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(54) Benevnelse **CYCLOSPORIN A-BASED AQUEOUS OPHTHALMIC SOLUTION**

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# CYCLOSPORIN A-BASED AQUEOUS OPHTALMIC SOLUTION

## FIELD OF THE INVENTION

This invention proposes an aqueous ophthalmic solution containing an immunosuppressive agent such as ciclosporin A as the active ingredient. The said solution has toxicological and allergenic characteristics which are acceptable for treatment of eye surface disorders which have an immune inflammatory ground, such as dryness of the eye or loss of corneal sensitivity.

More precisely, the invention concerns an aqueous ophthalmic solution in which the active ingredient, in particular ciclosporin A, is formulated with a ternary polymeric system so that it can be solubilised in a therapeutically effective quantity and with great stability, even without any preservative.

## STATE OF THE ART

Dry eye syndrome, also called ocular dryness or keratoconjunctivitis sicca (KCS), is a condition in which the lachrymal glands do not produce enough tears. It results in eye discomfort accompanied by itching, smarting and/or burning sensations.

Ocular dryness can result from an abnormality of the lachrymal glands, inflammation of the eyelids, eye inflammation due to an allergy, refractive surgery (particularly using lasers), deficiency in certain lipids in the daily diet, prolonged wearing of contact lenses, hormonal changes, an auto-immune disease, or side effects of certain medicinal products.

Loss of corneal sensitivity may for example result from the sequels of surgery, ulceration or a viral infection (viral keratitis) of the cornea.

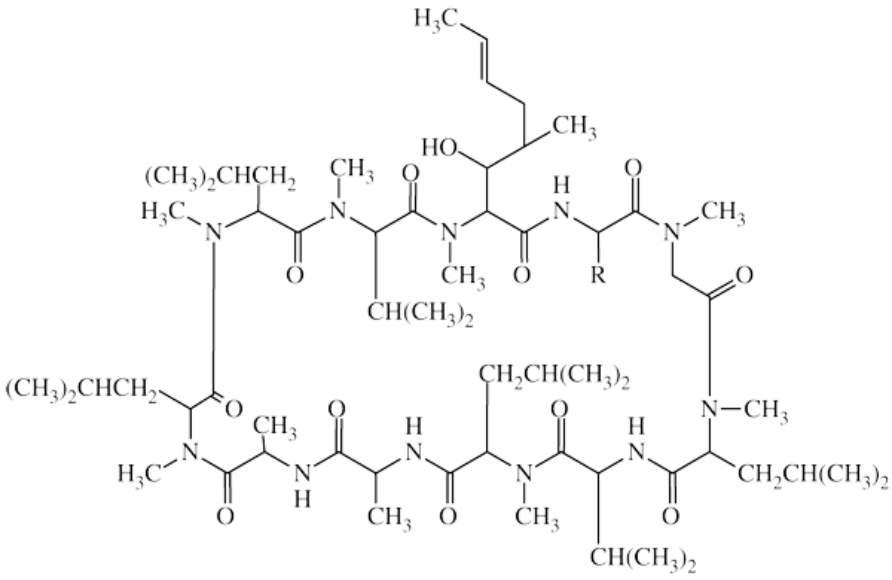
In the treatment of ocular dryness, the application of artificial tears, also known as lubricating or moistening eye drops, provides short-term local relief but does not solve the problem from a systemic point of view: they do not help the organism to improve the quantity or quality of the tears and are only a temporary substitution solution. Low or medium viscosity artificial tears are generally based on polyvinyl alcohols or cellulose derivatives, while those with higher viscosity contain carbomers or hyaluronic acid.

A more promising treatment for these two types of conditions is based on the topical application of ciclosporin A (or cyclosporin A).

Ciclosporin A, generally called ciclosporin, is an effective immunosuppressive agent, particularly used in organ transplantation and to prevent the acute rejection of allografts.

It seems to be agreed that ciclosporin acts by inhibiting calcineurin, a phosphatase involved in the transcription of the interleukin 2 gene, normally secreted by T-lymphocytes. In addition, ciclosporin A inhibits the production of lymphokines and the release of interleukins, which lead to a substantial reduction in the activity of effector T-lymphocytes. In other words, ciclosporin A is a molecule which can inhibit or prevent the activity of the immune system.

Ciclosporin A is a cyclic peptide of eleven amino acids, synthesised by a microscopic fungus *Tolypocladium inflatum*. Ciclosporin A has the formula [R-[[R*,R*-(E)]]-cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L-α-amino-butyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl) (CAS number 59865-13-3), with the following structure:



CYCLOSPORINE

A practical difficulty with ciclosporin A is that it is only very slightly soluble in aqueous media, in the order of 20-30 μg/l at 25°C, i.e. 0.002 to 0.003% by weight (w/v). On the other hand, this molecule is soluble in alcohols (e.g. ethanol or methanol), acetonitrile, ethyl acetate and oils (e.g. olive oil, maize or castor oils).

This same problem of solubility occurs with other ophthalmic immunosuppressive agents such as tacrolimus (CAS number: 104987-11-3) or rapamycin (CAS number: 53123-88-9).

Considerable efforts have been made to obtain an eyewash formulation, for ocular treatment of dry eyes and loss of sensitivity of the cornea, with acceptable tolerance to instillation and bioavailability of ciclosporin A in the target tissues.

Tolerance is measured by the irritation caused by the eyewash in the eye following its instillation, e.g. by using the scale produced by Draize *et al*. (Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. and Exp. Therapeutics,* 1944; 82: 377–390).

Moreover, the efficacy of eyewash penetration reflects the bioavailability of ciclosporin A in the various tissues encountered after instillation, i.e. the conjunctiva (bulbar and palpebral) and the cornea.

Given the very low solubility of ciclosporin A in water, developments in the ophthalmic area have focused on eyewashes in the form of emulsions or hydro-alcoholic solutions.

Thus, ciclosporin A is commercially available in the United States in the form of a 0.05% emulsion in castor oil. This product, sold in preservative-free single doses under the name of Restasis™, is recommended for the treatment of keratoconjunctivitis sicca.

In Europe, and more particularly in France, the administration of ciclosporin A relies essentially on hospital preparations. However, these hospital preparations, in which the concentration varies between 0.05 and 2% as an active formula in an oily medium (olive oil, castor oil or maize oil) and/or in the presence of alcohol, are only stable for a few weeks.

Moreover and in both cases, the tolerance to instillation is not good, which detracts from good compliance with the treatment, and thus its efficacy.

Nourry *et al.* (Etude de la cytotoxicité de différents collyres à base de ciclosporine A buvable (Sandimmun$^{TM}$). *J. Fr. Ophtalmol*., 2006 ; 29, 3, 251-257) suggested that the excipients (oil and/or alcohol) of these eyewashes cause side effects, such as irritation of the conjunctiva or hyperaemia, toxicity of the corneal epithelium, itching, or burning sensations. The same authors compared the tolerance of hydro-alcoholic and oily (emulsion) eyewashes and concluded that oily eyewashes are less irritant on instillation than hydro-alcoholic eyewashes.

This is the reason why most recent developments in eyewashes based particularly on ciclosporin A aim to improve the efficacy of oily or emulsion eyewashes, in order to increase their tolerance on instillation while at the same time overcoming the problems of solubility.

There is nevertheless an obvious need to develop immunosuppressant-based ophthalmic formulations, in particular using ciclosporin A, with the following characteristics:

- Presented in the form of aqueous solutions,
- Stable over time,
- Well tolerated by the eye following instillation,
- Good penetration of the cornea,
- Containing a suitable dose for treating, in particular, dry eye syndrome and the loss of corneal sensitivity.

Document WO2009/099467 describes compositions based on cyclosporin A, optionally in the form of solutions formulated using 2 polymers. Document US2002/173516 discloses tacrolimus-based compositions, optionally in the form of solutions formulated with 2 polymers.

**DESCRIPTION OF THE INVENTION**

In this invention, despite the low solubility of ophthalmic immunosuppressants, the Applicant has developed a stable aqueous solution, possibly preservative-free, in which the concentration of ciclosporin A ensures that the product is effective and the pharmaceutical formulation guarantees very good tolerance on instillation.

- According therefore to a first embodiment, this invention concerns an aqueous ophthalmic solution containing ciclosporin A as an immunosuppressive agent ;
- a cellulose derivative as a first polymer ;
- a polyvinyl derivative as a second polymer ;

- a macrogolglycerol hydroxystearate as a third polymer.

Due to the combined use of 3 judiciously chosen polymers, an effective therapeutic quantity of the immunosuppressant, preferably ciclosporin A, can be dissolved in water while the said composition is provided with suitable viscosity for topical ocular application. In other words the 3 polymers play a role in the solubilisation and/or gelification and are defined as solubilising agents and/or gelling agents, even cosolubilising and/or cogelling agents.

The aqueous ophthalmic solution according to the invention thus includes, as its active substance, a slightly soluble immunosuppressive agent, preferably ciclosporin A. Nevertheless it is possible to apply the same formulation principle to other topical ocular immunosuppressive agents which are only slightly soluble in water, such as the other forms of ciclosporin, tacrolimus (CAS number: 104987-11-3) or rapamycin (CAS number: 53123-88-9).

The concentration of active substance in the solution, particularly ciclosporin A, is appropriately between 0.01 and 0.2% by weight of the solution (weight/volume or w/v), preferably between 0.05 and 0.1%. These are effective therapeutic quantities and they are compatible with the solubilisation limits of the proposed formulation system. According to a preferred embodiment, particularly for ciclosporin, the concentration is greater than or equal to 0.05% by weight, preferably greater than 0.05%, more preferably greater than or equal to 0.1% by weight, which corresponds to a solubilized quantity never reached in the context of an aqueous solution. For immunosuppressive agents other than ciclosporin, especially for those having a greater solubility in water, the weight/volume ratio in the solution according to the invention can be greater than or equal to 0.1%, preferably greater than or equal to 0.2%, more preferably greater than or equal to 0.5% or even 1%.

It should be noted that a solution according to the invention can also comprise other active substances from the same category (i.e. immunosuppressive agents) of from another category. In an adapted manner, said other active substance(s) is (are) also under a solubilized form. According to a preferred embodiment, the other active substance(s) is (are) not a corticosteroid.

The composition according to the invention is characteristically presented as an aqueous solution. By definition, an aqueous solution is a liquid phase containing several chemical species, of which one, i.e. water ($H_2O$), is the major species and acts as solvent (or vehicle) for the minor species forming the solutes or "dissolved chemical species". Advantageously, the solution according to the invention contains no organic solvent, particularly any alcohol such as ethanol.

In the invention, ciclosporin is thus dissolved in the solution, and not as micelles or in an emulsion (in an oily phase) as in the prior art. Preferably, the solution according to the invention therefore contains no oil, particularly any of plant origin such as castor oil.

According to another preferred embodiment, the solution according to the invention is essentially free of particles, especially as defined by the method 2.9.19 in the European Pharmacopoeia. Especially, the solution is advantageously free of micelles.

According to the invention, this solubilisation is provided by a ternary polymer system:

The aqueous ophthalmic solution according to the invention contains a first polymer, preferably selected from cellulose derivatives, more precisely from cellulose ethers. Surprisingly, while these derivatives are known for their gelling properties, the Applicant has observed that they allow presolubilisation of the immunosuppressant, preferably ciclosporin A. This first polymer can therefore be defined as a cosolubiliser/cogelling agent.

Preferably, the cellulose derivative is chosen from the group including: methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, or their salts. More preferably, it is sodium (Na) carboxymethylcellulose.

The concentration of this first polymer, preferably a cellulose derivative, is appropriately between 0.1 and 3% by weight of the solution (weight/volume or w/v), preferably between 0.5 and 1.5% and preferably equal to 0.8%.

According to a preferred embodiment, the immunosuppressive agent, preferably ciclosporin A, is presolubilised in a first step in the presence of this first polymer in water. This first step results in a suspension, which is then mixed with the other two polymers of the solution.

The aqueous ophthalmic solution according to the invention includes a second polymer, preferably a polyvinyl polymer, more preferably polyvinyl alcohol.

Polyvinyl alcohol (CAS number 9002-89-5) is known for its gelling properties, hence its use as a gelling agent. Remarkably, this polyvinyl derivative participates notably in the cosolubilisation of ciclosporin A, potentially through the use of its hydroxyl groups.

Another polyvinyl derivative which can be used is polyvinyl pyrrolidone or povidone (PVP) (CAS n° 9003-39-8).

The concentration of the second polymer, preferably a polyvinyl derivative, is appropriately between 0.25 and 3% by weight of the solution (weight/volume or w/v), preferably equal to 0.5%.

These 2 polymer categories are selected notably from the family of excipients widely used for the formulation of eyewashes intended for the treatment of ocular dryness. Thus, whereas for treatment with Restasis$^{TM}$, concomitant application of artificial tears is recommended, the ophthalmic solution according to the invention acts as a "2 in 1" product.

The aqueous ophthalmic solution according to the invention characteristically includes a third polymer, playing the role of a major solubilising agent, preferably a non-ionic polymer. Preferably it is a polymer with a lipophilic functional group, such as macrogolglycerol hydroxystearate. Macrogolglycerol hydroxystearate is a non-ionic solubilising agent, suitable for cosolubilising for example ciclosporin A, , particularly because of its apolar part .

These polymers are composed of a fatty acid molecule ( in C18, omega-9 hydroxylated, ricinoleic acid or (*R*)-(+)-12-hydroxy-9*Z*-octadecenoic acid (apolar part ) and 35 molecules of polyethylene glycol H-(0-CH=CH)$_n$-OH, with varying lengths of carbon chain ($C_7$, $C_{25}$, $C_{40}$ or $C_{60}$) defining the grades 7, 25, 40 or 60.

Still more preferably, the third polymer is macrogolglycerol hydroxystearate 40, also called PEG 40 hydrogenated castor oil, its CAS number being 61788-85-0.

The concentration of the third polymer, preferably macrogolglycerol hydroxystearate, is appropriately between 0.5 and 20% by weight of the solution (weight/volume or w/v), preferably between 5 and 15%, and more preferably equal to 10%. It is thus the major polymer of the ternary system.

Preferably, the solution according to the invention contains no cyclodextrin. According to another preferred embodiment, it contains no penetration enhancer such as BAK or DMSO, known to help the solubilisation of active substances and to destabilize the membrane of the cells constituting the corneal epithelium.

Preferably, combining these three polymers provides the solution according to the invention with viscosity suitable for the required topical ophthalmic application.

Thus, the appropriate viscosity of the solution according to the invention is lower than or equal to 50 mPa.s (cP), preferably between 5 and 50 mPa.s, and more preferably between 5 and 15 mPa.s, when measured using a Brookfield RVDV III rotational viscometer at 25°C.

In practice, this viscosity is preferably close to the viscosity of human tears, evaluated at approximately 6.5 mPa.s at 20°C and with a velocity gradient close to zero. Application of the solution according to the invention thus causes no sensation of discomfort or irritation. Indeed, it has been determined that easy elimination with no discomfort is only possible if the viscosity of the ophthalmic solution/tears mixture is lower than 50 mPa.s.

The aqueous ophthalmic solution naturally may, in addition, contain an additive selected from the group of non-ionic isotonifying agents, antioxidant agents and/or buffer systems.

The solution according to the invention preferably has a neutral pH, preferably between 6.5 and 7. Preferably, it is an isotonic formulation, with osmolality preferably between 290 and 350 mosmol/kg.

In a particular embodiment, the solution according to the invention is composed of the ingredients listed in the table below, preferably according to the centesimal formula shown:

| PRODUCT | FUNCTION | CENTESIMAL FORMULA (g/100 g) |
|---|---|---|
| Ciclosporin A | Active product | 0.01 to 0.10 g |
| Macrogolglycerol hydroxystearate 40 | Cosolubilising agent/Cogelling agent | 10.0 g |
| Sodium carboxymethylcellulose | Cosolubilising agent/Cogelling agent | 0.80 g |
| $NaH_2PO_4.H_2O$ | Buffer | 0.520 g |
| $Na_2HPO_4.2H_2O$ | Buffer | 0.652 g |
| PVA | Cosolubilising agent/Cogelling agent | 0.50 g |
| Water | - | qs 100 g |

As indicated in the application and preferably, the solution according to the invention is free of antimicrobial preservative, especially free of ammonium quaternary type of preservative such as for example benzalkonium chloride (BAK).

In this invention, "antimicrobial preservative" or "antimicrobial" means a preservative agent with antimicrobial properties, i.e. a compound capable of guaranteeing the protection of the ophthalmic solution from a possible microbial contamination. Compounds of the ammonium quaternary type, particularly benzalkonium chloride (BAK) or polyquad, possibly associated with EDTA (e.g. disodium edetate), are usually used as preservatives.

Other possible preservatives include but are not limited to:
- Guanidine-based preservatives (PHMB, Chlorhexidine, …);
- Benzyl alcohol;
- Parabens (methyl, propyl, …);
- Mercury-based preservatives, such as Thimerosal;
- Chlorbutanol;
- Benzethonium chlorides;
- Oxidative-type preservatives (Purite, …);
- ...

The invention's formulation can be supplied as single-use (unidose) or multidose vials such as Abak®, Comod® or equivalent, to allow the ophthalmic solution to be delivered without preservatives for several days.

The invention therefore also concerns a single-use (unidose) or multidose vial containing the ophthalmic solution previously described, e.g. made in LDPE, preferably, of additive-free EP quality.

In addition, the solution according to the invention is stable for at least 24 months, or for at least 36 months, at ambient temperature (25°C – 30°C).

The invention also concerns the use of this solution in treating disorders of the surface of the eye which have an immune inflammatory ground.

In human ophthalmology, this solution is particularly suited for treatment of dry eye syndrome and/or loss of corneal sensitivity.

Indications relating to loss of corneal sensitivity are for example related to:

- an operation affecting the cornea;
- a viral infection (e.g. herpes HSV-I, HSV-II or VZV viruses);
- keratorefractive surgery or penetrating keratoplasty;
- a patient presenting a radial keratotomy;
- a photorefractive keratotomy;
- a LASIK (laser-assisted in situ keratomileusis) operation, in particular EPI-LASIK (epithelial LASIK) or LASEK (laser sub-epithelial keratomileusis).

Moreover, a solution according to the invention can be used in veterinary medicine, particularly in the following cases:

- Keratoconjunctivitis sicca in dogs;
- Problems of corneal neovascularisation, corneal pigmentation and cellular infiltration in chronic superficial keratitis in dogs (dog CSK), cats and horses;
- Lymphoplasmocytic infiltration of the nictitating membrane in dogs;
- Eosinophilic keratitis in cats and horses;
- Punctate keratitis in dogs (Teckel, etc.) and horses;
- Keratouveitis, endothelitis, certain torpid ulcers and periodic fluxion of horses;
- In combination, in the treatment of inflammatory reactions of immune origin in dogs, cats and horses;
- In the prevention of corneal graft rejection in dogs, cats and horses.

In practice, the solution according to the invention is administered to humans or animals via the topical route, in the form of one or more drops per day in each eye.

In another embodiment and particularly relating to the use of a cellulose derivative, the invention concerns a process for preparing the solution according to the invention, comprised of the following steps:

- Presolubilising the immunomodulator agent, preferably ciclosporin A, in the presence of at least a fraction of the first polymer (preferably 25% by weight), preferably a cellulose derivative, in the presence of water and preferably while stirring;
- Mixing the remaining fraction if any of the first polymer (preferably 75% by weight), preferably a cellulose derivative, with the two other polymers, water and possibly the buffer systems and isotonifying agents, preferably while stirring and/or heating, e.g. to a temperature of 60°C;
- Mixing the products obtained in the previous steps, preferably while stirring and/or heating, e.g. to a temperature of 60°C.

At the end of said process, in view of its conservation and its use in ophthalmology, said solution can be sterilized by heat, preferably by autoclave.

As is evident from the application, the aqueous ophthalmic solution according to the invention has several original features and advantages:

- It is stable over time;
- It is better tolerated by instillation than an oily emulsion and/or a hydro-alcoholic solution;
- It penetrates the cornea well, without accumulating in the conjunctiva or passing into the aqueous humor or into the blood;
- It contains a dose of ciclosporin A suitable for treating ocular surface disorders with an inflammatory immune ground, such as ocular dryness and/or the loss of corneal sensitivity.

**EXAMPLES OF EMBODIMENTS**

The invention and the advantages ensuing from it are better illustrated by the following non-exhaustive examples, given for information purposes, supported by the attached figures, in which:

Figure 1 illustrates ocular penetration over time, in the conjunctiva (A) and cornea (B), of ciclosporin A at three concentrations (0.01, 0.05 and 0.1% respectively).

Figure 2 illustrates the pharmacokinetic profile, in the conjunctiva (Cj) and cornea (Co), of a solution according to the invention containing 0.1% ciclosporin A, following repeated instillation (3 doses per day for 9 days (multidose), and analysis of the 28$^{th}$ dose on D10).

**I- Preparation of a solution of 0.1% (w/w) ciclosporin A (100 kg batch)**

*I-1/ Preparation of fraction A:*

200 g of sodium carboxymethylcellulose powder (the first polymer; 25% of the final quantity) are mixed with 100 g of ciclosporin A powder (the active substance, of a quality complying with the European Pharmacopoeia monograph) then 3 litres of water are added. This is mixed by stirring for an hour.

Ciclosporin A is in the form of a fine powder, toxic for the operator. This first pre-solubilisation step produces a milky suspension of ciclosporin A, much safer for the operator.

*I-2/ Preparation of fraction B:*

500 g of polyvinyl alcohol (or PVA, the second polymer) are added to approximately 65 litres of cold water (75% of the final volume of water). This is mixed while stirring, gradually increasing the temperature to 60°C.

When the PVA has dissolved (after a minimum of 30 minutes at 300 rpm), 10 kg of macrogolglycerol hydroxystearate 40 (or PEG 40 hydrogenated castor oil, the third polymer) are added. The solution is mixed while stirring for approximately 20 minutes at 60°C.

The buffers ($NaH_2PO_4.H_2O$ and $Na_2HPO_4.2H_2O$), are then incorporated and the solution is mixed while stirring for 20 minutes at 60°C. Under the same conditions (approximately 20 min at 60°C), 600 g of sodium carboxymethylcellulose are subsequently added (the first polymer; 75% of the final quantity).

*I:-3/ Preparation of the final solution:*

Fraction A is added to fraction B. Following homogenisation, the final volume is made up with water and mixed for 10 minutes at 60°C.

The aqueous ophthalmic solution thus obtained is sterilised at 121°C for 20 minutes while stirring. It may be packaged without any preservative, particularly an antimicrobial preservative, either in single-use LDPE (low density polyethylene) vials or in multidose ABAK or COMOD vials or another type.

It should be noted that the preparation time of the sterile aqueous ophthalmic solution according to the invention is markedly shortened (maximum 8 hours) compared with prior art protocols, particularly document WO 2009/099467 in which at least one of the mixing steps occurs overnight.

This manufacturing process allows a conventional approach for the industrial production of sterile ophthalmic products, without prolonged immobilisation of the industrial equipment, a risk factor in terms of microbial growth.

*I-4/ Composition of the solution obtained:*

| **PRODUCT** | **FUNCTION** | **CENTESIMAL FORMULA (g/100 g or %)** |
|---|---|---|
| Ciclosporin A | Active substance | 0.10 |
| Macrogolglycerol hydroxystearate 40 | Solubilising agent | 10.0 |
| Sodium carboxymethylcellulose | Cosolubilising agent/gelling agent | 0.80 |
| $NaH_2PO_4.H_2O$ | Buffer | 0.520 |
| $Na_2HPO_4.2H_2O$ | Buffer | 0.652 |
| PVA | Gelling agent | 0.50 |
| Water | Vehicle | qs 100 |

It should be noted that an identical protocol can be applied for the preparation of a solution with a ciclosporin A concentration between 0.01 and 0.1% (w/v), e.g. at 0.05%.

*I-5/ Characteristics of the solution obtained:*

| **Tests** | **Specifications** |
|---|---|
| Appearance | Slightly opalescent, colourless solution |
| pH | 6.79 |
| Osmolality (mOsmol.kg) | 303 |
| Opalescence (NTU) | 3.65 |
| Coloration (method 2.2.2 Ph. Eur.: A solution is said to be *colourless* if it has the appearance of water or of the solvent, or if it is no more coloured than control solution $B_9$) | < B9 |
| Particles (method 2.9.19 Ph. Eur.)<br>Particles >= 10µm<br>Particles >= 25µm | Complies<br>570 (<6000)<br>17 (<600) |
| Viscosity (mPa.s) | 7.55 |
| Active substance concentration (g/100 ml) | 0.099 |

It is apparent that the solution obtained has the following advantages:

- Neutral pH;
- Isotonic formulation;
- Viscosity close to that of human tears. Indeed, the viscosity in human tears at 20°C would be approximately 6.5 mPa.s with a velocity gradient close to zero. The force exerted by the eyelids during normal blinking is approximately 0.2 N and during forced blinking approximately 0.7 - 0.8 N. In the presence of the solution according to the invention, the patient does not experience any uncomfortable sensation or irritation, which appears during blinking when the eyelids have to move a force greater than or equal to 0.9 N, since this sensation of discomfort is caused by reflex blinking to induce rapid elimination by means of tears.

This solution is a high tolerance formulation, obtained despite the very poor solubility of the active substance in water.

## II- Stability of the solution according to the invention

Stability tests at 25°C (Table 1) and 40°C (Table 2) have demonstrated very good stability of the solution according to the invention, so that shelf life can be claimed of 36 months in storage conditions at ambient temperature.

| TESTS | NORMS | Initial | 6 months | 12 months | 24 months | 36 months |
|---|---|---|---|---|---|---|
| **Appearance** | Viscous, opalescent, colourless to slightly yellow solution | Complies | Complies | Complies | Complies | Complies |
| **pH** | 6.5-7.0 | 6.79 | 6.72 | 6.70 | 6.71 | 6.58 |
| **Osmolarity (mosmol/kg)** | 290-350 | 303 | 300 | 303 | 296 | 301 |
| **Opalescence (NTU)** | <5.1 NTU (sol III Ph. Eur.) | 3.65 | 4.33 | 4.03 | 4.37 | 4.81 |
| **Coloration** | <B9 | <B9 | <B9 | <B9 | <B9 | <B9 |
| **Particles** | Absent | Absent | Absent | Absent | Absent | Absent |
| **Viscosity (mPa.s)** | >5 mPa.s | 7.55 | 7.55 | 7.51 | 7.17 | 7.25 |
| **Ciclosporin A content** | 0.090%-0.110% | 0.099 | 0.100 | 0.100 | 0.099 | 0.100 |
| **Total impurities content** | <2.5% | 1.15 | 1.22 | 1.03 | 0.92 | 1.14 |
| **Sterility test** | Complies | Complies | Complies | Complies | Complies | Complies |

Table 1: Stability of the aqueous ophthalmic solution according to the invention at 25°C and 40% relative humidity

| TESTS | NORMS | Initial | 3 months | 6 months | 12 months |
|---|---|---|---|---|---|
| **Appearance** | Viscous, opalescent, colourless to slightly yellow solution | Complies | Complies | Complies | Complies |
| **Osmolarity (mosmol/kg)** | 290-350 | 303 | 304 | 311 | 318 |
| **Opalescence (NTU)** | <5.1 NTU (sol III Ph. Eur.) | 3.65 | 4.04 | 4.60 | 4.62 |
| **Coloration** | <B9 | <B9 | <B9 | <B9 | <B9 |
| **Particles** | Absent | Absent | Absent | Absent | Absent |
| **Viscosity (mPa.s)** | >5 mPa.s | 7.55 | 7.17 | 7.08 | 6.66 |
| **Ciclosporin A content** | 0.0900%-0.1100% | 0.0994 | 0.0991 | 0.0992 | 0.0981 |
| **Total impurities content** | <2.5% | 1.15 | 1.12 | 1.23 | 1.04 |

Table 2: Stability of the aqueous ophthalmic solution according to the invention at 40°C and 25% relative humidity

**III- Ocular tolerance of the solution according to the invention**

A solution of 0.1% by weight of ciclosporin A, prepared as described above, was tested in the rabbit, with 8 daily instillations of 50 µl for 7 days.

The following ocular examinations were made:

- Observation of both eyes using an ophthalmoscope to assess them on the Draize scale, before treatment and following the last instillation of the day;
- Ocular examinations of both eyes using a slit lamp to assess them using the McDonald-Shadduck scale, before treatment, on day 1 just before the last administration, then on day 8;
- Collection and fixation of all the eyes.

Good ocular tolerance of the solution according to the invention was seen in the rabbit compared with other formulations, particularly compared with an alcoholic preparation with a non-ionic surface-active agent such as MODUSIK-A®.

**IV- Kinetic study of a solution according to the invention compared with a commercial emulsion (Restasis™)**

The ocular distribution of ciclosporin A was analysed on a pool of conjunctivas (bulbar and palpebral) and on the cornea, after a single instillation of 50 µl into the right eye of rabbits of:

- an aqueous solution according to the invention, containing 0.05% (by weight) of ciclosporin A, prepared as described in point I and labelled T1580;
- Restasis™, a commercial emulsion containing 0.05% (by weight) of ciclosporin A.

To do this, 48 pigmented rabbits (Fauve de Bourgogne - ref: HY R NZ 104) were divided into 2 groups of 24 animals. Each sub-group was then subdivided into 6 groups of 4 animals, corresponding to 6 analysis times (20 minutes, 40 minutes, 1 hour, 4 hours, 12 hours and 24 hours respectively). The animals received a single instillation of 50 µl of T1580 or Restasis™ into the right eye. At each analysis time the animals were sacrificed to obtain the samples of conjunctiva (bulbar and palpebral) (Cj) and cornea (Co), preserved at -20°C until measurement. The ciclosporin A content was determined in the conjunctivas and corneas by HPLC-MS (high performance liquid chromatography combined with mass spectrometry).

The results are given in Table 3 below, in which:

- $C_{max}$ is the maximum concentration observed;
- $T_{max}$ is the time required to reach the maximum concentration; and

- AUC is the area under the curve for the time between time zero and the time corresponding to the last measurable concentration. For this calculation, the random method for non-sequential samples is used.

| | **Ciclosporin A in the conjunctiva (Cj)** | | | **Ciclosporin A in the cornea (Co)** | | |
|---|---|---|---|---|---|---|
| | **$C_{max}$ (ng/g)** | **$T_{max}$ (h)** | **AUC** | **$C_{max}$ (ng/g)** | **$T_{max}$ (h)** | **AUC** |
| **T1580** | 453 | 0.33 | 966 | 769 | 0.66 | 8189 |
| **Restasis™** | 257 | 0.33 | 924 | 504 | 1 | 6516 |

Table 3: Pharmacokinetic comparison of the aqueous ophthalmic solution according to the invention and the commercial emulsion Restasis™, each containing 0.05% of ciclosporin A.

It can be seen that after an instillation of 50 µl of T1580 or Restasis™ into the right eye of pigmented rabbits, ciclosporin A is mainly found in the conjunctiva between 20 minutes and 4 hours, and in the cornea between 20 minutes and 24 hours later. The animals treated with T1580 had a larger quantity of ciclosporin A in the conjunctiva and the cornea between 20 minutes and 12 hours, in particular in the cornea, compared with animals treated with Restasis™. This shows better availability of the active substance formulated according to the invention.

**V- Pharmacokinetic study of a solution according to the invention administered as a single dose at three different concentrations**

The ocular penetration of a single dose of solution according to the invention was determined in the conjunctiva and the cornea following instillation of 25 µl into each eye of pigmented rabbits as a function of time (0.33, 0.66, 1, 2, 4, 8, 12 and 24 h after instillation). Three ciclosporin A concentrations were tested: 0.01, 0.05 and 0.1% w/w. The study was made on 72 rabbits, i.e. 3 rabbits per time period.

Figure 1A, for the conjunctivas, and figure 1B, for the corneas, show a dose-dependent effect between the ciclosporin A concentration and the pharmacokinetic profile obtained for the conjunctivas and corneas.

The AUC obtained for the corneas is 4 to 5 times the size of that measured for the conjunctivas and the ciclosporin A remains longer in the corneas than in the conjunctivas, which demonstrates a particular tropism of ciclosporin A for the cornea.

**VI- Pharmacokinetic study of a solution according to the invention administered as repeated instillations**

In this study, pigmented rabbits received an instillation in each eye (right and left eyes) of a dose (25 µl) of a solution according to the invention containing 0.1% (w/w) of ciclosporin A three times a day for 10 days.

More precisely, instillation was continued for 9 days (i.e. 27 instillations), then the pharmacokinetic profile was determined on the 28$^{th}$ instillation, i.e. on the morning of day 10 (D10). A sample was taken at time 0 (C0 trough estimation) then after 0.33 h and 0.66 h corresponding to Cmax for the conjunctivas and the cornea, then at 1, 2, 4, 8 and 24 hours.

The results are given in Figure 2. At this concentration and dose:

- There was no accumulation of ciclosporin A in the conjunctivas, nor did the concentration persist. In this structure, the nycthemeral immunomodulator cover over a 24-hour period seems to be limited in time;
- Accumulation occurred in the cornea and a dose of 1,000 to 2,000 ng of ciclosporin A /g of cornea persists for 24 hours in the cornea. In the cornea, the 24-hour nycthemeral immunomodulator cover is therefore satisfactory.

Moreover, no passage into the aqueous humor was observed and passage into the blood was insignificant.

In conclusion, the efficacy of the solution according to the invention has been demonstrated since the values obtained are of the same order as the partial data available for Restasis™ and are compatible with those in the literature which recommends a concentration of active substance in the target tissues of 100 to 400 ng/g to obtain an immunomodulator effect.

Patentkrav

1. En vandig oftalmisk løsning innbefattende:
- ciklosporin A som et immunosuppressivt middel;
- et cellulosederivat som en første polymer;
- et polyvinyl derivat som en andre polymer;
- an makrogolglyserol hydroksystearat som en tredje polymer.

2. Løsning i henhold til krav 1, **karakterisert ved** at ciklosporin A representerer minst 0,05 masse-% av løsningen, fortrinnsvis mellom 0,05 og 0,1 %.

3. Løsning i henhold til et av de foregående krav, **karakterisert ved** at den er fri for benzalkonium klorid (BAK).

4. Løsning i henhold til et av de foregående krav, **karakterisert ved** at den har en Brookfield viskositet ved 25 °C på mindre enn 50 mPa.s, fortrinnsvis mellom 5 og 15 mPa.s.

5. Løsning i henhold til et av de foregående krav, **karakterisert ved** at cellulosederivatet er en cellulose ester, fortrinnsvis valgt fra metylcellulose, etylcellulose, hydroksyetylcellulose, hydroksypropyl-cellulose, karboksymetylcellulose, hydroksypropylmetylcellulose eller et salt derav, mer foretrukket natrium karboksymetylcellulose.

6. Løsning i henhold til et av de foregående krav, **karakterisert ved** at polyvinyl derivatet er polyvinyl alkohol.

7. Løsning i henhold til et av de foregående krav, **karakterisert ved** at makrogolglyserol hydroksystearatet er makrogolglyserol hydroksy-stearat 40.

8. Løsning i henhold til et av de foregående krav, **karakterisert ved** at cellulose derivatet representerer mellom 0,1 og 3 masse-% av løsningen, fortrinnsvis mellom 0,5 og 1,5 5, enda mer foretrukket 0,8 %.

9. Løsning i henhold til et av de foregående krav, **karakterisert ved** at polyvinyl derivatet representerer mellom 0,25 og 3 masse-% av løsningen, fortrinnsvis 0,5 %.

10. Løsning i henhold til et av de foregående krav, **karakterisert ved** at makrogolglyserol hydroksystearatet representerer mellom 0,5 og 20 masse-% av løsningen, fortrinnsvis mellom 5 og 15 %, enda mer foretrukket 10 %.

11. Engangs- eller flerdoseflaske fremstilt av HDPE innbefattende løsningen i henhold til et av kravene 1 til 10.

12. Løsning i henhold til et av kravene 1 til 10 for anvendelse ved behandling av okular overflate lidelse med en inflammatorisk immunkomponent, fortrinnsvis tørt øye syndrom og/eller tap av hornhinnesensitivitet.

13. Løsningen for anvendelse i henhold til krav 12, **karakterisert ved** at en til flere dråper per dag blir administrert til mennesker eller dyr, i hvert øye.

14. Fremgangsmåte for fremstilling av en løsning i henhold til et av kravene 1 til 10, innbefattende følgende trinn:
- oppløse det immunosupressive middelet i nærvær av minst en fraksjon av den første polymeren, i nærvær av vann og fortrinnsvis under omrøring;
- blande den mulig gjenværende fraksjonen av den første polymeren med de andre to polymerene, vann og eventuelt buffersystemene og isotoniseringsmidlene, fordelaktig med omrøring og/eller under oppvarming, for eksempel ved en temperatur på 60 °C;
- blande produktene erholdt i foregående trinn, fordelaktig under omrøring og/eller under oppvarming, for eksempel til en temperatur på 60 °C.

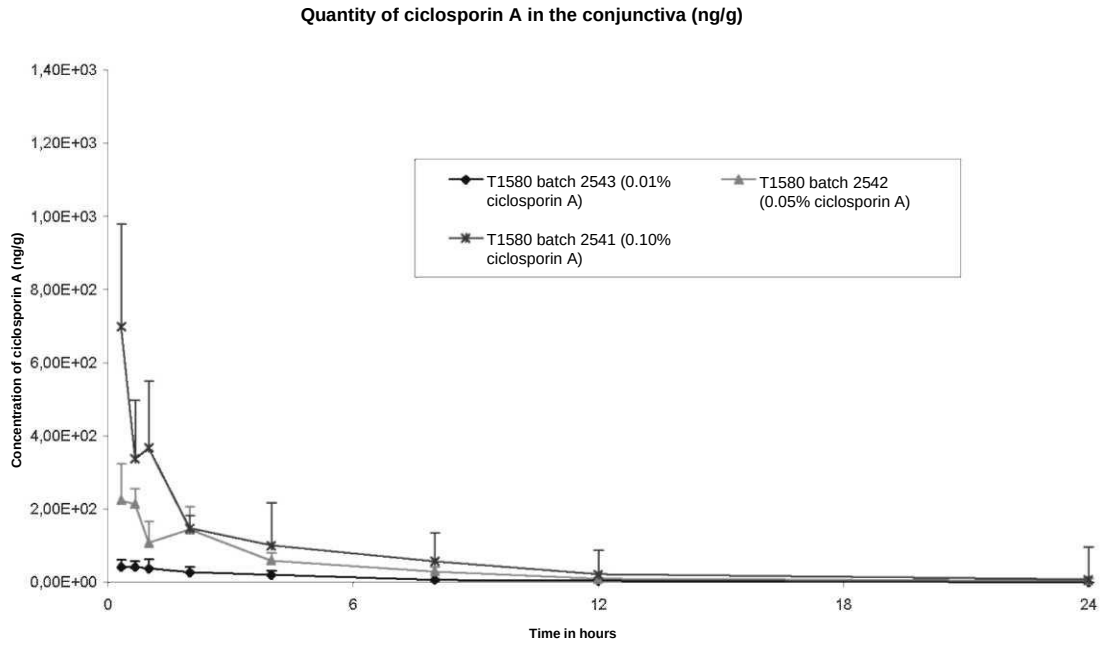



**Figure 1A**

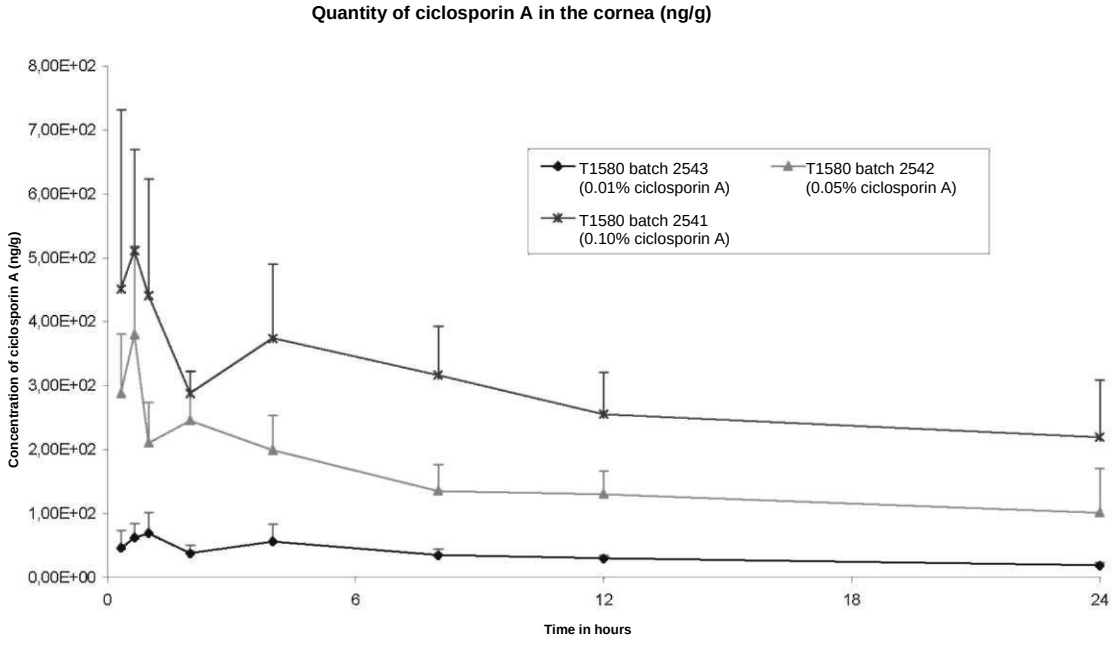



**Figure 1B**

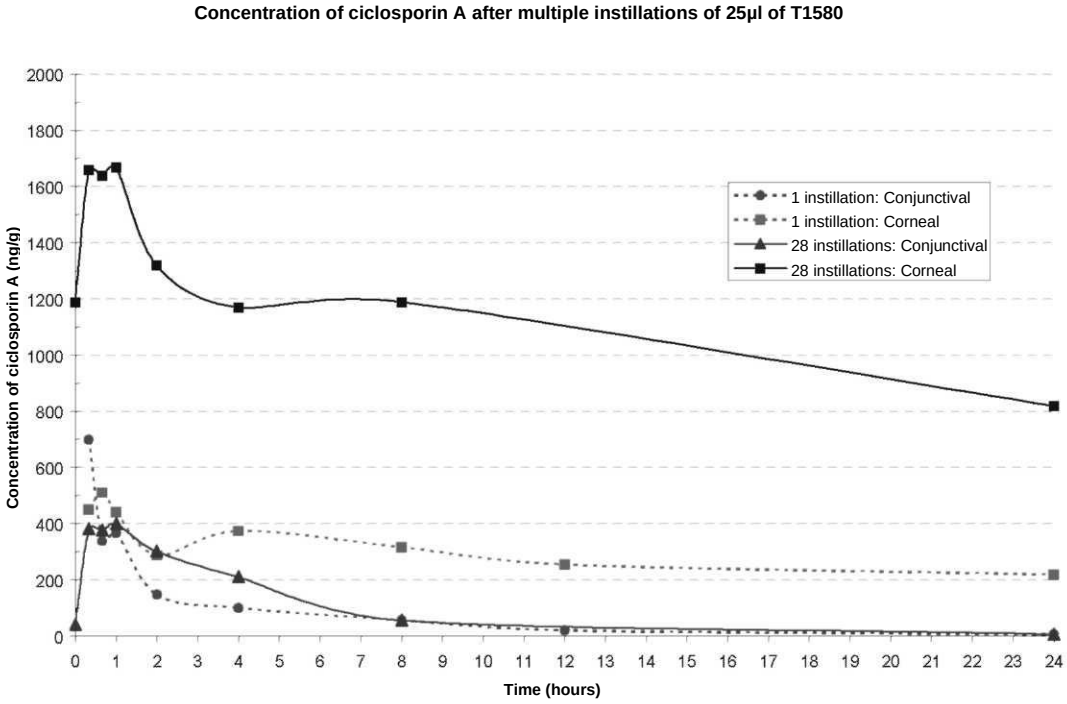

Concentration of ciclosporin A after multiple instillations of 25µl of T1580
Concentration of ciclosporin A (ng/g)
Time (hours)
1 instillation: Conjunctival
1 instillation: Corneal
28 instillations: Conjunctival
28 instillations: Corneal
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

**Figure 2**