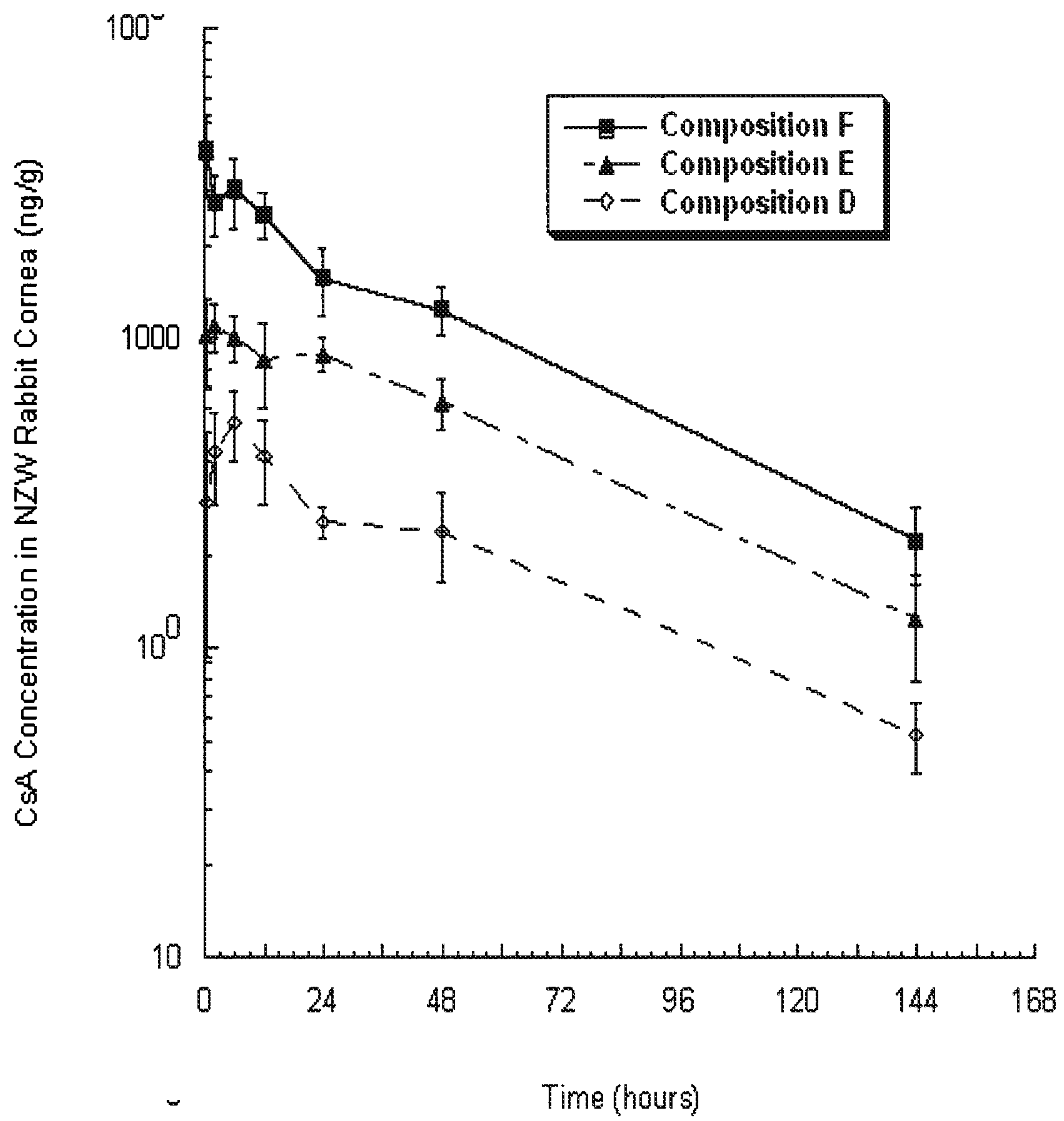
Fig. 1

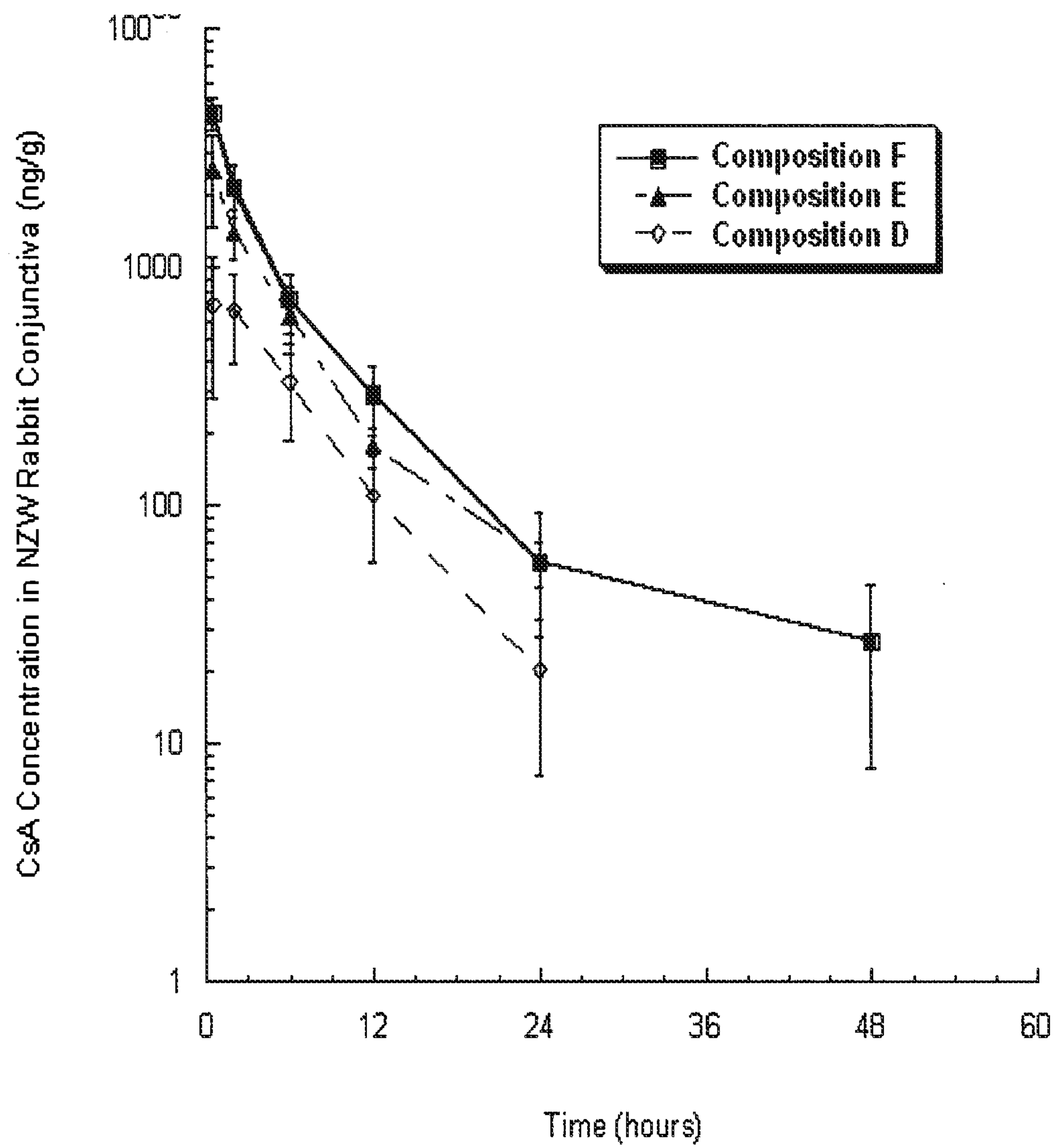
Fig. 2

Fig. 3

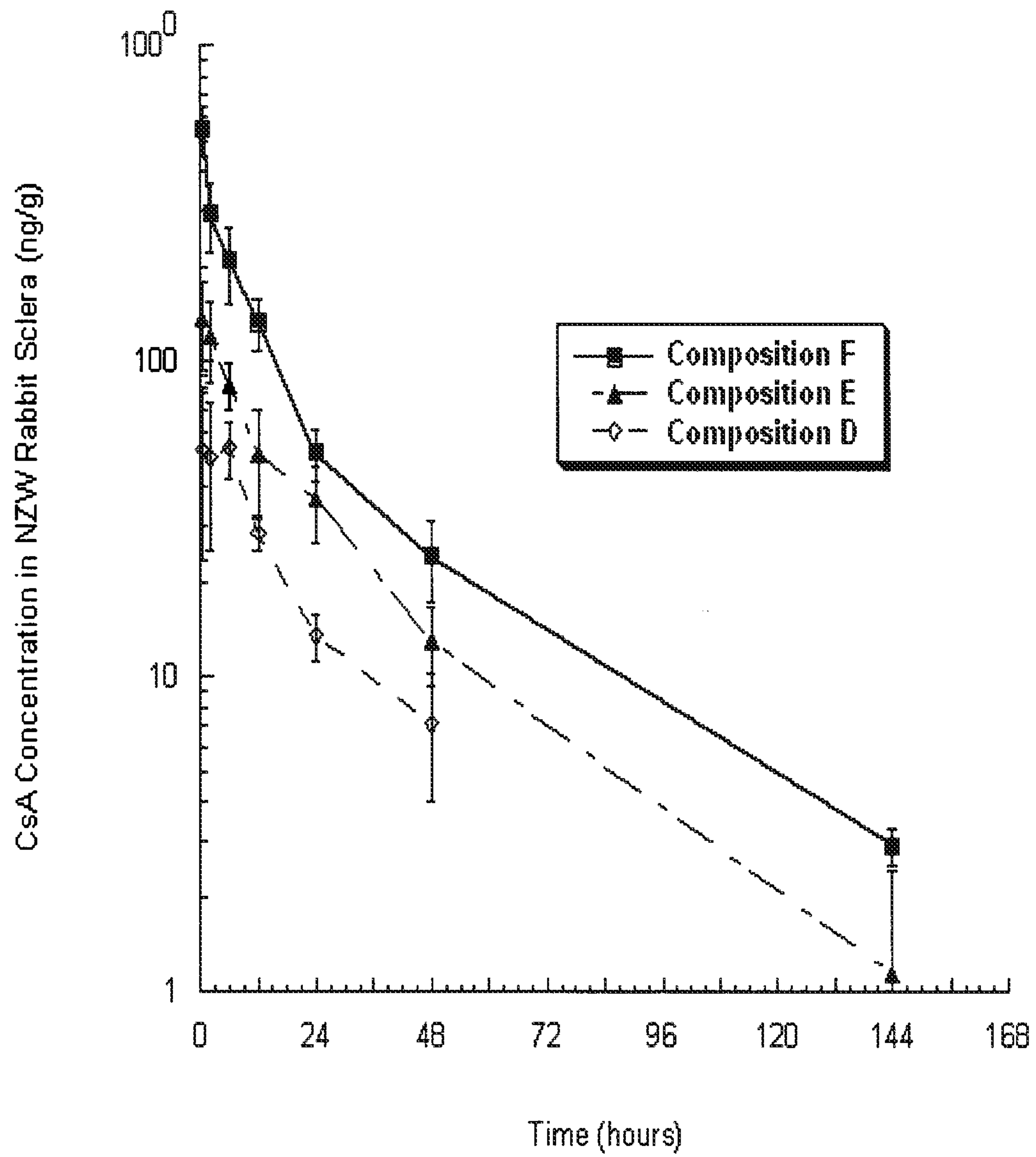


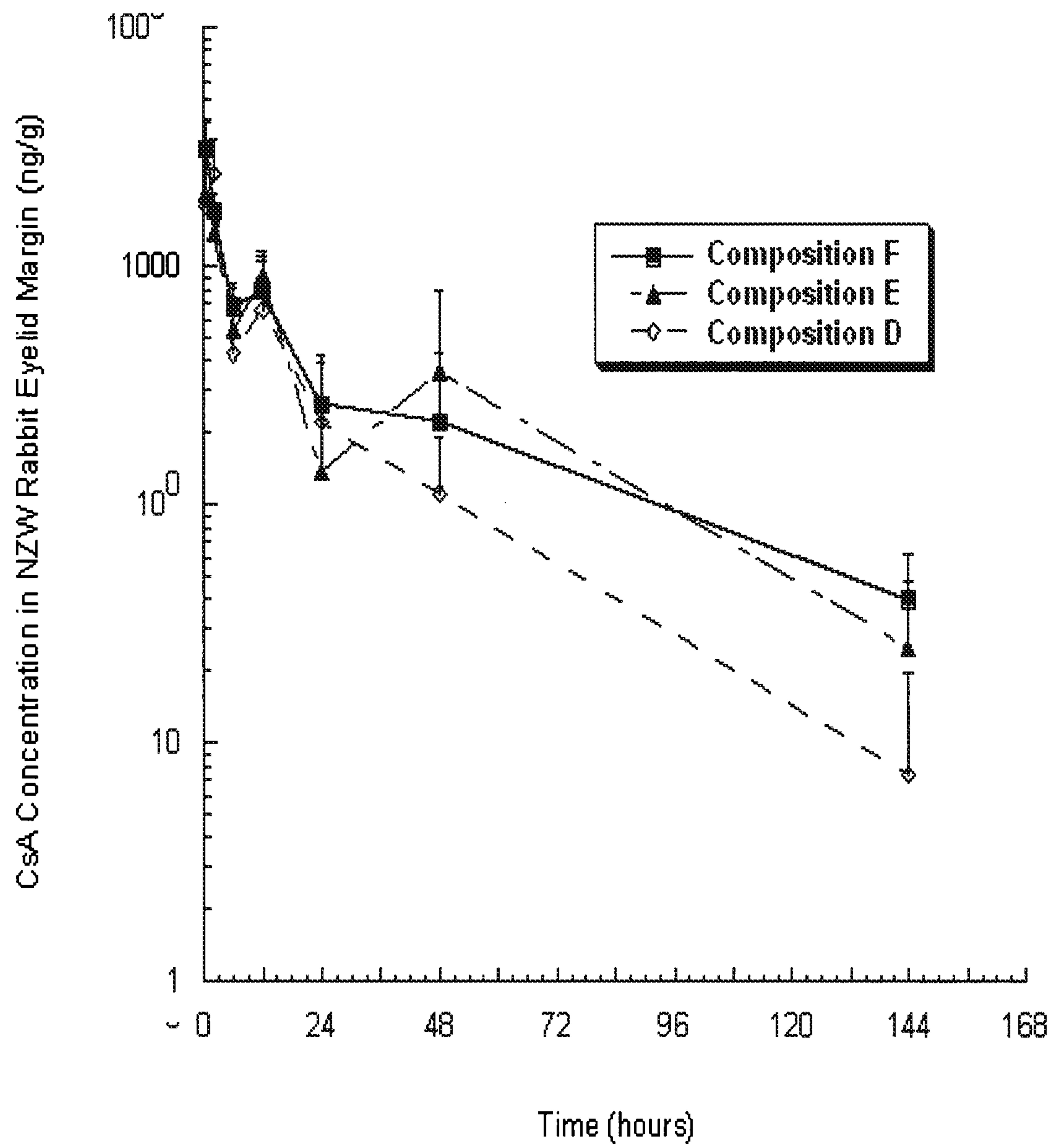
Fig. 4

Fig. 5

