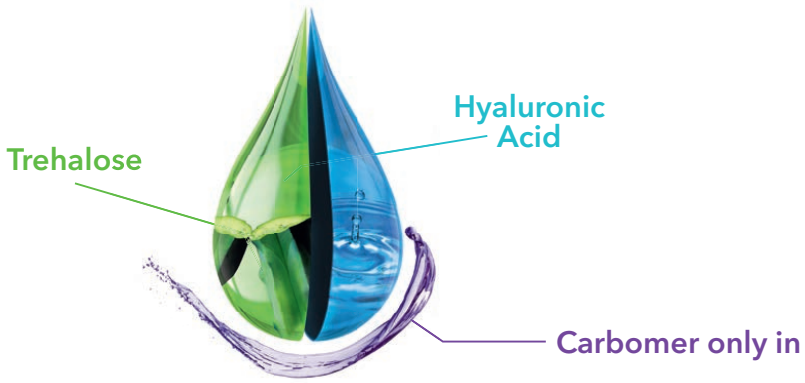
For patients with dry eyes

THEALOZ® DUO

Trehalose 3% | Sodium Hyaluronate 0.15%

The difference is clear

Mechanism of action
Experimental and clinical evidence



THEALOZ® DUO GEL
Trehalose 3% | Sodium Hyaluronate 0.15% | Carbomer 0.25%

Théa
let's open our eyes

Chapter I:
Mechanism of action

Table of contents

Chapter I overview

Trehalose is a naturally occurring bioprotective disaccharide that is present in numerous non-mammalian species, allowing cell survival in unfavourable environments. For example, it has an important role in anhydrobiosis.[1]

It has been demonstrated that Trehalose works as a bioprotective molecule. It has dual properties of bioprotection and osmoprotection, as described in the TFOS DEWS II report released in 2017.[2]

Trehalose acts by maintaining the integrity of cell membranes, while it protects proteins as well as the lipid bilayer. In addition, Trehalose may act as a signalling or regulatory molecule by activating autophagy[3,4] *and reducing cell death associated with apoptosis and inflammation.*[5]

Trehalose is also an osmoprotectant, the uptake of which is associated with a reduced concentration of intracellular inorganic salts,[6] *which can accumulate in cells; in this way, Trehalose helps control the osmotic gradient between the extra- and intra-cellular environments by maintaining homeostasis and protecting cells against desiccation.*[7]

That's why Trehalose has numerous medical applications, such as cryopreservation and organ transplant, as well as others such as improving antibody stability.[8] *The bioprotective effect offered by Trehalose is now being applied to DED (Dry Eye Disease). Due to its bioprotectant and osmoprotectant properties, it could be an important advance to counter tear hyperosmolarity and corneal cell apoptosis – a key step in DED pathophysiology – and so contribute to an exit from the vicious circle of DED.*[5,9,10,11,12,13]

THEALOZ® DUO is an eye drop formulation that contains both Sodium Hyaluronate and Trehalose. It has been developed to capitalise on both the lubricant properties of HA and the bioprotectant properties of Trehalose. This new formulation is preservative-free and available in a multi-dose format, allowing frequent use for extended periods, without causing harm to the ocular surface[14] *as recommended by the TFOS DEWS II report.*[2] *THEALOZ® DUO GEL is a derived formula in a unit dose vial, with the addition of carbomer, allowing an extended precorneal residence time.*[15]

List of figures

Product information

THEALOZ® DUO

Trehalose 3% | Sodium Hyaluronate 0.15%

THEALOZ® DUO GEL

Trehalose 3% | Sodium Hyaluronate 0.15% | Carbomer 0.25%

Indication

Protection, hydration and lubrication of the eye for treatment of moderate-to-severe Dry Eye Disease (DED)

Dosage and administration

- 1 drop in each eye, from 4 to 6 times a day
- Can be used when wearing contact lenses

- 1 drop in each eye, from 1 to 4 times a day
  Gels can be particularly suitable for use in the evening, just before going to bed. Shake well before opening.
- Contact lenses to be removed before using THEALOZ® DUO GEL; and a 30-minutes minimum waiting time is needed before putting them back

Product presentation

- Preservative-free - ABAK® bottle
- 6 months use, after opening
- Phosphate-free
- Hypotonic: 170 – 230 mOsm/kg

- In unit doses

Ingredients per 100 ml /100 g

- Trehalose: 3.00 g
- Sodium Hyaluronate: 0.15 g
- Sodium Chloride, Trometamol, Hydrochloric Acid concentrated, water for injection: to 100 ml

- Trehalose: 3.00 g
- Sodium Hyaluronate: 0.15 g
- Carbomer (Carbopol 974P): 0.25 g
- Sorbitol, Sodium Hydroxide, water for injection: up to 100 g

I. Trehalose: A natural bioprotectant

Introduction

How does the Rose of Jericho *(Selaginella lepidophylla)*, or resurrection plant, manage to survive without water for years, shrivelling to 3% of its mass, and become green again within less than one hour of being watered?

This phenomenon, known as anhydrobiosis, is linked to a molecule with remarkable properties, known as Trehalose. Trehalose temporarily suspends the vital functions, so that an animal or plant organism is able to tolerate an extended period of intense desiccation without sustaining structural damage.

Trehalose is a naturally-occurring disaccharide, and acts as a natural bioprotectant[8,13]. Like all sugars, it is a store of carbon and energy for the organisms containing it. But even more importantly, it provides bioprotection for cells and their organelles against desiccation, heat, cold and the oxidising effects of stress. In this way, Trehalose is synthesised by numerous bacteria, especially in response to osmotic shock *(E. Coli)*[1]; in yeast and fungus, it can account for up to 10% of the mass of some spores;[16] in plants, it sustains organisms exposed to desiccation for prolonged periods (anhydrobiosis)[8]; in the tardigrade or 'water bear,' Trehalose replaces water in the cells, enabling this invertebrate to survive in extreme conditions (cryptobiosis);[17] it enables shrimp embryos to enter a dormant state (brine shrimp eggs); and is the main sugar in the haemolymph of numerous insects, including bees.[1]

Owing to its exceptional properties, including its potential for multiple applications in numerous therapeutic areas, Trehalose has generated considerable interest among the scientific community over the past decade: more than 7,600 articles are available in the PubMed database in 2018. We will focus mainly on its applications in ophthalmology, particularly in Dry Eye Disease.

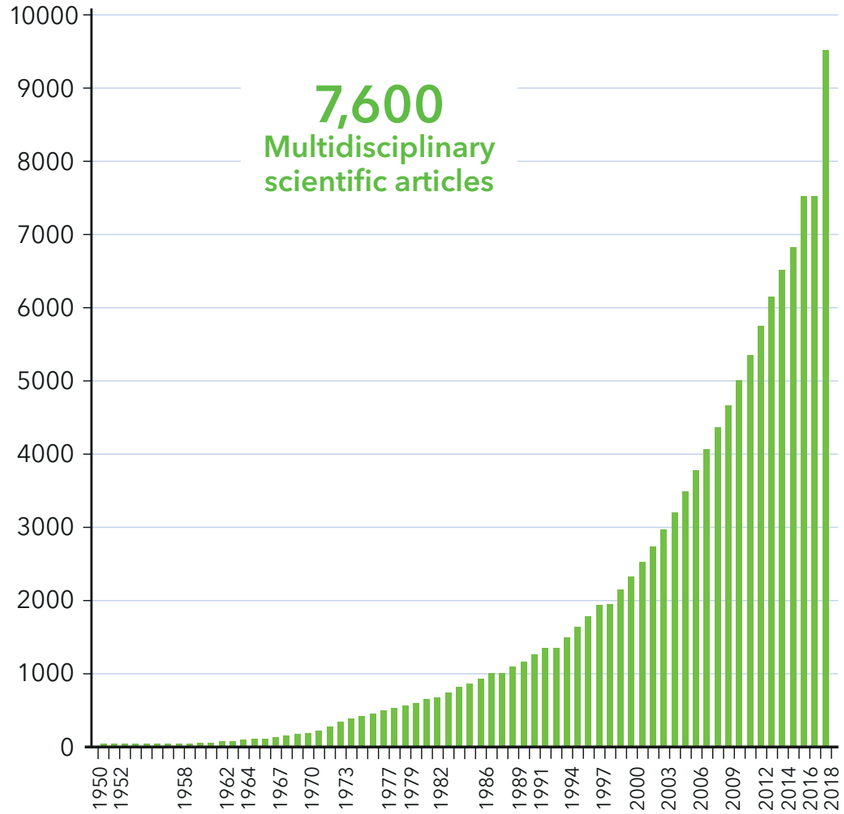


Figure 1: Publications on Trehalose from 1950 to 2018.

Trehalose: From its discovery to its ophthalmological applications

1. The Trehalose molecule

Isolated for the first time in 1859 by Marcellin Berthelot, Trehalose is a natural non-reducing disaccharide. It comprises two glucose molecules, linked together by a highly stable and hydrolysis-resistant α,α-1,1 bond. Compared to other sugars, Trehalose demonstrates superior stability, in longer pH and temperature intervals. It has a low viscosity (5.7 centipoises for a 40% solution).[18]

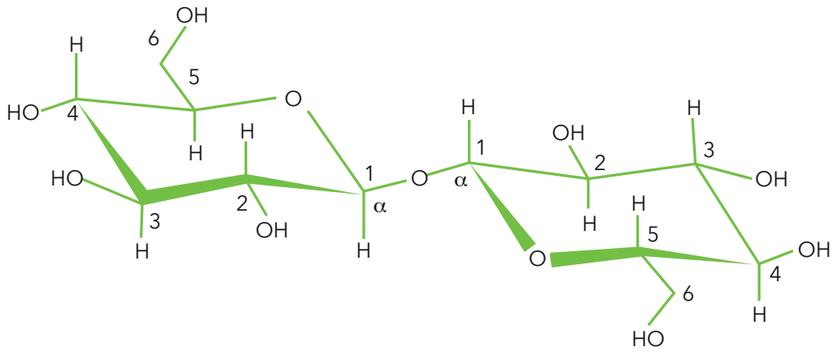


Figure 2: The Trehalose molecule.

2. Application to living organisms[8,19]

Trehalose has been identified in over 80 species of plants (particularly cactuses), algae, fungi, yeasts, bacteria, insects and other invertebrates, and is probably present in many other organisms. It is the main sugar in the haemolymph of insects (80 to 90% of all insects).

The biological functions of Trehalose are many and varied, depending on the species. For the least evolved organisms, Trehalose appears to be a source of energy during certain developmental stages, such as spore germination. In mycobacteria, Trehalose is a structural component of glycolipids. For other microorganisms, Trehalose appears to be a metabolic intermediate or a structural molecule. It is also an energy source used by numerous insects for flight and, as such, could be a specific evolutionary adaptation in flying insects.

One of the most fascinating functional aspects of Trehalose is its role in the survival of certain species in conditions of extreme dehydration or in extreme temperatures. Most organisms that survive at low temperatures owe this ability to the presence of glycerol or other molecules acting as natural 'antifreeze.' For example, a sawfly larva is capable of survival at temperatures below 40°C, even though it does not contain glycerol, but Trehalose in high concentration. In yeast, accumulated Trehalose endows these organisms with their resistance to heat and desiccation.

Even more remarkable are revival plants, such as the Rose of Jericho, which has a Trehalose content accounting for 12.5% of its dry weight. These plants have developed impressive strategies for tolerating periods of drought lasting up to several years. They can endure virtually total desiccation without suffering damage to their structures (anhydrobiosis) and are fully restored to life with normal cellular activity when rehydrated.

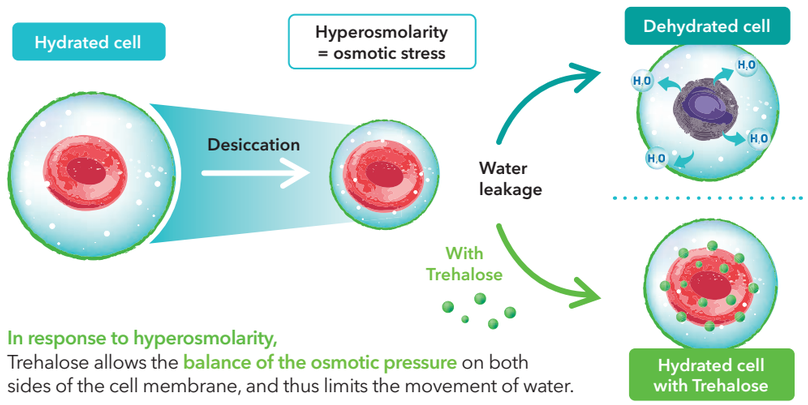
Figure 3: The Rose of Jericho, an example of anhydrobiosis.

Experimental studies have confirmed that it is indeed Trehalose that is responsible for protecting certain plants from dehydration. It has been reported that introducing Trehalose-producing genes into rice plants enables the plants to produce three to eight times more Trehalose than the control plants. When these modified plants are subjected to substantial hydric stress in laboratory conditions, they demonstrate the potential for revival, whereas the control plants subjected to the same regimen suffer severely inhibited growth. Furthermore, these same high-yield Trehalose cell lines survive at temperatures ten degrees lower than those tolerated by their non-modified cousins.[19]

The mechanisms by which Trehalose provides biological molecules with better bioprotection than other oligosaccharides during dehydration can be divided into 4, not mutually exclusive, categories: osmoregulation; a stabilising effect of membranes; preservation of protein structure; and autophagy induction. All of these mechanisms help to protect the structures and functions of the cell, thereby *increasing* cell viability by *decreasing* cell apoptosis.

2.1. Osmoregulation: Trehalose counters the efflux of water from the cells

In instances of desiccation, hyperosmolarity in the cell environment attracts water for the purpose of balancing osmotic pressure on both sides of the cell membrane. This creates an efflux of water from the cell towards the extracellular environment, resulting in cell dehydration. Trehalose balances the osmotic pressures on both sides of the cell membrane, thereby reducing the movement of water.[20] *E. Coli*, for example, synthesises substantial quantities of Trehalose in conditions of hyperosmolarity.[21]



In response to hyperosmolarity,
Trehalose allows the **balance of the osmotic pressure** on both sides of the cell membrane, and thus limits the movement of water.

Figure 4: Osmoregulating action of Trehalose.

2.2. Trehalose has a stabilising effect on membranes

The protective effect of Trehalose during dehydration also occurs in lipid membranes.

When water is removed from biological membranes, massive irreversible structural damage is commonly caused through separation of the phases of the membrane's components. Water accounts for 25% of phospholipid membranes, as each phosphate molecule is surrounded by 10 to 12 water molecules linked together by hydrogen bonds. When the water evaporates, the remaining lipids congeal. When rehydrated, the disorganised membrane, now unable to recover its initial structure, becomes permeable. Effects such as this are prevented whenever the membranes become dehydrated and are then rehydrated with Trehalose present. Trehalose is therefore able to create an amorphous gel as a substitute for the water molecules and preserve the membrane's structure during the desiccation phase.[22-24]

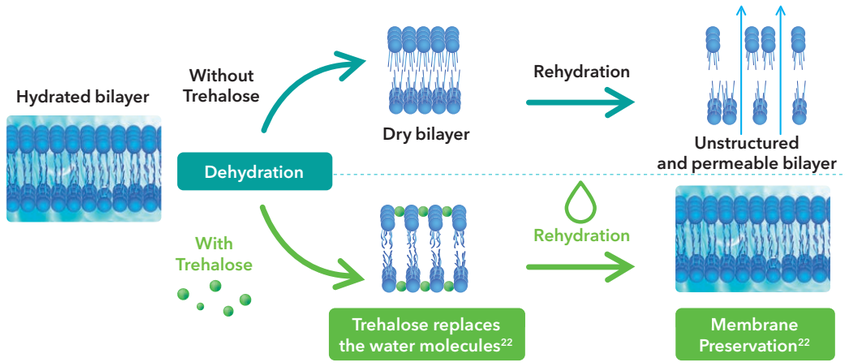


Figure 5: Stabilisation of the phospholipid bilayers by Trehalose.

In this way, the dehydration of biological membranes causes phase separation and a change in state, resulting in vesicle fusion during rehydration. This occurrence is totally inhibited by Trehalose, enabling the vesicles to preserve 100% of their internal environment. Because of this, Trehalose is able to prevent adjacent liposomes from fusing together when desiccated, thereby losing their contents, and is also able to maintain the fluidity and functionality of lipid membranes (ion transport, ATPases, etc.) in the absence of water. Among various sugars tested, Trehalose exerts the greatest stabilising effect by far on membranes.[25]

2.3. Trehalose protects the three-dimensional structure of proteins

Numerous molecules are able to stabilise proteins during a freezing process, but only carbohydrates, and disaccharides in particular, are effective in dehydration.

Among the enzymes tested, phosphofructokinase, purified from rabbit-derived skeletal muscle, is one of the enzymes most susceptible to dehydration, as it is totally and irreversibly deactivated during the freezing-desiccation process.[25] By administering Trehalose or maltose, nearly 80% of enzyme activity can be recovered after rehydration. This type of effect is not observed in the case of glucose or galactose, indicating that spatial orientation of the sub-units of certain disaccharides is probably an important element in this action. When dehydrated without freezing, with the use of a nitrogen stream at ambient temperature, this same enzyme is totally deactivated, yet able to recover 50% of its activity when pre-treated with Trehalose.[25]

In fact, the proteins inside a normally hydrated cell have a three-dimensional spatial structure essential to their biological actions, which is maintained by water-dependent hydrogen bonds. During dehydration, the concentrated state of the intracellular environment causes the hydrogen bonds to be broken and the spatial organisation to be denatured, resulting in the loss of functional properties. Trehalose protects the proteins from denaturation by sticking to them on contact and encapsulating them.

Three hypotheses have been advanced on the effects of this encapsulation: direct replacement of the water molecules by Trehalose molecules so that the hydrogen bonds are retained; trapping of the water molecules at the protein surface; and mechanical trapping of the protein's spatial structure in a highly viscous Trehalose matrix.[26-28] The beneficial effects of Trehalose were very tangibly demonstrated on animal models reproducing neurodegenerative diseases involving protein abnormalities: Trehalose maintains the three-dimensional structure of these proteins and exerts a neuroprotective effect in Huntington's disease,[28,29] or in Alzheimer's disease.[30,31]

Trehalose is incorporated into the formulation of several drugs made up of monoclonal antibodies or recombinant coagulation factors, in order to preserve these protein molecules and retain their properties.[8,32]

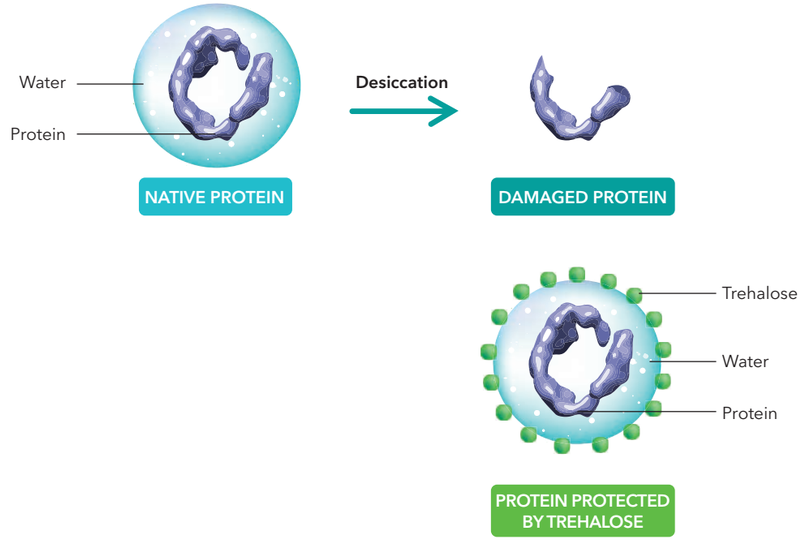


Figure 6: Trehalose protection of the three-dimensional structure of proteins.

2.4. Trehalose is an autophagy inducer

Autophagy is the protective physiological degradation of part of the cell's cytoplasm by its own lysosomes. As an indispensable element of cell survival, autophagy maintains cell homeostasis by destroying damaged proteins and mitochondria and by aiding in the recycling of cell material.[32] Autophagy is equivalent to an adaptive process: it contributes to cell viability by eliminating altered proteins likely to cause tissue degeneration, genome instability, cancer or aging; and to recycling of cell contents, if its environment is changed. This mechanism, demonstrated by Japanese Yoshinori Ohsumi, won him the Nobel Prize for Medicine in October 2016.[33]

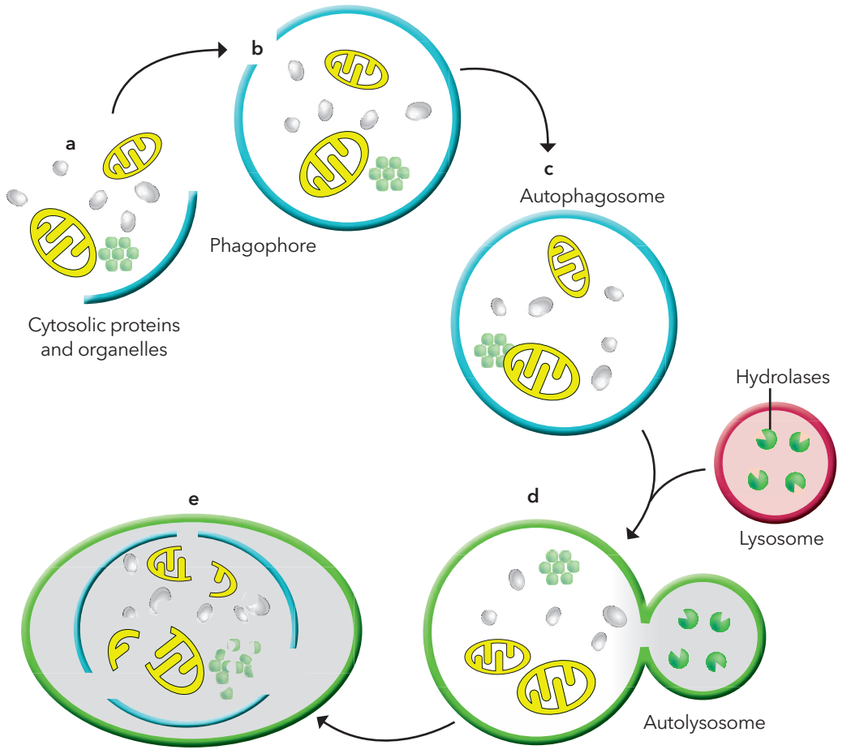


Figure 7: Autophagy. (a) and (b): the altered cytosolic material is confined by a membrane, the phagophore, which stretches to form a sac: the autophagosome (c). The autophagosome fuses with a lysosome, exposing its contents to the hydrolases (d). The contents of the autolysosome are degraded, as a result.[34]

Trehalose is autophagy-inducing, and this results in extension of cellular lifespan.[34] In an *in vitro* study on corneal cells, Trehalose activates autophagy and significantly improves cell viability in systemic inflammatory response after administering TNFα and cycloheximide, two apoptosis-inducing molecules.[35]

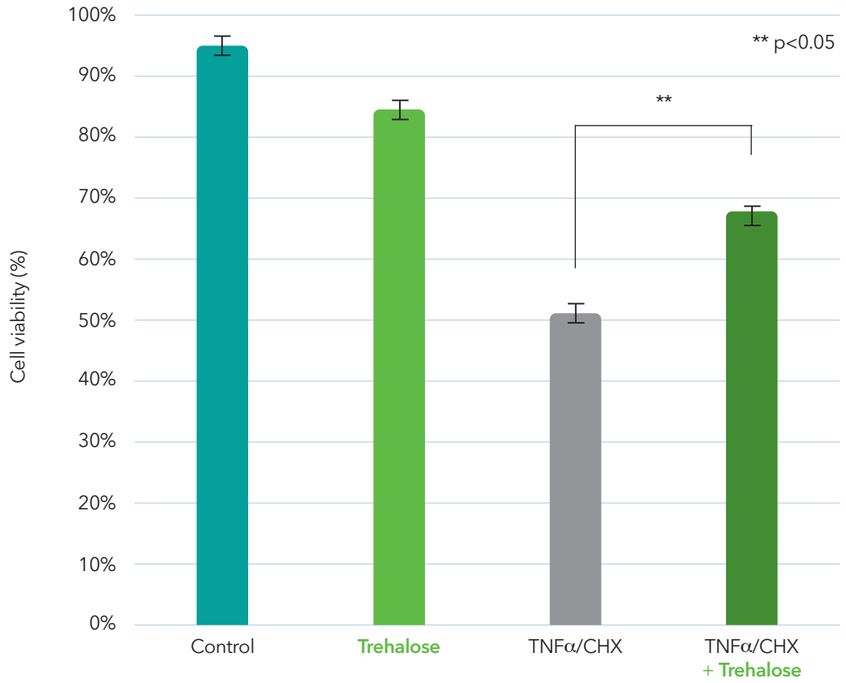


Figure 8: When TNFα and cycloheximide are present, cell viability is significantly improved by Trehalose.[35]

2.5. As a result of the 4 actions, Trehalose reduces apoptosis

All of the properties of Trehalose – osmoregulation, membrane stabilisation, protection of protein structure and autophagy induction – counter water movement and protect the mechanisms of cell physiology, thereby reducing apoptosis and preserving cell viability.

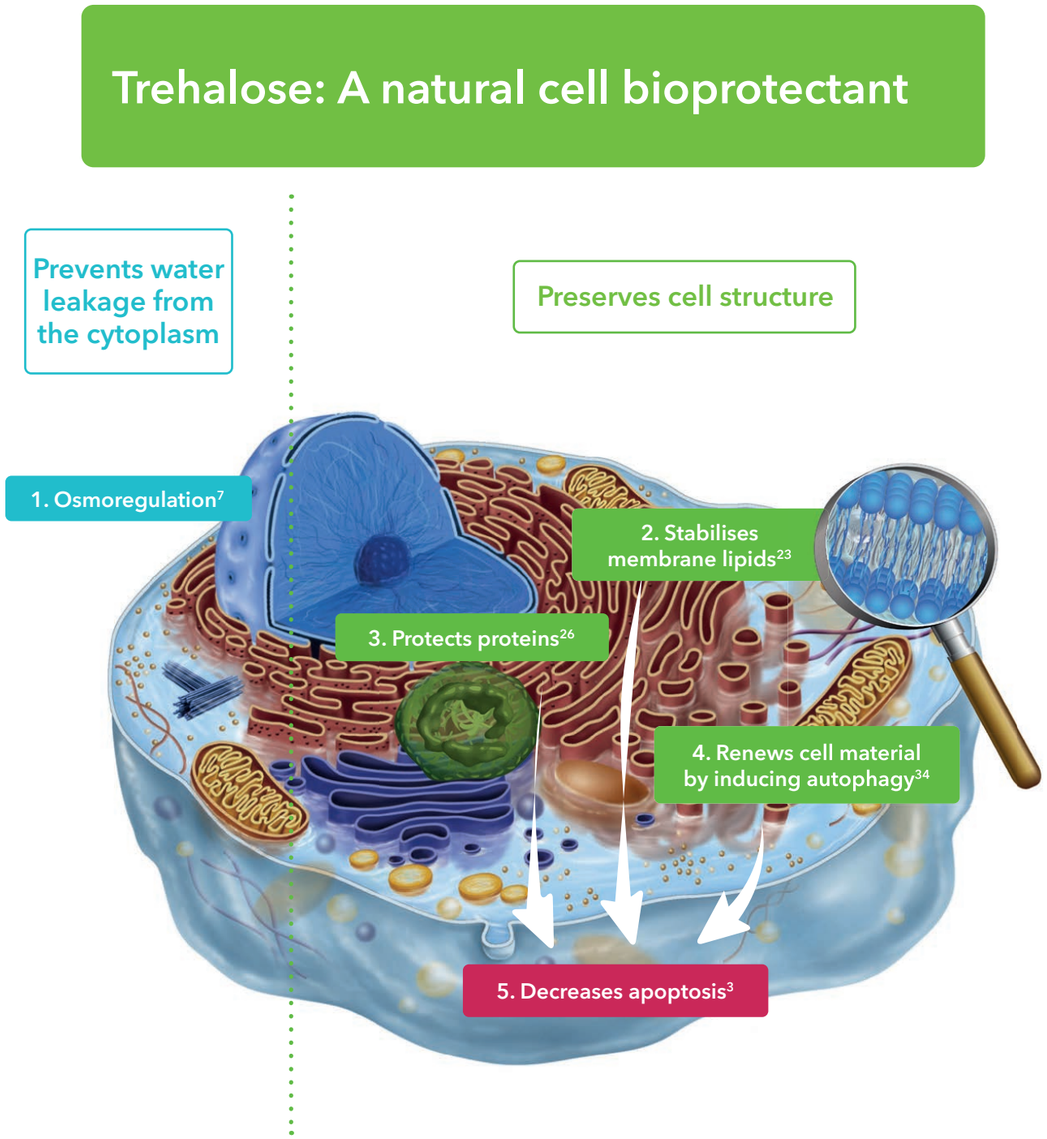


Figure 9: Synthesis of the natural bioprotective properties of Trehalose.

3. Applications in medicine and transplantation

Various Trehalose applications are currently being used or studied.

Trehalose enhances the preservation of dehydrated RNA stored at 4°C,[36] and this could open up valuable prospects for the manufacture and preservation of vaccines, antibodies and immunology diagnostic kits.[37]

Due to the action of Trehalose, lyophilisation of sperm or oocytes will perhaps soon replace freezing, which is a storage method requiring space and equipment. In fact, preliminary studies have shown that Trehalose can be incorporated into sperm, enabling these cells to remain viable after dehydration and rehydration.[38] Similarly, adding Trehalose to oocytes during freezing provides significant protection against stress linked to cold, and very substantially improves the proportion of oocytes surviving the thawing process[39]. It has also been reported that the addition of Trehalose to the conventional freezing medium optimises cryoprotection of frozen umbilical cord blood and foetal liver, two sources of human hematopoietic stem cells.[40]

Human red blood cells can also be loaded with Trehalose, which can later be removed simply by washing or suspension in an iso-osmotic medium, for the purpose of preservation by freezing-dehydration.[41]

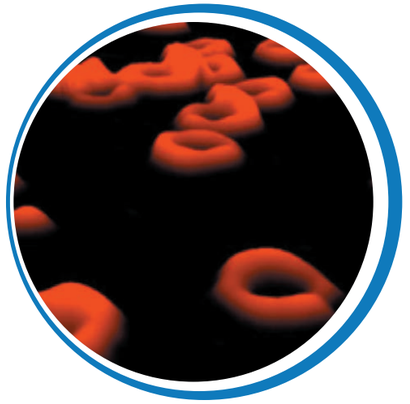


Figure 10: Trehalose enhances preservation by freezing and/or dehydration of cell and organ samples.

In the area of organ preservation prior to transplantation, Trehalose has been proven superior to the monosaccharides traditionally used for this purpose, maintaining the functional capacity of ischaemic lungs in animals.[42] In humans, the preservation solution known as 'ET-Kyoto' which includes Trehalose, has consequently been successfully used prior to transplantation of lungs from living donors or from cadavers, after an ischemia time of close to 10 hours in the latter case.[43,44] The use of Trehalose as an additive when freezing human hepatocytes increases cell viability and culture yields after thawing, and this is an indication of cytoprotection.[45] In the pancreas, when ET-Kyoto solution is introduced by intraductal injection, the yield from sampled islet cells and the transplantation success rate in diabetic patients is improved.[46] A cryopreservation protocol for human adipose cells showed better preservation when Trehalose is combined with tissue freezing.[47]

4. Applications in ophthalmology

Whether in corneal transplantation, glaucoma surgery or Dry Eye Disease, ophthalmological applications of Trehalose are rapidly expanding. In fact, while water retention is related to sugars in plant metabolism, sugars also appear to be fundamental to the physiology of the eye surface.[48]

The corneal epithelium is covered with a coating of glycoprotein 300 nm thick. This film is made up primarily of mucins, glycoproteins characterised by their high glycation level: as much as 80% of the mass of mucins is made up of sugars. These o-glycans form highly organised networks that trap water in their meshes, thereby creating a very effective barrier. Deficiencies in this hydrated glycoprotein barrier have now been identified in Dry Eye Disease.[48]

Recent studies have shown, for example, that Rose Bengal is able to penetrate healthy corneal epithelial cells, but that inducing the production of glycosylated mucins prevents Rose Bengal from being captured by the cells. So, we may assume that when the corneal surface is altered, stain uptake could affect not the dead or diseased cells, but the altered areas in the mucin network.[48] More recently, the same authors demonstrated that glycosylated mucins protect the epithelial cells in the human cornea by making them anti-adhesive to their apical membranes; this involved the insertion of sugar clusters into the protein matrix, stiffening the structure of the mucins. In this way, sugars play a physiological role in protecting the ocular surface.[48]

Dry Eye Disease is a condition in which the cornea is altered through desiccation: Trehalose , a truly bioprotective compound, unlike synthetic lubricants, constitutes a biological approach to Dry Eye Disease, restoring the physiology of corneal epithelial cells, and thereby re-establishing normal tear film hydration and osmolarity.

Conclusion

Trehalose has unique bioprotective properties: it is osmoprotective, membrane-stabilising, preserves protein structure, and induces autophagy. Owing to these properties, Trehalose has the ability to preserve physiological mechanisms and cell structures, making it possible to maintain cell viability during osmotic shock. These properties have already been proven clinically valuable and are already being applied in various medical fields – in particular, organ preservation prior to transplantation. Its use in ophthalmology has also been validated through pre-clinical and clinical studies recognised in the most recent TFOS DEWS II report,[2] and these are described in detail in the following chapter: Experimental and clinical evidence.

II. Sodium Hyaluronate: A long-lasting lubricating and hydrating agent

Sodium Hyaluronate is a natural compound with viscoelastic and hygroscopic properties.

It is one of the most hydrophilic molecules in living organisms and can be described as 'nature's hydrating agent.' Owing to its rheological properties, its function in the body is, among other things, water-binding and lubrication of the body's moving parts, such as joints and muscles.[49] In the eye, it is found in the cornea and conjunctiva, as well as in the lens and – in lower concentrations – in the aqueous humour.

Sodium Hyaluronate is a high-molecular-weight biological polymer, characterised by a repetition of disaccharides, glucuronic acid and N-acetyl-β-glucosamine. This glycosaminoglycan is a commonly-occurring component of the extracellular matrix, known to influence water distribution in conjunctival tissue, including the stroma of cornea. It has a large hydrodynamic volume and, at very high concentrations, its long spirals intertwine to form a continuous, flexible, three-dimensional network.[50]

Its viscosity and mucoadhesive properties depend on its concentration, as well as on its molecular weight. Sodium Hyaluronate differs from most other tear substitutes (except for carbomers) by its non-Newtonian pseudo-plastic behaviour. In fact, like the glycoproteins in mucous, its viscosity is high between blinks; then lower under shear stress during blinking; followed by an instant return to the initial value once the stress is no longer present. This property increases precorneal residence time, while at the same time keeping visual comfort at an acceptable level.[51]

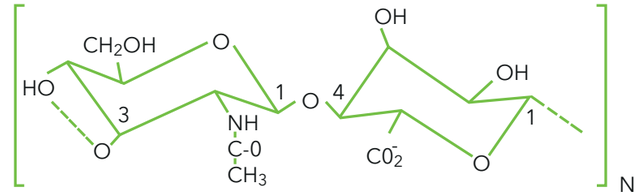


Figure 11: Hyaluronic acid chemical structure.

Besides its viscoelastic properties, Sodium Hyaluronate, like all polysaccharides, is highly hygroscopic, as each disaccharide unit is capable of bonding to about 15 water molecules.[52] Consequently, Sodium Hyaluronate can capture about 1,000 times its own weight in water.[50] This property is considered to be one of the mechanisms involved in keeping the skin hydrated.[53]

In the field of ophthalmology, endogenous Sodium Hyaluronate probably contributes to corneal hydration by retaining the water on the cornea.

Due to these properties, Sodium Hyaluronate reduces tear evaporation by increasing water retention at the corneal surface.[54,55] Several studies have shown that Sodium Hyaluronate, when applied at a minimum concentration of 0.1%, lubricates the ocular surface by thickening and stabilising the tear film.[56] Its residence time is longer than that of other compounds such as hydroxypropyl methylcellulose (HPMC) or polyvinyl alcohol (PVA).[51]

A hyaluronate receptor, CD44, is expressed on the cells of the cornea and the conjunctiva and, when activated, boosts interaction with cytoskeletal proteins, suggesting that hyaluronate may play a role in cell adhesion and mobility.[57] *In vitro*, it stimulates cell migration, stabilises the epithelial barrier on the ocular surface,[57] and boosts epithelial repair and the cicatrisation process.[58] In fact, clinical studies have quantified the enhancing effects of Sodium Hyaluronate on the function of the cornea's epithelial barrier, evaluated by its permeability to fluorescein, in patients with Dry Eye Disease[59] and its protection of the cornea.

An increase in CD44 expression was reported in patients suffering from mild-to-moderate Dry Eye Disease with superficial keratitis; and Sodium Hyaluronate, administered for two months, was associated with a decreased expression of this adhesion molecule. Hence, Sodium Hyaluronate may play a direct role in controlling inflammation at the ocular surface in patients with Dry Eye Disease.[56,60]

Conclusion

Thanks to its viscoelastic properties, Sodium Hyaluronate spreads out over the surface of the eye like a protective film. It lubricates the eye's surface by thickening and stabilising the tear film, and improves corneal and conjunctival hydration.

Sodium Hyaluronate is a perfect complement to Trehalose: the two products, used together, provide combined mechanical protection and cellular bioprotection.

III. Carbomer: Gelling agent

Carbomers are high-molecular-weight hydrophilic macromolecules with properties similar to those of Sodium Hyaluronate: the carbomer macromolecules form a highly hydroscopic three-dimensional network. However, whereas hyaluronate on its own retains a fluid consistency, the carbomers have high gelling and mucoadhesive potential at weak concentrations, thereby lengthening the precorneal residence time of eye drops.[61] Viscosity is increased without modifying hypotonicity. Hypotonicity, in combination with increased viscosity, allows the main active ingredient to remain longer at the ocular surface and increases patient relief.[62]

Not only are carbomers used as vehicles for various active ingredients in the form of eye drops, but numerous clinical trials have also demonstrated the efficacy of carbomer gels in Dry Eye Disease.

Conclusion

Carbomers are Dry Eye Disease-tested lubricating agents, with a high viscosity that lengthens the precorneal residence time of eye drops, while preserving their tonic effect, hence making the patient more comfortable. The gel form of THEALOZ® DUO, which is a combination of Trehalose and Sodium Hyaluronate in a carbomer gel, is an alternative or complement to the solution form, and provides each patient with optimal eye relief according to the situation or clinical circumstances.

IV. Conclusion

THEALOZ® DUO and THEALOZ® DUO GEL are a new advance in treatment for Dry Eye Disease. They consist of a unique combination of Trehalose, a natural bioprotective agent with properties that aid in preserving cell viability in cases of osmotic shock; and Sodium Hyaluronate, considered the second generation as a lubricating treatment for Dry Eye Disease. With the addition of a carbomer, the gel form combines another lubricating agent that lengthens precorneal residence time.[59]

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Chapter II: Experimental and clinical evidence

Table of contents

Summary of the first chapter

Mechanism of action

THEALOZ® DUO and THEALOZ® DUO GEL are eye drop formulations (Medical Devices) that contain both Sodium Hyaluronate and Trehalose 3%, to capitalise on both the lubricant properties of hyaluronic acid (HA) and the bioprotectant properties of Trehalose. THEALOZ® DUO GEL is a derived formula with the addition of carbomer, allowing an extended precorneal residence time.

The properties and mechanism of action of Trehalose 3%, Sodium Hyaluronate and carbomer have been detailed previously in Chapter I.

Trehalose 3% is a naturally occurring bioprotective disaccharide that is present in numerous non-mammalian species, allowing cell survival in unfavourable environments. For example, it has an important role in anhydrobiosis.[1]

It has been demonstrated that Trehalose 3% works as a bioprotective molecule. Trehalose 3% has dual properties of bioprotection and osmoprotection:

Trehalose 3% acts by maintaining the integrity of cell membranes while it protects proteins, as well as the lipid bilayer. In addition, Trehalose 3% may act as a signalling or regulatory molecule by activating autophagy,[2,3] and reducing cell death associated with apoptosis and inflammation.[4] Trehalose 3% is also an osmoprotectant, the uptake of which is associated with a reduced concentration of intracellular inorganic salts,[5] which can accumulate in cells; in this way, Trehalose 3% helps control the osmotic gradient between the extra- and intra-cellular environments by maintaining homeostasis, protecting cells against desiccation.[6]

That's why Trehalose 3% has today numerous medical applications, such as cryopreservation and organ transplantation, as well as others such as improving antibody stability.[7] The bioprotective effect offered by Trehalose 3% is now being applied to Dry Eye Disease (DED) and included in the treatment guidelines mentioned by the TFOS DEWS II report.[7b] Due to its bioprotectant and osmoprotectant properties, it could be an important advance to counter tear hyperosmolarity and corneal cell apoptosis a key-step in DED pathophysiology – and so contribute to an exit from the vicious circle of DED.[4,8,9,10,11,12]

Chapter II overview

This chapter covers the preclinical and clinical scientific data available on each of the individual components of THEALOZ® DUO and THEALOZ® DUO GEL.

Trehalose 3%, a natural bioprotective agent, has been studied in various experimental models of cellular stress at the ocular surface.

In cultured models of human corneal epithelium subjected to desiccation, Trehalose 3% preserved cell viability better than any other tear substitute tested and preserves tight junctions as well as tissue thickness.[10,13]

On Dry Eye Disease models in rats, Trehalose 3% improves corneal discomfort significantly better than autologous serum. Moreover, it preserves mucous-secreting cells, reduces the number of apoptotic cells and eliminates over-expression of inflammation markers.[4,9]

In a model of keratitis caused by UV radiation exposure, Trehalose 3% markedly reduces apoptosis and speeds up corneal healing, restores transparency to the cornea, and eliminates neovascularisation.[14]

The efficacy of Trehalose 3% has been confirmed in several clinical studies.

In a randomised study on moderate-to-severe Dry Eye Disease patients (n=34), Trehalose 3% improved symptoms for a longer period than artificial tears, significantly lowered the vital staining scores, and lengthened TBUT.[15]

In another randomised cross-over trial on moderate-to-severe Dry Eye Disease patients (n=36), Trehalose 3% proved to be significantly more effective than Sodium Hyaluronate and hydroxyethyl cellulose on objective symptoms. In another randomised cross-over trial on moderate-to-severe Dry Eye patients (n=36), Trehalose proved to be significantly more effective than Sodium Hyaluronate and hydroxyethyl cellulose the Ocular Surface Disease Index (OSDI) measures: Break-up time (BUT), fluorescein test and Rose Bengal test. It was also effective on symptoms experienced by patients, who preferred it.[16]

In Dry Eye Disease linked to chronic GVH disease, Trehalose 3% reduces corneal and conjunctival discomfort and improves quality of life.[17]

In THEALOZ® DUO, Trehalose 3% is combined with Sodium Hyaluronate 0.15%; and in the THEALOZ® DUO GEL form, with the further addition of carbomer gel 0.25%.

Several clinical trials have been conducted on Sodium Hyaluronate as a hypotonic solution. It is at least as effective as the other lubricant treatments for Dry Eye Disease, and even more effective than some of them. It is currently the leading lubricating treatment for Dry Eye Disease.[18-24] As excipients, carbomers lengthen the precorneal residence time of an active ingredient. They are also inherently effective in Dry Eye Disease.[25-27]

THEALOZ® DUO (Trehalose 3% + Sodium Hyaluronate) and THEALOZ® DUO GEL (Trehalose 3% + Sodium Hyaluronate + carbomer) were evaluated in five randomised comparative studies.[28-32]

THEALOZ® DUO proved to have the same effectiveness as Sodium Hyaluronate on keratitis, quantified by the Oxford staining score. THEALOZ® DUO is significantly superior to Sodium Hyaluronate alone for decreasing the severity of eye symptoms. It is considered more effective than Sodium Hyaluronate by both investigators and patients. It improves the quality of the tear film for a longer period than Sodium Hyaluronate and has a more significant impact on quality of life than does a lubricating eye drop or Sodium Hyaluronate.

Because of the extended precorneal residence time due to the presence of a carbomer, THEALOZ® DUO GEL produces longer-lasting increases in tear film thickness than THEALOZ® DUO or other tear substitutes, allowing for a reduction in instillation frequency.

THEALOZ® DUO and THEALOZ® DUO GEL eye drops are preservative-free, in order to avoid any iatrogenic cytotoxic effects.[33,34]

Described by the TFOS DEWS II report as both osmoprotectant and bioprotectant, THEALOZ® DUO meets all the requirements expressed by the DEWS for a tear substitute: THEALOZ® DUO improves both hyper-osmolarity and inflammation, reduces signs and symptoms, is long-acting, preservative-free and easy to use.[35]

List of figures

List of tables

List of abbreviations

DED	Dry Eye Disease
UV	Ultraviolet
TBUT	Tear break-up time
GVH	Graft-versus-host
TFOS DEWS	Tear Film & Ocular Surface Society Dry Eye Workshop
TNFα	Tumour necrosis factor alpha
°C	Degrees celsius
OCT	Optical coherence tomography
PBS	Phosphate buffered saline
IL	Interleukin
MMP	Matrix metalloproteinase
UVB	Ultraviolet B
HA	Hyaluronic acid (= Sodium Hyaluronate)
HEC	Hydroxyethyl cellulose
LASEK	Laser-Assisted Subepithelial Keratomileusis
OSDI	Ocular Surface Disease Index
CMC	Carboxy methylcellulose
PVA	Polyvinyl alcohol
HPMC	Hydroxypropyl methylcellulose
S	Systane
PEG	Polyethylene glycol
PPG	Propylene glycol
BAK	Benzalkonium chloride

Product information

THEALOZ® DUO

Trehalose 3% | Sodium Hyaluronate 0.15%

THEALOZ® DUO GEL

Trehalose 3% | Sodium Hyaluronate 0.15% | Carbomer 0.25%

Indication

Protection, hydration and lubrication of the eye for treatment of moderate-to-severe Dry Eye Disease (DED)

Dosage and administration

- 1 drop in each eye, from 4 to 6 times a day
- Can be used when wearing contact lenses

- 1 drop in each eye, from 1 to 4 times a day
  Gels can be particularly suitable for use in the evening, just before going to bed. Shake well before opening.
- Contact lenses to be removed before using THEALOZ® DUO GEL; and a 30-minutes minimum waiting time is needed before putting them back

Product presentation

- Preservative-free - ABAK® bottle
- 6 months use, after opening
- Phosphate-free
- Hypotonic: 170 – 230 mOsm/kg

- In unit doses

Ingredients per 100 ml /100 g

- Trehalose: 3.00 g
- Sodium Hyaluronate: 0.15 g
- Sodium Chloride, Trometamol, Hydrochloric Acid concentrated, water for injection: to 100 ml

- Trehalose: 3.00 g
- Sodium Hyaluronate: 0.15 g
- Carbomer (Carbopol 974P): 0.25 g
- Sorbitol, Sodium Hydroxide, water for injection: up to 100 g

I. Current concept of Dry Eye Disease: Tear film instability, inflammation, hyperosmolarity due to cellular stress

Dry Eye Disease (DED) is one of the most commonly occurring age-related ophthalmologic diseases. After the age of 65, 15 to 33% of the population is believed to suffer from Dry Eye Disease, and tear substitutes are the key treatment for this. As defined by the TFOS DEWS II report (the Dry Eye Workshop second report of the Tear Film & Ocular Surface Society), published in July 2017: "Dry eye is a multifactorial disease of the ocular surface characterised by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play aetiological roles."[7,36]

This definition is rather different from the previous one:[37] the notion of 'loss of homeostasis of the tear film' is introduced, and the causal role of hyperosmolarity and inflammation is enacted, supported by research performed since the first TFOS DEWS report in 2007, as well as the neurosensory abnormalities, responsible for the lack of correlation between signs and symptoms.

Indeed, neuropathic pain may explain significant symptoms associated with moderate or absent objective signs. On the contrary, a reduced corneal sensitivity corresponds to a minimal symptomatology, despite pronounced objective alterations.[7b]

This new report also stresses the complexity of the mechanisms underlying DED: the separation between hyperevaporation and production deficiency now appears rather artificial, since 30% to 70% of the patients may have a combination of both components.[7b]

Hence, as a result of these multiple intercurrent factors, the distinction between the stages of severity in DED are much less clear-cut than in the previous report.[7b,37]

Dry Eye severity level	1	2	3	4*
Discomfort, severity and frequency	Mild and/or episodic, occurs under environmental stress	Moderate episodic or chronic, stress or no stress	Severe frequent or constant, without stress	Severe and/or disabling and constant
Visual symptoms	None or episodic, mild fatigue	Annoying and/or activity-limiting episodic	Annoying, chronic and/or constant, limiting activity	Constant and/or possibly disabling
Conjunctival injection	None to mild	None to mild	+/-	+/++
Conjunctival staining	None to mild	Variable	Moderate to marked	Marked
Corneal staining (severity/location)	None to mild	Variable	Marked central	Severe punctate erosions
Corneal/tear signs	None to mild	Mild debris, ˅ meniscus	Filamentary keratitis, mucus clumping, ^ tear debris	Filamentary keratitis, mucus clumping, ^ tear debris, ulceration
Lid/meibomian glands	MGD variably present	MGD variably present	Frequent	Trichiasis, keratinisation, symblepharon
TFBUT (sec)	Variable	≤ 10	≤ 5	Immediate
Schirmer score (mm/5 min)	Variable	≤ 10	≤ 5	≤ 2

* Must have signs AND symptoms. TBUT: fluorescein tear break-up time. MGD: meibomian gland disease

Table I: Dry Eye Disease grading scheme (TFOS DEWS I).[37]

While there are multiple points of entry in Dry Eye Disease, its fundamental pathophysiological origins can invariably be broadly classified into: hyperosmolarity of the tear film; apoptosis of the ocular surface cells; and nerve stimulation causing inflammation and activation of inflammatory mediators at all levels of the ocular surface.[38] Hyperosmolarity is responsible for cellular stress, which produces increased apoptosis of conjunctival cells and mucocytes. Nerve stimulation, induced by ocular surface damage, eventually results in inflammation – which, in turn, leads to the release of proinflammatory cytokines, tumour necrosis factor alpha (TNF-α), interleukins, interferon gamma (IFN-γ), and the abnormal expression of class II

antigens and cytokines from the TH1-lymphocytic system, as well as the activation of metalloproteinases. These metalloproteinases convert inactive inflammatory cytokine precursors into their active forms which, along with the other effects, result in an inflammatory chain reaction, with a cytotoxic effect on the ocular surface. The destruction of mucocytes increases tear film instability and the resultant cellular stress.[38] Furthermore, an unstable or inadequate tear film can lead to alterations in the local microbiota, and the consequent release of lipases and bacterial toxins, thereby contributing to inflammation of the eyelid and meibomian glands, and leading to changes in lipid composition. These changes, in turn, increase tear film instability.[38]

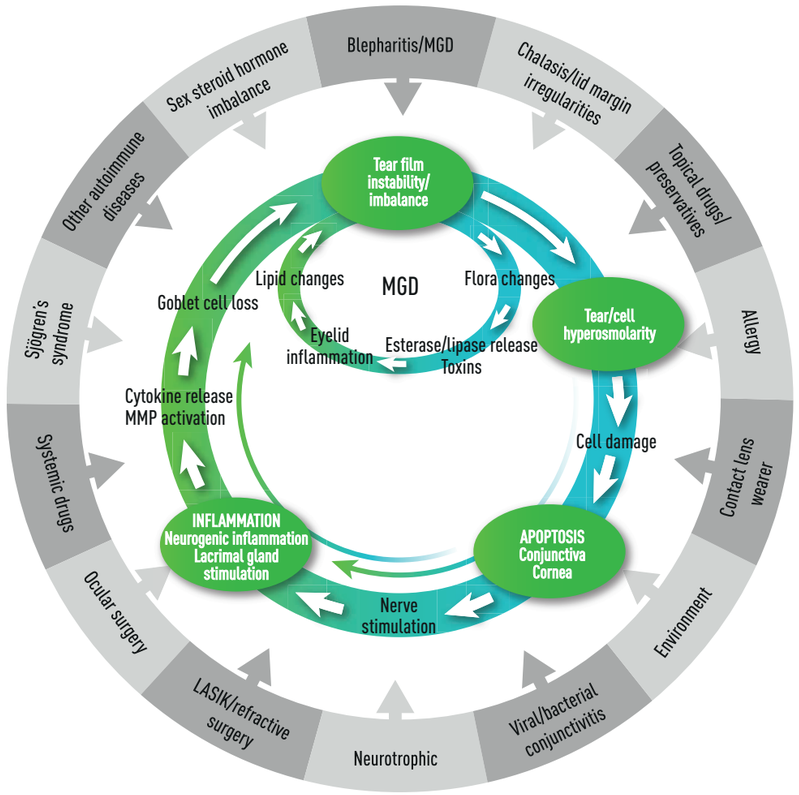


Figure 1: Pathophysiological loop leading to a 'Vicious Cycle' in Dry Eye Disease (according to Baudouin *et al*).[39]

Therefore, cellular stress is an inevitable consequence of this self-aggravating circle.

According to the TFOS DEWS II report, Dry Eye Disease treatment cannot be only palliative: it must also target the main pathophysiologic mechanisms.[7b]

Step 1	Step 2 if previous options are inadequate, consider:	Step 3 if previous options are inadequate, consider:	Step 4 if previous options are inadequate, consider:
- Education regarding the condition, its management, treatment and prognosis - Modification of local environment - Education regarding potential dietary modifications (including oral essential fatty acid supplementation) - Identification and potential modification/ elimination of offending systemic and topical medications - Ocular lubricants of various types (if MGD is present, then consider lipid-containing supplements) - Lid hygiene and warm compresses of various types	- Non-preserved ocular lubricants to minimise preservative-induced toxicity - Tea tree oil treatment for Demodex (if present) - Tear conservation • Punctal occlusion • Moisture chamber spectacles/goggles - Overnight treatments (such as ointment or moisture chamber devices) - In-office, physical heating and expression of the meibomian glands (including device-assisted therapies, such as LipiFlow) - In-office intense pulsed light therapy for MGD • Prescription drugs to manage DED • Topical antibiotic or antibiotic/steroid combination applied to the lid margins for anterior blepharitis (if present) • Topical corticosteroid (limited-duration) • Topical secretagogues • Topical non-glucocorticoid immunomodulatory drugs (such as cyclosporine) • Topical LFA-1 antagonist drugs (such as lifitegrast) • Oral macrolide or tetracycline antibiotics	- Oral secretagogues. - Autologous/ allogeneic serum eye drops - Therapeutic contact lens options • Soft bandage lenses • Rigid scleral lenses	- Topical corticosteroid for longer duration - Amniotic membrane grafts - Surgical punctal occlusion - Other surgical approaches (eg tarsorrhaphy, salivary gland transplantation)

Table II: Staged management and treatment recommendations for Dry Eye Disease (adapted from the TFOS DEWS II report).[7b]

'Ocular lubricants' are the mainstay of Dry Eye Disease treatment. Products aiming only to replace the tear film, or 'artificial tears,' are considered as insufficient, since they have no action on etiological mechanisms.[40]

Thus, the ideal tear substitute should have an effect on both hyperosmolarity and inflammation, and:

- Induce no ocular surface toxicity
- Allow a long-acting hydration and lubrication
- Mimic the normal tear film behaviour, with good stability
- Protect the ocular surface
- Be easy to use

THEALOZ® DUO is currently one of the most complete responses to this specification: preservative-free and hypotonic, it combines two complementary ingredients:

- Trehalose, classified as an osmoprotectant in the TFOS DEWS II, has very high water retention capabilities, and also has the dual properties of bioprotection and osmoprotection.[7b] Trehalose is a natural bioprotectant, which protects the ocular surface cells from consequences of hyperosmolarity and inflammation. This mechanism of action is particularly suited to the treatment of Dry Eye Disease and its complications, with remarkable experimental and clinical results
- Sodium Hyaluronate, a lubricant with a high viscosity at rest that decreases with increasing shear rate, as occurs during a blink, and which is used into numerous tear substitute formulations

Thus, THEALOZ® DUO capitalises on the lubricant properties of Sodium Hyaluronate *and* the bioprotectant action of Trehalose.[7b]

II. Experimental and clinical data on each component

(A table summarising the trials described below is shown on p64)

1. Trehalose 3%: Experimental data

1.1. *In vitro* models

Evaluation of the effectiveness of Trehalose 3% on the viability of human corneal epithelial cells subjected to desiccation.

Trehalose 3% (THEALOZ®) was subjected to a comparative evaluation with various commercial tear substitutes, for its effectiveness on a human corneal epithelium model in a monolayer culture:[13] HIALEYE (hyaluronic acid 0.2%, BAK), SYSTANE (hydroxypropyl guar, Polyquad), KERATOSTILL (hydroxylethyl cellulose 0.3%, dexpanthenol, EDTA), LACRIMAL (polyvinyl acetate, BAK) and NaCl 0.9%. The products were applied so that they covered the cell cultures and, after incubation, these cell cultures were subjected to desiccation under an ambient air flow for 5 to 45 minutes.

	5 Min	15 Min	30 Min	45 Min
Thealoz®	98.8%	90.6%	51.7%	10.2%
Hialeye 0.2 %	92.6%	35.1%	8.1%	0.6%
Hialeye 0.4 %	87.4%	32.1%	11.2%	0%
Keratostill	92%	50.0%	23.5%	4.4%
Lacrimal	40.1%	10.2%	0%	0%
Starazolin	91.5%	44.0%	7.8%	0%
Systane	92.9%	50%	6.8%	0.6%
NaCl 0.9 %	88.4%	2.4%	0%	0%
Not treated	89.6%	0%	0%	0%

Mean % of living cells: 0%-10% | 10.1%-50% | 50.1%-90% | 90.1%-100%

Table III: The level of cell viability is about twice as high with Trehalose 3% compared to the other products.[13]

Trehalose 3% keeps the cell viability level significantly higher, compared with all of the other products tested. At the end of 5 minutes of desiccation, even the cells not receiving any treatment remain over 90% viable; however, from 15 minutes of desiccation onwards, cell viability is lost in the absence of treatment and is only just higher when treated with physiological serum, showing intense cellular stress. Cell viability does not exceed 50% with the tear substitutes tested; however, cell viability is maintained at over 90% with Trehalose 3%. After 30 minutes of desiccation, 50% of the cells remain viable with Trehalose 3%, as compared with a mere 25% for the best performing tear substitute. Overall, after 15 minutes, Trehalose 3% keeps twice as many cells alive compared to the standard tear substitutes producing the best results. A functionality test shows that cells pre-incubated with Trehalose 3% keep cross-membrane transport active as well as cytosolic transport by endosomes; whereas, with most of the other products, the membrane transport functions are eliminated.

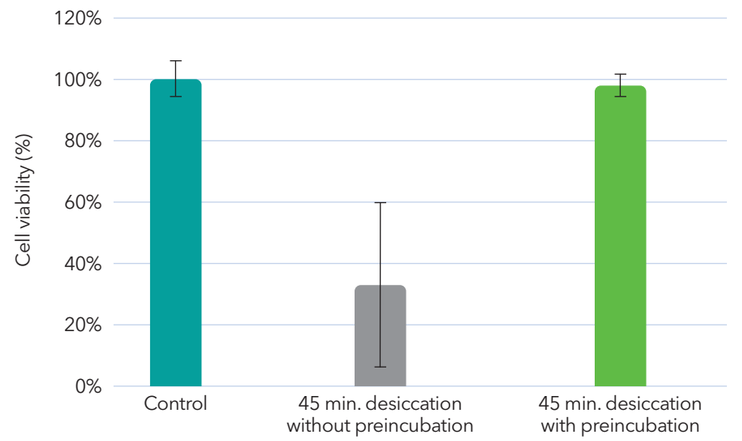


Figure 2: Cell viability after desiccation without treatment, and with pre-treatment with Trehalose 3%.[10]

Evaluation of the effectiveness of Trehalose 3% on cell viability, tight junctions and thickness of human corneal epithelial tissue subjected to desiccation.

The bioprotective properties of Trehalose 3% were also evaluated on a reconstituted three-dimensional model of human corneal epithelium.[10] The samples were divided into 3 groups: control, desiccation without treatment, and desiccation after 1-hour pre-treatment with Trehalose 3%. Desiccation was performed by complete aspiration of the medium, followed by 45 minutes of exposure at ambient temperature (24°C) and 34% humidity.

Without treatment, the level of cell viability fell to 32% after desiccation, while a 98% level of cell viability was maintained with Trehalose 3% pre-treatment. The confocal immunofluorescence analysis shows that the tight junctions in the samples pre-treated with Trehalose 3% are preserved. Trehalose 3% maintains OCT-measured tissue thickness at the same level as the control group, while non-treatment desiccation reduced the same thickness by half.

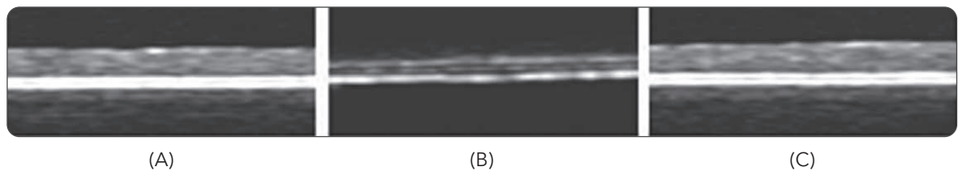


Figure 3: Pre-treatment with Trehalose 3% maintains corneal epithelium at the same thickness whereas desiccation without treatment reduces it by half.

Key points

Out of two cultured models of human corneal epithelial cells subjected to desiccation:

- **Trehalose 3% preserves cell viability better than any other tear substitute tested**
- **Trehalose 3% preserves tight junctions and tissue thickness**

1.2. *In vivo* models

Trehalose 3% versus analogous serum in Dry Eye Disease induced by accelerated evaporation.

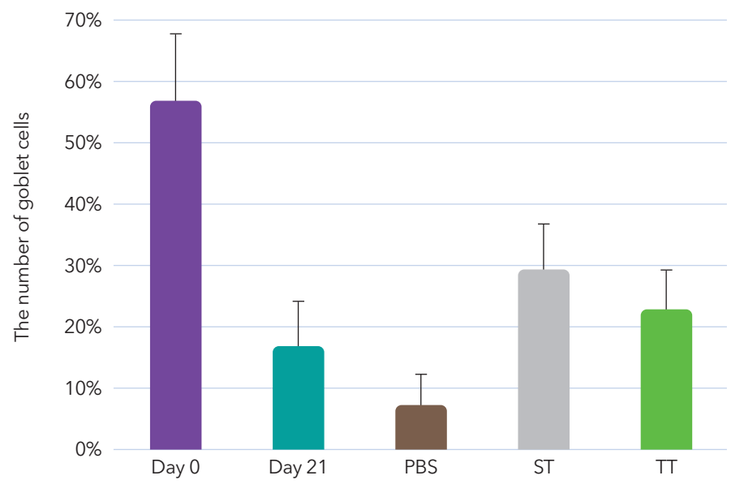
Trehalose 3% was studied in a mouse Dry Eye Disease model,[9] and compared to the leading treatment in this type of model, murine analogous serum, the effectiveness of which is well documented. Alterations at the ocular surface were induced by means of a continuous dehumidified air flow in a controlled environment, applied for 21 days, causing accelerated tear evaporation. The animals were randomly divided into 3 treatment groups: neutral control solution; 20% autologous murine serum; or Trehalose 3%, (30 mg/ml 87.6 mM). Each treatment was administered at a rate of 10 µl every 6 hours for 14 days.

After 14 days of treatment, Trehalose 3% appeared to improve corneal damage significantly more than the serum or the neutral solution:

- The fluorescein-stained surface is significantly lower in the Trehalose 3% group compared to the two other treatments
- The thickness of the corneal epithelium is increased in the Trehalose 3% and autologous serum groups
- Epithelial disorganisation and desquamation are reduced, to a marked degree in the Trehalose 3% group
- The number of goblet cells is significantly increased and the thickness of the conjunctival epithelium is reduced in the Trehalose 3% and autologous groups
- The number of apoptotic cells is sharply reduced in the autologous serum and Trehalose 3% groups
- Tear production is significantly increased with autologous serum and Trehalose 3%

	PBS	Autologous Serum (ST)	Trehalose (TT)
After 7 days of treatment	11.2 ± 2.7 p<0.01*	7.6 ± 1.6 p<0.01	6.8 ± 2.1 p>0.05**
After 14 days of treatment	12.6 ± 3.2 p<0.01*	6.6 ± 2.0 p<0.01	**4.5 ± 1.7** p<0.01**

Table IV: Trehalose significantly reduces fluorescein-quantified damage.



PBS: Control solution - ST: Autologous Solution - TT: Trehalose 3%

Figure 4: Trehalose 3% increases the number of mucous-secreting cell.[9]

Numbers of apoptotic cells on ocular surface:

	D0	D21	Autologous serum	Trehalose 3%	Control (PBS)	p (Trehalose 3% vs Serum)
Cornea	4 ± 2	32 ± 7	21 ± 6	11 ± 4	39 ± 11	<0.01
Conjunctiva	6 ± 3	59 ± 14	34 ± 8	15 ± 6	67 ± 14	<0.001

Table V: Trehalose 3% reduces the density of apoptotic cells at the ocular surface.[4]

A similar Dry Eye Disease model was used by Li *et al* in an *in vivo* study. This study showed that eye dryness was induced in mice with Intelligently Controlled Environmental System (ICES). Trehalose 3% produces a marked decrease in inflammation marker expression in the conjunctiva: IL-1β, IL-2, IL-6, IL-17, tumour necrosis factor-alpha (TNF-α), and MMP-9 mRNA. Trehalose 3% almost totally blocks over-expression of pro-inflammatory interleukins (IL), metalloproteinases and TNF-α.[4]

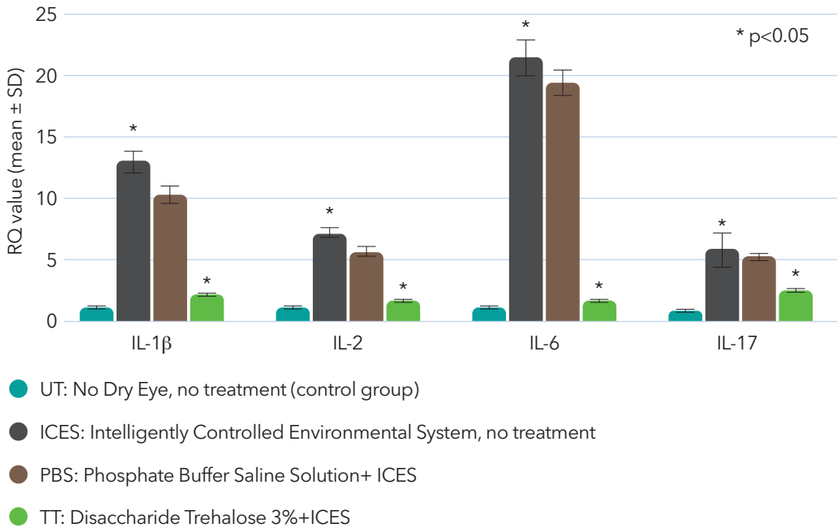


Figure 5: Trehalose 3% reduces the expression of inflammation markers.

Key points

On mouse Dry Eye Disease models, Trehalose 3%:

- **Improves corneal damage significantly more than autologous serum**
- **Preserves goblet cells**
- **Reduces the number of apoptotic cells**
- **Eliminates over-expression of inflammation markers**

Evaluation of Trehalose 3% in corneal healing on a keratitis model induced by UVB radiation exposure in rabbits[14]

Corneal exposure to radiation from UVB rays produces oxidative stress, which diminishes re-epithelialisation, and stimulates corneal neovascularisation, as well as cell death by apoptosis. For this model, the eyes of anaesthetised rabbits were kept open and exposed to a UVB lamp placed at a standardised distance from the eyes, with the lamp plane positioned perpendicularly to the optical axis. The UVB dose was 0.5 J/cm^2 per day for 4 days. After radiation exposure (D4), 3 drops of Trehalose 3% were instilled into the right eye and 3 drops of PBS into the left eye, 6 times a day for 1 week (3 animals), 2 weeks (3 animals) or 3 weeks (3 animals).

As a result of the UV radiation, the control corneas developed significant neovascularisation and became white, losing their transparency. Trehalose 3% accelerated corneal healing, restored corneal transparency and eliminated neovascularisation. There was a sharp reduction in the ratio of apoptotic cells from the first week of treatment.

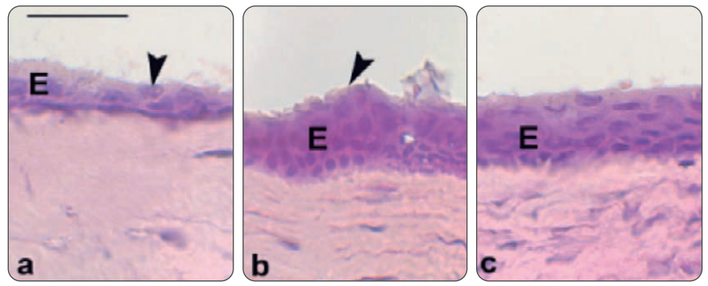


Figure 6: Trehalose 3% promotes re-epithelialisation of UVB-damaged corneas.[14]
a. Irradiated cornea after two weeks of buffered saline treatment. In the central part of the cornea, the superficial epithelial layers are missing *(arrow)*.
b. Irradiated cornea on which Trehalose drops were applied for two weeks after irradiation. The cornea is re-epithelialised *(arrow)*.
c. Normal cornea. Scale bar: 10 µm.

Key points

In UV radiation-induced keratitis, Trehalose 3%:

- **Sharply reduces apoptosis**
- **Accelerates corneal cicatrisation, restores corneal transparency and eliminates neovascularisation**

Conclusion

Trehalose 3% is more effective than traditional tear substitutes with hyaluronic acid, such as SYSTANE, HIALEYE or KERATOSTILL, in protecting the cells of the corneal epithelium and maintaining the function of their membranes.

In Dry Eye Disease conditions, Trehalose 3% restores the integrity of the corneal epithelium and the ocular surface, reduces apoptosis and inflammation, and increases the number of goblet cells.

2. Trehalose 3%: Clinical data

2.1. Comparative study of Trehalose 3% and physiological serum[15]

This randomised, double-blind study conducted by Matsuo *et al*[15] included patients suffering from moderate-to-severe bilateral Dry Eye Disease, with each patient as their own control, having one eye treated with Trehalose 3% (100 to 200 mM) and the other with sodium chloride, 6 times a day for 4 weeks. All of the patients who previously used tear substitutes underwent a period of therapeutic wash-out with a preservative-free buffered saline solution. At inclusion, the patients had a Rose Bengal test score of at least 3 and a positive score in the Schirmer and/or BUT test.

Thirty-four patients who met the inclusion criteria and completed the study were analysed. These included 26 (76%) with Gougerot-Sjögren syndrome.

After 2 and 4 weeks of treatment, the Rose Bengal and fluorescein scores on ocular surface damage were significantly improved by Trehalose 3% at the two concentrations tested, compared with the saline solution. Tear film break-up time (TBUT) was significantly extended in both groups of patients receiving Trehalose 3%. The symptoms score was significantly improved in the patients receiving Trehalose 3%. In fact, while the methodology using the contralateral eye as the control is suitable for measuring objective parameters, the patients found it difficult to individually evaluate the symptoms of each eye. Overall, however, the patients mentioned a longer-lasting effect in the eye administered with Trehalose 3%, compared with the one administered with sodium chloride.

No adverse effects were recorded in the group treated with Trehalose 3%.

Key points

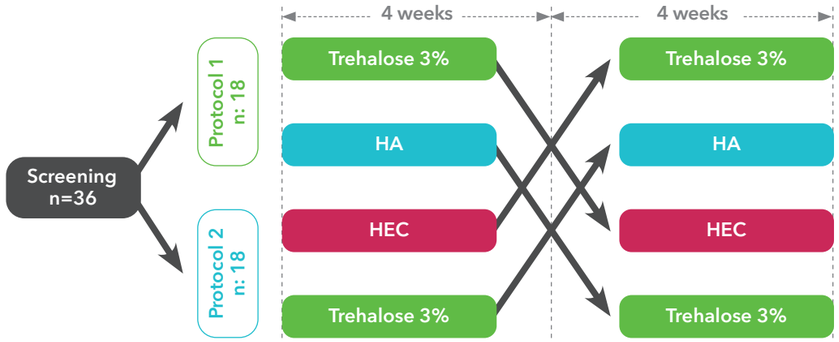
In this randomised comparative study with artificial tears in moderate to severe Dry Eye Disease, Trehalose 3%:

- **Improves symptoms for a longer period**
- **Significantly lowers vital staining scores**
- **Extends the Tear Break-Up Time (TBUT)**

2.2. Study comparing Trehalose 3% 100 mM to Sodium Hyaluronate and hydroxyethyl cellulose[16]

This randomised cross-over study conducted by Matsuo *et al* included 36 patients with moderate-to-severe Dry Eye Disease (including 23 with primary or secondary Gougerot-Sjögren syndrome) who were treated with Trehalose 3% or with one of the 2 comparative treatments (Sodium Hyaluronate and hydroxyethyl cellulose), with permutations after 4 weeks of treatment.[16]

The treatments were administered in both eyes at a rate of 4 instillations per day for 8 weeks.



HA: Hyaluronic acid HEC: Hydroxyethyl cellulose

Figure 7: Study design[16]

Trehalose 3% proved to be significantly more effective than Sodium Hyaluronate and hydroxyethyl cellulose (HE Cellulose) on the objective signs; BUT, fluorescein and Rose Bengal tests.

Scores	Median value (interval)		*p*	Median value (interval)		*p*
	Trehalose 3% 100 mM	Sodium Hyaluronate		**Trehalose 3% 100 mM**	HE-cellulose	
TBUT	2.75 (0-5)	0 (0-2.5)	*0.0005*	3 (1-5)	0 (0-1)	*0.0002*
Fluorescein	1 (0-1.5)	2 (1-3)	*0.0003*	0.5 (0.1-1.5)	1.5 (0-3)	*0.0007*
Rose Bengal	3 (0.5-4)	5.75 (1-9)	*0.0003*	2.5 (2-5)	5.5 (2-9)	*0.0004*

Table VI: Ocular surface and tear production scores after 4 weeks of treatment.[16]

The symptom scores for Trehalose 3% 100 mM and the two comparison products were statistically comparable. However, at the end of 8 weeks of treatment, each patient had to state which of the 2 treatment sequences had brought them the greatest relief. 66.7% of the patients in the group treated with cellulose and 55.6% in the group treated with Sodium Hyaluronate preferred Trehalose 3%.

Key points

In this randomised cross-over study, Trehalose 3%:

- **Is significantly more effective than Sodium Hyaluronate and hydroxyethyl cellulose on the objective signs; BUT, fluorescein and Rose Bengal tests**
- **Is also effective on the symptoms**
- **Is preferred by most patients**

2.3. Study of Trehalose 3% in relation to alcohol-induced alterations in the epithelium[17]

LASEK refractive surgery involves chemical separation of the corneal epithelium from the Bowman's membrane by application of a diluted ethanol solution to the corneal surface. Ethanol is substituted for intra- and extracellular water, causing the tissue to retract and stiffen. It produces epithelial cell alterations linked to water efflux and protein denaturation.

In this study, the right eye of twelve patients requiring LASEK surgery was pre-treated with one drop of Trehalose 3% every 15 minutes for one hour prior to the surgical procedure. The left eye remained untreated and served as the control.[17]

The morphometric analysis showed significantly increased thickness in epithelial and surface basal surface cells, and clearer cytoplasm in the Trehalose 3%-treated eyes. Desmosomes and hemi-desmosomes distribution was significantly greater in the Trehalose 3%-treated eyes.

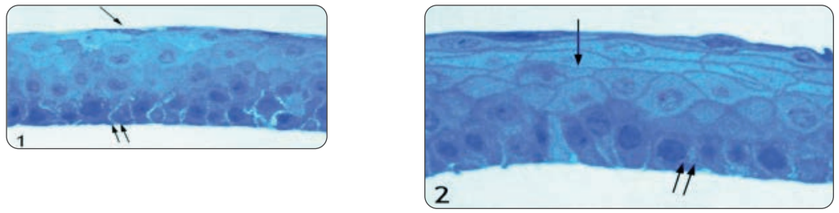


Figure 8: Epithelial cells from eyes treated with Trehalose 3% (2) are better preserved, and retain a normal shape and distinct intercellular boundaries.

Key points

In this experimental, controlled study, Trehalose:

- **Better preserves morphological and morphometric features of alcohol-treated corneal epithelium for LASEK surgery**
- **Significantly increases thickness in epithelial and surface basal surface cells**
- **Has a protective role on the corneal epithelium**

2.4. Study of Trehalose 3% in relation to chronic graft-versus-host reaction[41]

Chronic Graft-Versus-Host (GVH) reaction produces ocular events, most common among them Dry Eye Disease, which occurs in more than half of cornea recipients.

A prospective study was conducted to evaluate the benefits of administering Trehalose 3% eye drops, 3 to 6 times a day, for 4 weeks, to patients with severe Dry Eye Disease linked to chronic GVH.[41] The Trehalose 3% treatment reduced conjunctival and corneal staining, as well as the OSDI (Ocular Surface Disease Index) score.

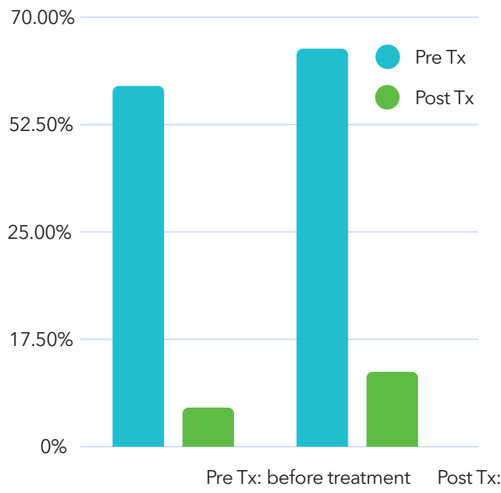


Figure 9: Conjunctival and corneal staining.[41]

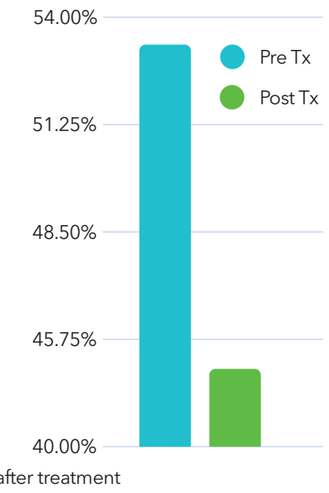


Figure 10: OSDI Score.

Key points

In Dry Eye Disease linked to chronic GVH, Trehalose 3%:

- **Reduces corneal and conjunctival damage**
- **Improves quality of life**

Conclusion

Trehalose 3% improves ocular signs and symptoms more effectively than hyaluronic acid and hydroxyethyl cellulose in patients with Dry Eye Disease, in terms of both ocular surface alterations and tear film stability.

In addition, Trehalose 3% improves the objective parameters measuring ocular surface integrity and tear film quality, preserving the morphology of corneal epithelia subjected to stressful conditions.

3. Sodium Hyaluronate

The efficacy of Sodium Hyaluronate at various concentrations is supported by numerous clinical trials, many of which are controlled and randomised. Sodium Hyaluronate relieves burning and stinging sensations for a longer period than physiological serum, in patients suffering from Dry Eye Disease,[18,19] including Sjögren's syndrome.

In mild-to-moderate Dry Eye Disease, Sodium Hyaluronate solution 0.1% also proved to be as effective as, or even more effective than, carboxymethyl cellulose (CMC) 0.5%; more effective than polyvinyl alcohol (PVA); and comparable to hydroxypropyl methylcellulose (HPMC).[20-23] Moreover, Sodium Hyaluronate hypotonic formulas provide more effective treatment of symptoms, objective signs and cytological markers than isotonic formulas, probably owing to their additional action on tear hyperosmolarity.[24]

Key points

- Sodium Hyaluronate is at least as effective or more as other lubricating treatments for Dry Eye Disease, and is safer than some of them[20-23]
- It is currently the leading lubricating treatment in Dry Eye Disease
- Several clinical trials have been conducted on Sodium Hyaluronate in hypotonic solution[24]

4. Carbomers

The use of carbomers in the treatment of Dry Eye Disease has been widely studied since the 1980s. Open or placebo-controlled studies have revealed positive developments in terms of clinical symptoms, as well as functional tests (BUT, vital staining) in patients receiving a carbomer.[25]

Randomised studies were conducted to compare carbomers to chondroitin sulphate[31], hydroxypropyl methylcellulose (HPMC),[25] polyvinyl alcohol,[26] and even various textures of carbomers with one another.[27]

In all of these trials, the carbomers proved to be equivalent or superior to the comparison treatment. Interestingly enough, a liquid carbomer formulation achieved the same degree of efficacy on symptoms and ocular surface impairment as the less liquid standard gels in tubes.[27]

Carbomers are also used for their gelling and mucoadhesive properties, in order to extend the precorneal residence time of eye drops.[25]

Key points

- As excipients, carbomers are used to lengthen the precorneal residence time of an active ingredient
- Carbomers are also inherently effective in the treatment of Dry Eye Disease

Study	Methodology	Comparison product	Results
Pre-clinical Trehalose 3%			
Hill-Bator 2014 ***(in vitro)*** Ref 13	Human corneal epithelial model in a monolayer culture, subjected to desiccation	Hialeye 0.2 and 0.4 Systane Keratostill Lacrimal NaCl+Starazolin	Trehalose 3% maintains cell viability levels twice as high as standard tear substitutes, producing the best results.
Hovakimyan 2012 ***(in vitro)*** Ref 10	Reconstituted three-dimensional human corneal epithelial model in a culture, subjected to desiccation	Control not treated	Pre-treatment with Trehalose 3% results in cell viability being maintained at 98% (vs 32% for the control samples) and maintains tissue thickness (reduced by half in the case of the control samples).
Chen 2009 ***(animal)*** Ref 9	Mouse Dry Eye Disease model	Autologous serum Neutral solution	After 14 days of treatment, Trehalose 3% improves corneal damage significantly more than the serum and the neutral solution.
Li 2012 ***(animal)*** Ref 4	Mouse Dry Eye Disease model	Control not treated	Trehalose 3% achieves a marked reduction in conjunctival expression of inflammation markers: IL-1β, IL2, IL-6, IL-17, TNF-α, and MMP-9 mRNA.
Cejkovà 2012 ***(animal)*** Ref 14	UVB radiation-exposed rabbit keratitis model	Control not treated	Trehalose 3% sharply reduces the level of apoptotic cells, accelerates corneal healing, restores corneal transparency and eliminates neovascularisation.
Clinical Trehalose 3%			
Matsuo 2002 Ref 15	Randomised, double-blind study on moderate-to-severe Dry Eye (3/4 Gougerot-Sjögren)	NaCl 0.9%	Trehalose 3% relieves symptoms for a longer period than artificial tears, and significantly improves vital stain and TBUT scores.
Matsuo 2004 Ref 16	Randomised, cross-over study on moderate-to-severe Dry Eye (2/3 Gougerot-Sjögren)	Sodium Hyaluronate Hydroxyethyl cellulose	Trehalose 3% is significantly more effective than Sodium Hyaluronate on objective signs: BUT, fluorescein and Rose Bengal tests, and is also effective on symptoms.
Aragona 2014 Ref 17	Open study on alcohol-induced epithelial alterations during LASEK procedures	Control contralateral eye not treated	Trehalose 3% pre-treatment preserves the thickness of the corneal epithelium and prevents alterations in cell morphology.
Monteiro IPO PORTO 2012 Ref 41	Prospective study on Dry Eye Disease linked to chronic GVH	-	Trehalose 3% treatment reduces conjunctival and corneal staining, as well as the OSDI score.
Clinical Sodium Hyaluronate			
Vogel 2010 Ref 18	Randomised study on Dry Eye	Placebo	Sodium Hyaluronate 0.18% improves superficial keratitis and its symptoms.
Saeed 2013 Ref 19	Open study	-	Sodium Hyaluronate improves the symptoms associated with Dry Eye Disease.
Lee 2011 Ref 20	Randomised, double-blind study, on mild-to-moderate Dry Eye	Carboxymethyl cellulose	Sodium Hyaluronate and carboxymethyl cellulose are equally effective on the symptoms of Dry Eye, superficial keratitis and TBUT.
Brignole 2005 Ref 21	Prospective, randomised, masked observer, parallel on Dry Eye	Carboxymethyl cellulose	Both treatments are comparable in improving the symptoms and impairment associated with superficial keratitis, but do so more rapidly with Sodium Hyaluronate, which produces greater ocular relief.
Baudouin 2012 Ref 22	Prospective randomised, masked observer parallel on Dry Eye	Carboxymethyl cellulose	Both treatments are comparable in improving impairment from superficial keratitis, tear osmolarity, the Schirmer-I test and quality of life (OSDI).
McDonald 2002 Ref 23	Randomised cross-over study	Polyvinyl alcohol	Sodium Hyaluronate is superior to PVA in improving burning sensations and comparable in improving grittiness.
Aragona 2002 Ref 24	Randomised, double-blind study on moderate-to-severe Dry Eye	Artificial tears	Sodium Hyaluronate improves the condition of the ocular surface more effectively than the physiological serum, as evaluated by cytological marking.
Clinical carbomers			
Pouliquen 1999 Ref 25	Literature review	Chondroitin sulphate HPMC PVA	Identical efficacy on symptoms, TBUT and staining with a lesser frequency of instillation than Sodium Hyaluronate.
Marner 1996 Ref 26	Randomised, open crossover study	PVA	The carbomer proved more effective than PVA on symptoms and TBUT, with reduced instillation frequency.
Chiambaretta 2004 Ref 27	Randomised, blind, investigative study on Dry Eye	Carbomer fluid gel vs conventional carbomer gel	Both the fluid and conventional formulations are just as effective on Dry Eye symptoms, superficial keratitis and TBUT.

Table VII: Summary table of studies on Trehalose 3%, Sodium Hyaluronate and carbomers.

III. Thealoz® Duo: Clinical data

(A table summarising the trials described below is shown on pages 80-81.)

THEALOZ® DUO is a preservative-free eye drop combining Trehalose 3% and Sodium Hyaluronate 0.15%. Also available as a gel formulation, which adds a carbomer to the two ingredients mentioned. The two formulas are hypotonic and preservative-free.

Five randomised studies were conducted to evaluate the efficacy of THEALOZ® DUO and THEALOZ® DUO GEL compared to leading products: hyaluronic acid and various combinations of emollients. The evaluation criteria pertained not only to the signs and symptoms of Dry Eye Disease but also to patient quality of life.

1. Phase III study of THEALOZ® DUO compared with Sodium Hyaluronate 0.18%[28]

This non-inferiority clinical study was conducted according to a multi-centre, comparative, randomised, single-blind, parallel group methodology, on 105 patients suffering with moderate-to-severe Dry Eye Disease. The comparison product was Sodium Hyaluronate 0.18% (HA18, Vismed®). The two treatments were administered at a rate of one drop in each eye, 3 to 6 times a day, for 3 months. The average dosage was 4 drops a day.

Both treatments reduce the mean score for total corneal staining (Oxford score, the main evaluation criterion representing the degree of corneal ulceration) to similar levels: the latter score was 5.7 ± 1.5 (THEALOZ® DUO group) and 6.3 ± 1.5 (HA18 group: Vismed®) at inclusion. This decreased by 2.5 and 2.7, respectively, after 35 days of treatment, confirming the hypothesis of the THEALOZ® DUO non-inferiority compared to Sodium Hyaluronate 0.18% used on its own. After 3 months of treatment (D84), the reduction in the Oxford score compared to the base values was 4 and 3.9, similar for THEALOZ® DUO and HA18.

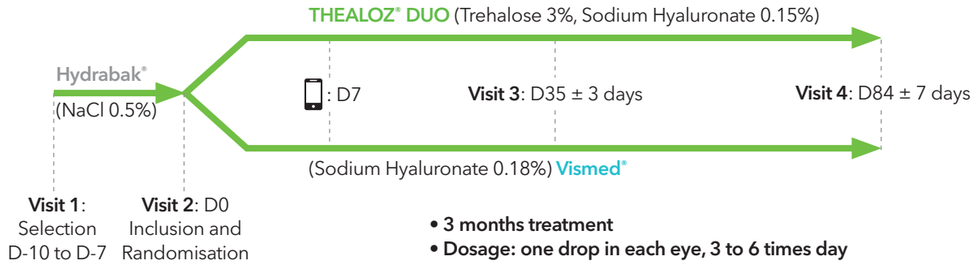


Figure 11: Phase III study design.

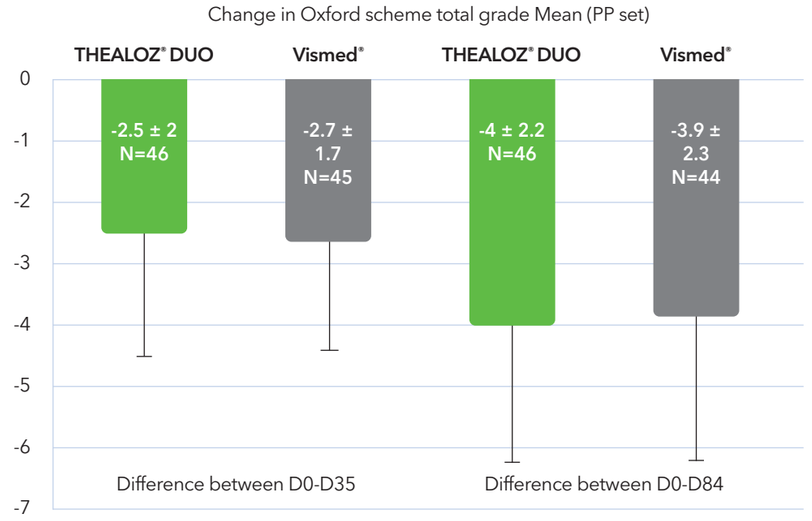
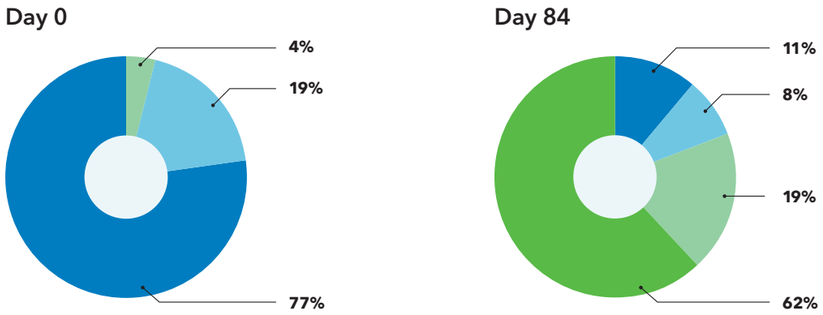


Figure 12: THEALOZ® DUO improves the mean corneal staining score.[28]

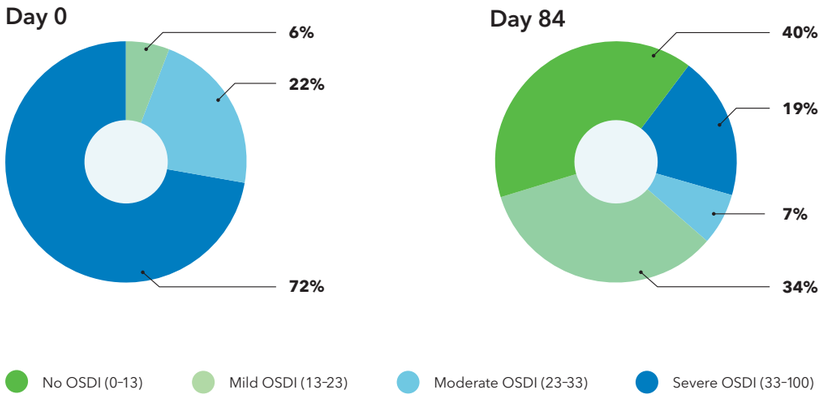
Most of the patients had an OSDI score of over 23 at inclusion; and 70% of patients even had severe Dry Eye Disease, and an OSDI score of over 33.

After 3 months of treatment, the number of patients below the pathological threshold (OSDI ≤ 18) was significantly higher on THEALOZ® DUO than on HA18 group Vismed® (p = 0.025).

Thealoz® Duo – 52 patients



Sodium Hyaluronate alone – 53 patients



n=105 patients with moderate-to-severe dry eye. Randomised, active-controlled, investigator-masked, multi-centric study. Patients received either Thealoz Duo (Trehalose + Sodium Hyaluronate) or Vismed® (Sodium Hyaluronate) 3–6 times a day for 84 days.

Adapted from Chiambaretta F, et al. Eur J Ophthalmol. 2016.[28]

The severity of ocular symptoms (burning, pain, stinging, itching, Dry Eye sensation, sensation of a foreign body in the eye, photophobia, and blurred vision) diminished at D35 and D84 in both treatment groups, with no significant difference between the treatments. However, this reduction in stinging and itching is more pronounced at D35 in the THEALOZ® DUO group compared to HA18, as well as for blurred vision at D35 and D84.

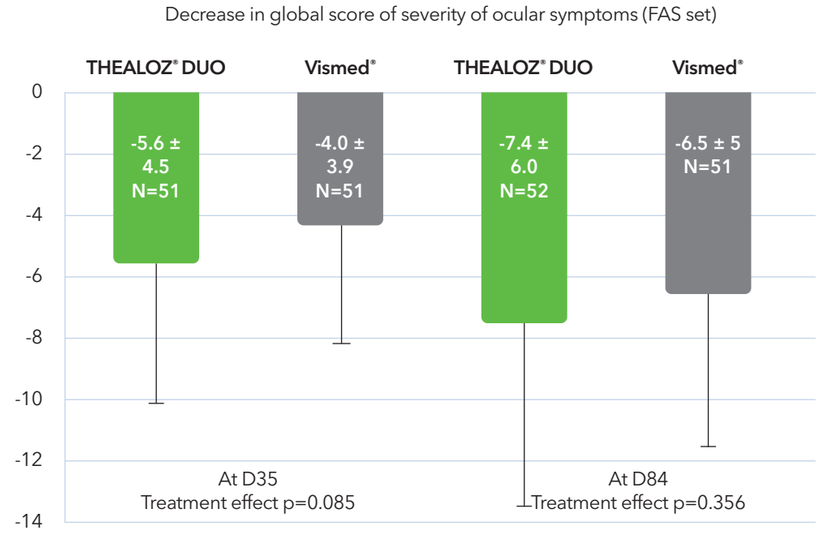


Figure 14: THEALOZ® DUO improves the symptom severity scores.[28]
(Base values: 11.1 ± 4.5, and 11.0 ± 4.7 respectively for the THEALOZ® DUO and HA groups).

Both treatments are comparable in improving tear film break-up time, the Schirmer test and hyperaemia. At D84, close to 85% of the patients had no, or very low, hyperaemia in the group treated with THEALOZ® DUO, versus 74% in the HA18 group (no significant difference).

The investigator considered efficacy to be higher for the THEALOZ® DUO group at D35 (p=0.015) and at D84 (p=0.043). Patient satisfaction was also significantly higher for the THEALOZ® DUO group at D35 (p=0.023) but not at D84.

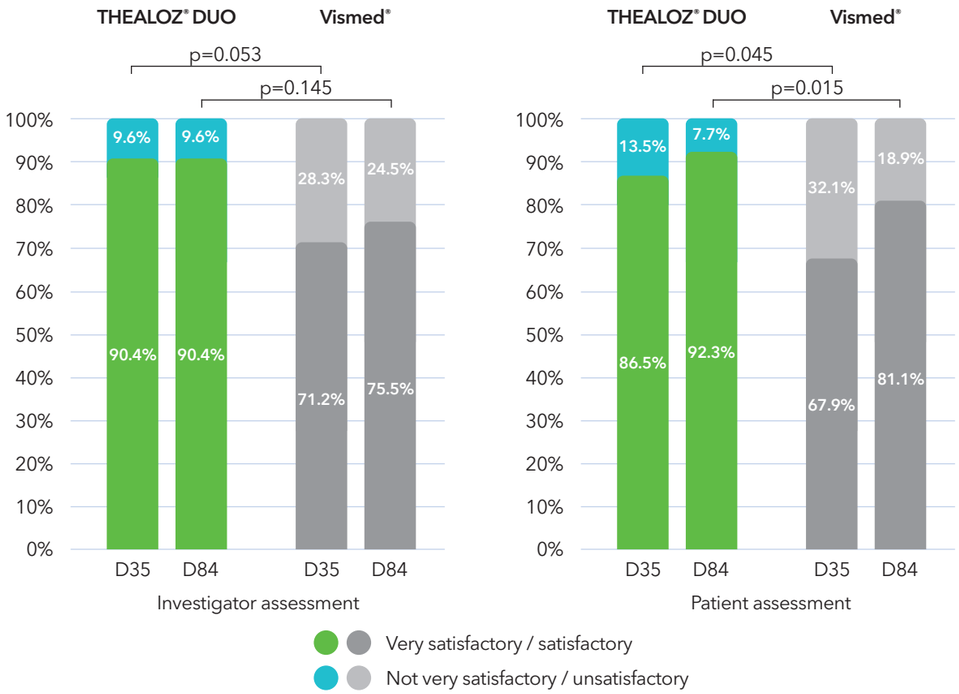


Figure 15: Global efficacy assessments by investigators and patients.

No serious adverse event linked to the treatments was reported during the study.

Key points

In this multi-centre, randomised, simple-blind, parallel group study:

- **THEALOZ® DUO is at least as effective, or even slightly more effective on some parameters, compared to 0.18% Sodium Hyaluronate, in vital staining, BUT, the Schirmer test, and ocular symptoms**
- **THEALOZ® DUO improves quality of life quantified by the OSDI score, significantly more than Sodium Hyaluronate**
- **Overall, THEALOZ® DUO is considered by the patients and the investigators to be significantly more effective**
- **Patient satisfaction is significantly better than existing HA**

2. Phase IV study comparing THEALOZ® DUO with Sodium Hyaluronate on tear film thickness[29]

Another randomised clinical study, conducted by Schmidl *et al*, compared THEALOZ® DUO (T3% + HA15) to Sodium Hyaluronate 0.15% (HA15, HYABAK®) with artificial tears (physiological serum NaCl, LARMABAK®)[29] given in a single dose on OCT-measured tear film thickness in 60 patients living with moderate Dry Eye Disease.

Tear film thickness was 2.4 to 2.6 ± 0.4 µm, depending on the inclusion groups, and as much as approximately 3 µm, ten minutes after instillation of THEALOZ® DUO and HA15; whereas no changes were observed with the artificial tears. This THEALOZ® DUO effect remained significant up to 2 hours after instillation, whereas it disappeared more rapidly with HA15.

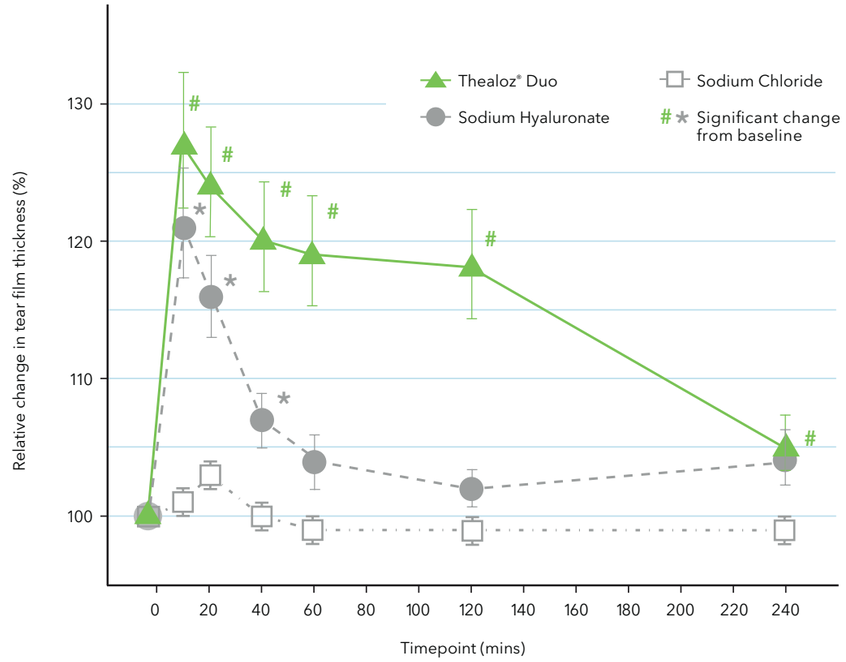


Figure 16: THEALOZ® DUO increases tear film thickness more effectively than HA15, up to 2 hours after instillation.

Key points

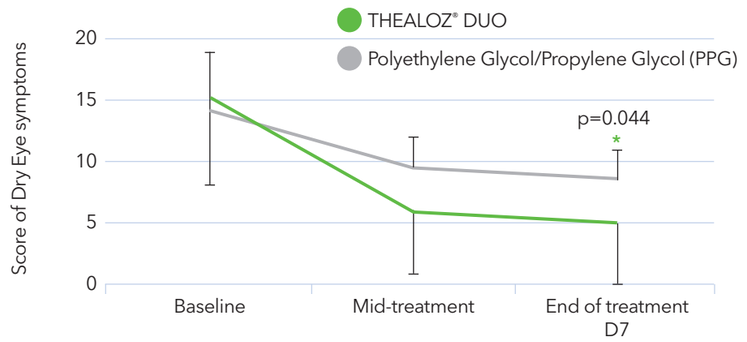
In this randomised parallel-group study on 60 patients, THEALOZ® DUO:

- **Increases tear film thickness to levels similar to Sodium Hyaluronate 0.15% in the early stages**
- **Maintains thickness of the tear film up to 120 minutes after instillation, whereas this film thickness shrinks rapidly with Sodium Hyaluronate**

3. Patient satisfaction study comparing emollient eye drops[30]

This prospective study was conducted as a randomised, open, cross-over investigation involving 17 patients suffering from moderate-to-severe Dry Eye Disease.[27] The patients received in each eye, 5 times a day for 7 days, one drop of THEALOZ® DUO or a lubricating eye drops containing a gelling agent (hydroxypropyl GUAR), and two emollients (polyethylene glycol 400 and propylene glycol) (SYSTANE, S).

The main evaluation criterion was patient satisfaction, measured by a 100 mm visual analogue scale (the main evaluation criterion). This measurement improved from 44.5 ± 19 to 70.2 ± 19.2 mm with THEALOZ® DUO and from 47.2 ± 23 to 57.1 ± 19.1 mm with the S. eye drops. The difference is statistically different in favour of THEALOZ® DUO (p=0.043).



17 adult patients with moderate-to-severe Dry Eye Disease were randomised to treatment with THEALOZ® DUO (combining Trehalose and hyaluronic acid) or PPG (Systane). Patients received 5 drops a day for 7 days of treatment.

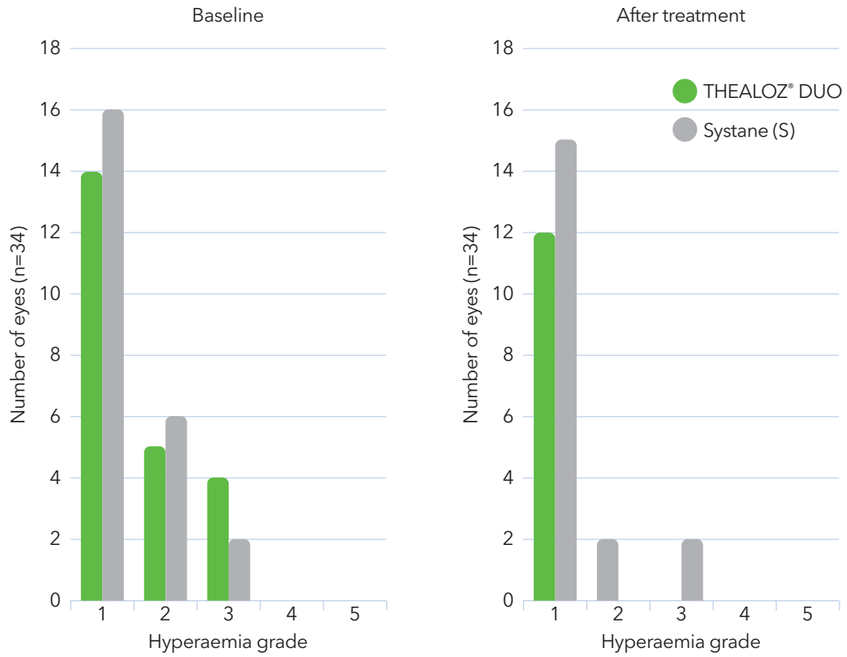


Figure 17: The OSDI score and hyperaemia are improved by THEALOZ® DUO.[30]

The symptoms linked to Dry Eye Disease were more significantly improved by THEALOZ® DUO, as well as their impact on professional life. Emotional impact, impact on daily activities and ocular relief were improved to a comparable degree by both treatments. There were also improvements in the Oxford score (staining), the Schirmer test, the TBUT and hyperaemia as a result of the two treatments, with no significant difference.

No adverse effects were reported.

Key points

In this randomised, open, cross-over study involving 17 patients:

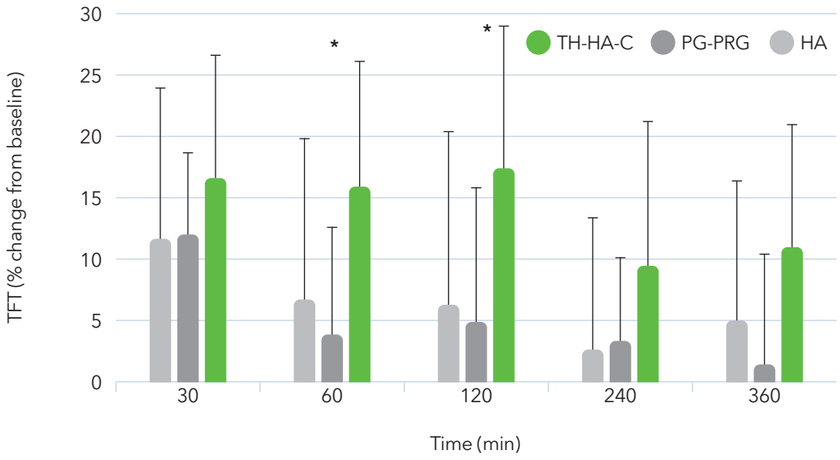
- **THEALOZ® DUO improves quality of life significantly more than a PEG/PPG combination: OSDI score, impact on day-to-day and professional life, emotional state, and eye relief**
- **Overall satisfaction is significantly greater with THEALOZ® DUO**

IV. Thealoz® Duo Gel: Clinical data

1. Comparative study of THEALOZ® DUO GEL versus hyaluronic acid or an emollient eye drops[31]

THEALOZ® DUO gel (T3% + HA 0.15% + Carbomer 0.25%) formulation in single-dose containers was compared by Schmidl *et al* to 0.2% Sodium Hyaluronate and a combination of hydroxypropyl GUAR + glycol 0.4% polyethylene + 0.3% propylene glycol (HYLOGEL®, SYSTANE GEL) in a randomised,[31] parallel-group, observer-masked trial, involving 60 patients suffering with Dry Eye Disease during a mean period of 7.6 ± 5.9 years. The included patients were required to have a TBUT below or equal to 10s, or Schirmer I test results between 2 and 5 mm, and an OSDI score above or equal to 22. The eye registering the shorter BUT was used for the study.

A single dose of each of the products studied was administered, after which tear film thickness was measured by OCT at instillation, and again after 10, 20, 40, 60, 120 and 240 minutes.



HA: Hyaluronic acid 0.2%, PG-PRG: Polyethylene glycol 0.4%, Propylene glycol 0.3%, TH-HA-Carbomer: Trehalose 3%, hyaluronic acid 0.15, carbomer 0.25.

Randomised, single masked, observer blinded parallel group design.

60 patients with history of DED for at least 3 months, BUT≤10s or Schirmer I test between 2 mm and 5 mm and OSDI≥22.

3 arms, patients received a single dose of either: Unpreserved Trehalose 3%+ HA 0.15% + Carbomer 0.25% (THEALOZ® DUO GEL) Hyaluronic acid 0.2% (Hylo Gel®).

Polyethylene glycol 0.4% + propylene glycol 0.3% (Systane Gel) Measurement of TFT.

*Significant vs baseline p<0.05

Figure 18: Relative changes from baseline of tear film thickness (TFT) over time after instillation of lubricant eye gels.

The results show a comparable increase in tear film thickness between the 3 groups in the early stages. However, from 60 minutes onwards, only THEALOZ® DUO GEL maintains a significant increase. The difference between the treatment groups is statistically significant (60 mm: p<0.021; 120 mm: p<0.037). No differences in BUT or Schirmer results were recorded, which is expected for a single-dose.

Hence, THEALOZ® DUO in gel form is an alternative or complement to the solution form, so that each patient is offered optimal ocular relief according to the situation (for example, at night) or the clinical circumstances.

Key points

In this randomised, parallel-group study involving 60 patients, THEALOZ® DUO GEL:

- **Achieves a comparable increase in tear film thickness compared with the comparison products (SYSTANE GEL, HYLO GEL®) up to 60 minutes**
- **However, only THEALOZ® DUO GEL maintains increased tear film thickness beyond 60 minutes**

2. Comparative study of THEALOZ® DUO GEL versus THEALOZ® DUO solution[32]

Forty patients with moderate-to-severe Dry Eye Disease were included in this randomised, cross-over study[29] and randomly followed two treatment sequences lasting one week each: either THEALOZ® DUO during the day and THEALOZ® DUO GEL at night; or THEALOZ® DUO GEL day and night.

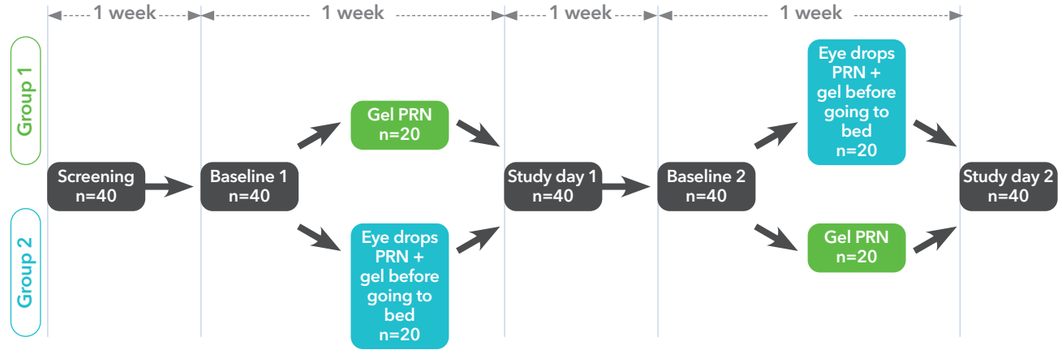
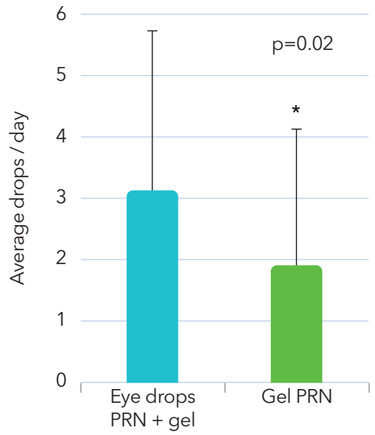


Figure 19: Study design[32]

The mean instillation frequency (the main evaluation criterion) was 3.1 ± 2.6 drops a day in the group using THEALOZ® DUO in the daytime; and 1.9 ± 2.2 drops a day in the group using THEALOZ® DUO GEL day and night (p=0.02).



N=40 patients with moderate-to-severe Dry Eye Disease. Randomised, observer-masked, cross-over study. Patients received either THEALOZ® DUO eye drops for use during the day combined with THEALOZ® DUO GEL before going to bed; or THEALOZ® DUO GEL only, to use as needed (PRN) for one week. Then, after another one week washout period, patients crossed over to the other treatment group.

*Significant vs baseline p=0.02

	Gel PRN		Drops PRN + gel before going to bed		p-value
	Baseline	Study day	Baseline	Study day	
Break-Up Time	3.5 ± 1.7	4.2 ± 1.7	3.4 ± 1.4	4.3 ± 1.9	*<0.001*
Corneal Fluorescein Staining	3.5 ± 2.3	2.7 ± 2.1	3.1 ± 1.7	2.2 ± 1.4	*<0.001*
Conjunctival Lissamine Green Staining	4.5 ± 2.8	3.5 ± 2.6	4.4 ± 2.5	2.8 ± 2.3	*<0.001*

Figure 20: Instillation frequency was significantly lower when using the gel compared to the artificial tears.

Significant lengthening of the BUT was observed in both groups, as well as a reduction in conjunctival vital staining, which was comparable in both groups.

Key points

In this randomised, cross-over study conducted on 40 patients with moderate-to-severe Dry Eye Disease, THEALOZ® DUO GEL:

- **Is as effective as THEALOZ® DUO on the objective signs of Dry Eye**
- **However, owing to a longer residence time linked to the presence of carbomers, THEALOZ® DUO GEL requires less frequent instillation**

Conclusion

THEALOZ® DUO is non-inferior to Sodium Hyaluronate on keratitis quantified by the Oxford staining score. THEALOZ® DUO is significantly more effective than Sodium Hyaluronate in relieving the severity of ocular symptoms. It is considered more effective than Sodium Hyaluronate by both the investigators and the patients. It improves tear film quality for longer periods than Sodium Hyaluronate and has a greater impact on quality of life than lubricating eyedrops and Sodium Hyaluronate. Owing to a longer precorneal residence time, due to the presence of carbomers, THEALOZ® DUO GEL increases tear film thickness for longer than THEALOZ® DUO or other tear substitutes, enabling a reduction in instillation frequency.

Study	Methodology	Comparison product	Results
Thealoz® Duo			
Chiambaretta 2016 Ref 28	A non-inferiority, multicentre, comparative, randomised, single-blind, parallel-group, 3-month study involving 105 patients with moderate-to-severe Dry Eye Disease.	Sodium Hyaluronate 0.18%	THEALOZ® DUO is at least as effective, or even slightly more effective in some parameters, as Sodium Hyaluronate 0.18% on vital staining scores, BUT, the Schirmer test and ocular symptoms. THEALOZ® DUO improves OSDI-quantified quality of life significantly more than Sodium Hyaluronate. THEALOZ® DUO significantly improves the symptoms and signs of Dry Eye Disease, without any significant difference when compared with Sodium Hyaluronate. Overall, THEALOZ® DUO is considered by the patients and the investigators to be significantly better.
Schmidl 2015 Ref 29	A randomised, single-dose, clinical study on OCT-measured tear film thickness, involving 60 patients suffering from moderate Dry Eye Disease.	Sodium Hyaluronate 0,15% Artificial tears	THEALOZ® DUO is similar to Sodium Hyaluronate 0.15% in increasing tear film thickness in the early stages, and maintains increased tear film thickness up to 120 minutes after instillation; whereas this thickness very rapidly diminishes with Sodium Hyaluronate.
Pinto-Bonilla 2015 Ref 30	A prospective, randomised, open, cross-over, 7-day study involving 17 patients suffering from moderate-to-severe Dry Eye Disease.	SYSTANE	THEALOZ® DUO is significantly better at improving quality of life than SYSTANE: OSDI score, impact on daily and professional life, and ocular relief. Overall satisfaction with THEALOZ® DUO is significantly greater.
Thealoz® Duo Gel			
Schmidl 2016 Ref 31	A randomised, parallel group, observer masked, single-dose study on OCT-measured tear film thickness involving 60 patients suffering from Dry Eye Disease	SYSTANE GEL	THEALOZ® DUO GEL is similar to the comparison products in increasing tear film thickness up to 60 minutes. But only THEALOZ® DUO GEL maintains increased tear film thickness *beyond* 60 minutes.
Schmidl 2016 Ref 32	A randomised, cross-over, 7-day study involving 40 patients suffering from moderate-to-severe Dry Eye Disease	THEALOZ® DUO GEL day + night vs THEALOZ® DUO in the daytime + THEALOZ® DUO GEL at night	THEALOZ® DUO GEL is as effective as THEALOZ® DUO on the objective signs of Dry Eye Disease. However, owing to a longer residence time linked to the presence of carbomers, THEALOZ® DUO GEL makes it possible for instillation frequency to be reduced.

Table VIII: Summary table of THEALOZ® DUO and THEALOZ® DUO GEL studies.

V. ABAK® system: Preservative-free

1. Impact of preservatives

Preservatives, especially benzalkonium chloride (BAK), have a harmful effect on the ocular surface.[33] They engage in non-specific biological activity aimed at destroying living cells by means of membrane solubilisation, increased ionic permeability and/or inhibition of cell metabolism. While this toxic effect is fortunately more pronounced in microorganisms, it is not non-existent in eukaryotic cells, particularly the very fragile, highly exposed cells of the cornea and conjunctiva. The toxicity of BAK has been demonstrated in numerous experimental and clinical studies: tear film instability, loss of goblet cells, rupture of the epithelial barrier, etc. The involvement of immunoinflammatory responses, with the leaching of proinflammatory cytokines, apoptosis, oxidative stress in addition to direct interactions with tear film and cell membrane lipid components, is clearly established.[33]

BAK is not the only compound at issue: other preservatives such as Polyquad are not toxicity-free, as underlined by the TFOS DEWS II report: "Some reports suggest that even these so-called 'disappearing preservatives' can show some negative effects on the ocular surface."[35] In fact, the overall damaging potential of commercial tear substitutes containing various preservatives was evaluated in an *in vitro* cultured corneal epithelial model, by acute and chronic administration (incubation for 24, 48 or 72H).[34] The results of this study indicate general toxicity in most of the preservatives, attended by a reduction in cell viability, increased interleukin 8 (IL-8) production, histological epithelial changes with damaging phenomena and tissue necrosis. The expression of occludin, a membrane protein associated with the tight junctions which when over-expressed represents an early marker of tissue damage, is increased in the early stages in the case of all preservatives, and is distributed in the epithelial layers.

It is important to note that the preservative-free products tested in this study do not produce any changes when compared to the control.

	24h	24h + 24h	72h
CONTROL	100% viability	100% viability	100% viability
ABAK®	Non-toxic	Non-toxic	Non-toxic
Perborate	Non-toxic	Non-toxic	Viability reduction (75%)
Polyquad	Non-toxic	Non-toxic	Viability reduction (70%)
Thiomersal	Non-toxic	Non-toxic	Toxic (residual 1%)
Oxyd®	Non-toxic	Viability reduction (residual 71%)	Toxic (residual 4.5%)
BAK 0.01%	Non-toxic	Severe viability reduction (residual 43% = toxic)	100% Toxic
BAK 0.1%	100% Toxic	100% Toxic	100% Toxic

Table IX: Effect of preservatives.[34]

The toxic effects of preservatives are time- and dose-dependent, and chronic use is generally inadvisable – more so where there are pre-existing alterations at the ocular surface,[33] as is the case in Dry Eye Disease.

To avoid preservatives toxicity, the TFOS DEWS II report recommends that 'ideally, all prescribed Dry Eye Disease products would be supplied in unit dose or unpreserved multi-dose bottles.'[7b]

For this reason, THEALOZ® DUO is preservative-free and packaged in an ABAK® bottle. The gel formulation of THEALOZ® DUO is available in single-dose containers, as carbomers do not allow the passage of membrane filters.

Key points

- **Preservatives, especially but not only benzalkonium chloride (BAK), have a harmful effect on the ocular surface**
- **The toxic effects of preservatives may be measured in vitro on cultured cornea epithelial cells by a reduction in cell viability**
- **The toxic effects of preservatives are time- and dose-dependent**
- **Preservatives are not recommended, especially in cases of pre-existing ocular surface alteration**

2. ABAK®: Sterile and preservative-free eye drops dispenser

The ABAK® bottle is an exclusive device developed by Théa. The bottle, with its fitted membrane filter to protect the solution from contamination by microorganisms, guarantees preservative-free solution sterility for up to 6 months, in addition to calibrating the drops dispensed.

The difficulty of instilling eye drops is a poor compliance factor limiting the therapeutic efficacy of treatment.[40,42-44] A French retrospective, multi-centric and transversal study involving 41 ophthalmologists with the aid of 654 patients, showed a good handling capability and overall acceptability of the ABAK® bottle compared to other methods of administration.[45]

Moreover, the ABAK® bottle is also economic: its drop-by-drop administration enables the patient to instil one drop after another, thus avoiding waste. A 10 mL ABAK® bottle contains 300 drops at least, or 150 instillations for 2 eyes, or the equivalent of 150 single-doses.

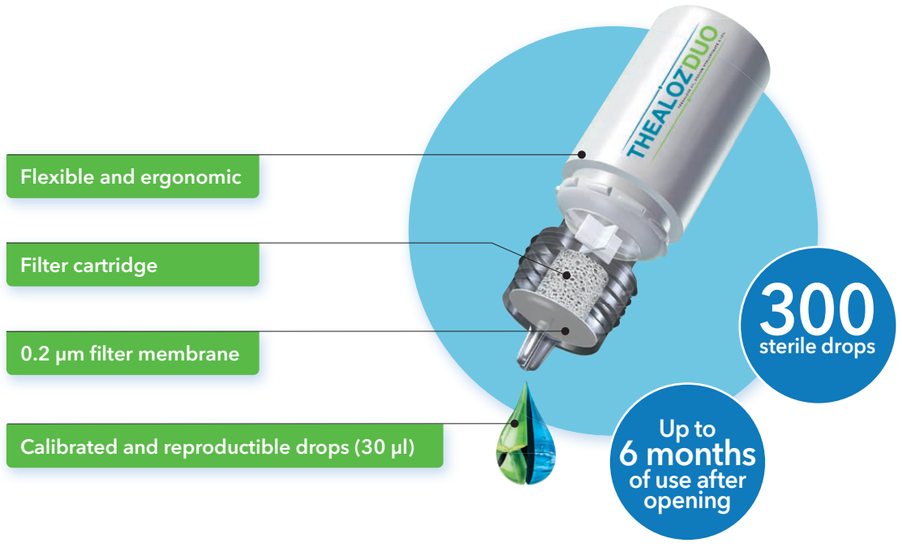


Figure 21: ABAK® system.

Key points

The ABAK® system is based on a membrane filter which:

- **Protects the eye drop from contamination by microorganisms**
- **Guarantees preservative-free eye drop sterility for up to 6 months**
- **Calibrates the drops dispensed**

The ABAK® bottle is patient-friendly: as small as a single-dose unit, in a supple, easy-to-use dispenser

Conclusion

THEALOZ® DUO is preservative-free, as should be all eye drops indicated in ocular surface diseases, to avoid the harmful effects of preservatives.[42]

THEALOZ® DUO is available in the ABAK® bottle, which guarantees sterility for up to 6 months and easy handling.

THEALOZ® DUO GEL is packaged in single-dose units and hence is also preservative-free.

VI. Conclusion: Thealoz® Duo meets all the requirements for an optimal Dry Eye Disease treatment

As stated by the 2017 TFOS DEWS II report, tear substitutes are a mainstay in DED (Dry Eye Disease) treatment.[7b] They aim not only to replace the deficient tear film with a lubricating effect, but also must target the etiopathogenic mechanisms involved in DED, i.e. reduce hyperosmolarity and inflammation.

THEALOZ® DUO introduces a new concept in DED management, by combining a well-known lubricant (Sodium Hyaluronate) with a natural bioprotective disaccharide: Trehalose.

The TFOS DEWS II report underlines the interest of this new formulation, which capitalises on both properties of lubricant and bioprotectant agents.[7b]

Indeed, according to the TFOS DEWS II recommendations, THEALOZ® DUO meets all the requirements for a tear substitute in DED management:

- *Lubricating effect with high stability, which mimics the normal tear film behaviour*
- *Ocular surface protection, by reducing the toxic pathophysiologic mechanisms that maintain the 'vicious circle,' i.e. hyperosmolarity and inflammation*
- *Long-acting*
- *Hypotonic*
- *Preservative-free*
- *Easy to use*

THEALOZ® DUO is currently one of the most advanced achievements in tear replacement for DED treatment.

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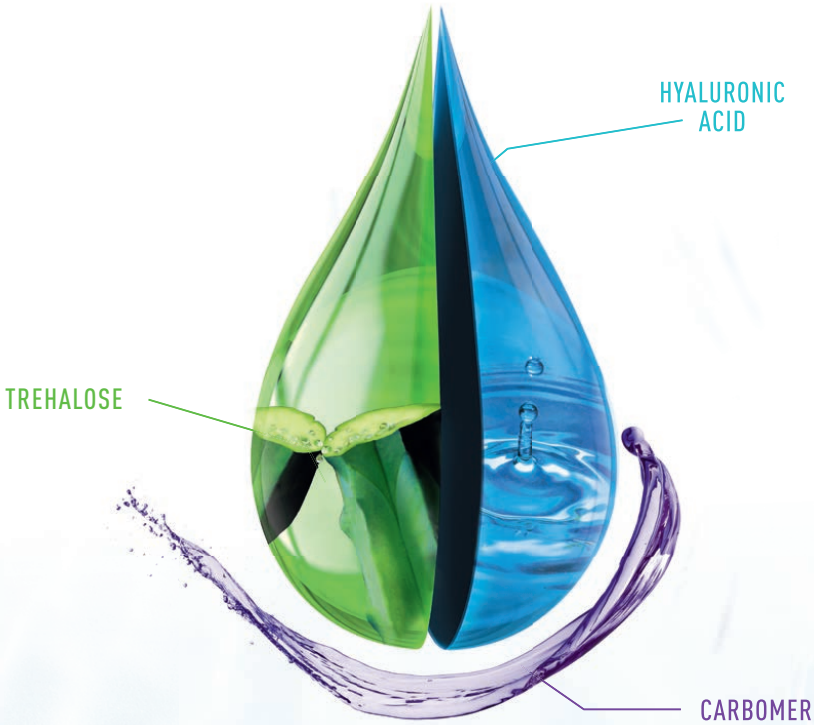

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