

EYESERYL[®]

REDUCE YOUR PUFFY EYEBAGS

CODE: PD070

Date: May 2007

Revision: 18

GENERAL DESCRIPTION

As skin loses its elasticity and muscles weaken through age, loose skin can accumulate around the eyes, forming folds in the eyelids. Fat, which cushions the eyes in their sockets moves forward out of the ocular cavities and accumulates in bulging bags around the eyelids.

Baggy eyelids are known medically as dermatochalasis and are commonly improved by performing a blepharoplasty, a surgical procedure which involves an inner or outer incision of the eyelid to extract the fat and excess skin. Blepharoplasty is the most common aesthetic procedure performed by plastic surgeons in America [Castro, E, Foster, JA (1999) Upper lid blepharoplasty. Facial Plast. Surg.15 (3): 173].

The other major reason for puffy eyes is water accumulation, known as eyelid oedema. Fluid may build up for several reasons, two of the major reasons being poor lymphatic circulation and increased capillary permeability.

EYESERYL[®] is a tetrapeptide with anti-oedema properties with a proven efficacy in reducing puffy eyebags.

PROPERTIES AND APPLICATIONS

- The draining effect of EYESERYL[®] achieves a reduction of puffy eyebags in only 15 days – fast anti-eyebag action

EYESERYL[®] can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where a reduction of puffiness under the eyes is desired.

TECHNICAL INFORMATION

PRODUCT SPECIFICATIONS

Code:	PD070
Appearance:	Transparent Solution
INCI name:	Water, Acetyl Tetrapeptide-5
Active ingredient content:	0.1% Acetyl Tetrapeptide-5
Solvent:	Demineralised water
Preservative:	0.3 % Phenonip

PROCESSING AND DOSAGE

EYESERYL[®] is presented as **EYESERYL[®] Solution**, an aqueous solution containing 1 g/L of the powder. It can be incorporated at the final stage of the manufacturing process, provided the temperature is below 40 °C. Taking into consideration the concentration of peptide in **EYESERYL[®] Solution**, it is recommended that 1 to 10% of the solution is present in the final formulation in order to obtain significant eyebag-reducing activity.

STORAGE AND SHELF LIFE

Keep in a clean, cool and dark place. If the product is stored as recommended it will remain stable for at least 24 months.

SAFETY

EYESERYL[®] has been tested in vitro for ocular irritation and is non irritant at the concentration of use. All the raw materials involved in the formulation are regarded as safe at the concentrations of use.

Results

The ACE inhibitory activity of EYESERYL[®] is shown in Fig. 1. EYESERYL[®] inhibited ACE activity at all tested concentrations, and the effects were dose dependent.

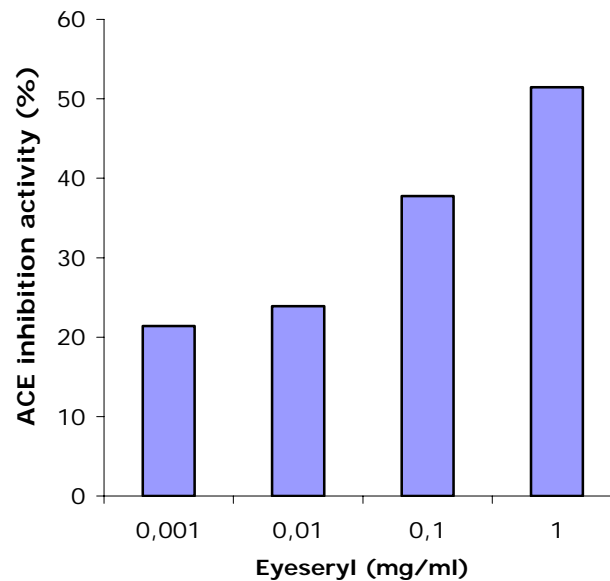


Figure 1. ACE inhibitory activity of EYESERYL[®]

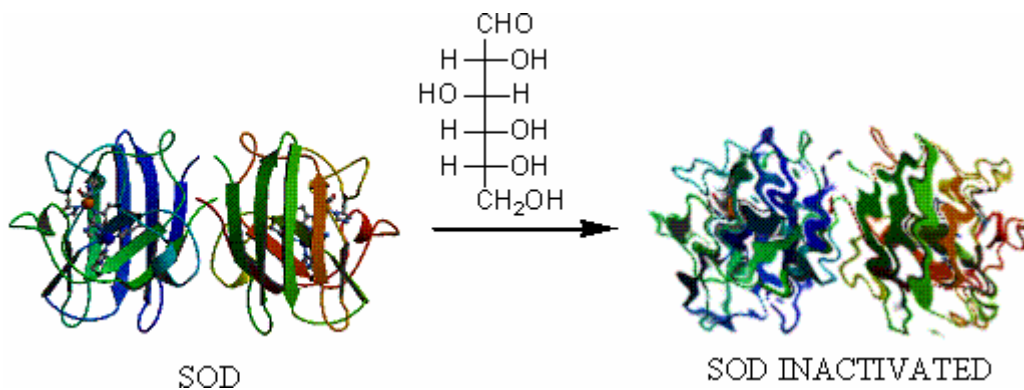
Glycation inhibitory activity of EYESERYL[®]

Glycation is a non enzymatic reaction between glucose or another monosaccharide and a protein. The aldehyde group of the monosaccharide reacts with the terminal amino groups or ϵ of the amines to produce a Schiff's base, forming fructosamine, or Amadori adduct.

At a second stage, the Amadori compound may react producing a group of irreversible reactions (oxidation, crosslinking...). Collagen cross-linking is one of the main causes of the formation of eyebags. This test was carried out to evaluate the ability of EYESERYL[®] to inhibit glycation.

It is known that some enzymes can suffer glycation *in vivo*. Among those enzymes we can find the Cu, Zn-Superoxide Dismutase (SOD). The SOD is an enzyme that converts superoxide radicals to hydrogen peroxide and oxygen.

The incubation of SOD with glucose or other monosaccharides gives rise to glycation, which inactivates the enzyme.

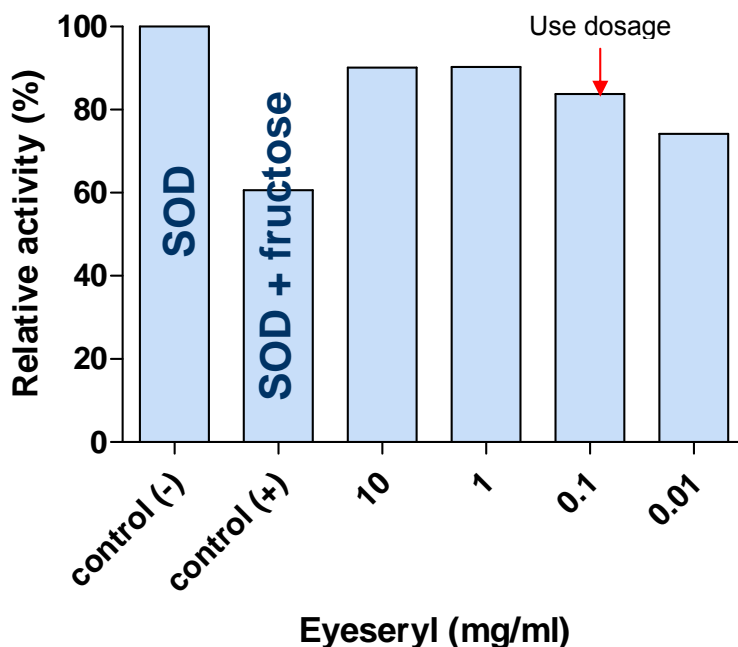


Some compounds can inhibit SOD glycation and, therefore, maintain its activity.

In this study, the inactivation of SOD by its reaction with fructose is used as a model of glycation. The effect of EYESERYL[®] as an inhibitor of glycation is evaluated. A method to assess the SOD activity by the inhibition of the transformation of xanthine to uric acid with the enzyme xanthine oxidase is used. With this reaction, the WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) is transformed to formazan, a compound which absorbs at 470nm. If SOD is added to this reaction, radical $O_2^{\cdot-}$ is captured and the formation of this coloured compound is avoided.

Results

The results show there is an increase in the SOD activity, which means that EYESERYL[®] inhibits its glycation.



Vascular permeability Assay Kit

The endothelial cell lining of the internal vasculature defines a semi-permeable barrier between the blood and the interstitial spaces of the body.

Endothelial cell monolayers cultured on semi-permeable membranes have been shown to form adherent tight junctions. The *in vitro* vascular permeability Assay Kit provides an efficient system for evaluating the effects of chemicals and drugs on endothelial cell adsorption, transport and permeability.

A multitude of vasoactive cytokines, growth factors, and signal modulators react with endothelial cell substructure components to control permeability. VEGF, Interleukin alpha and beta, TNF alpha, and IFN gamma have been shown to increase endothelial monolayer permeability.

The test is performed in a 24-well tissue culture plate with 12 cell culture inserts. The inserts contain 1.0 μm symmetrical pores within a transparent polyethylene membrane. The high pore density membranes permit amplified rates of basolateral diffusion with molecules of interest for permeability assays. The membrane is tissue culture treated on both sides for cell growth.

Endothelial cells are seeded onto collagen-coated inserts. The endothelial monolayer forms in several days, which occludes the membrane pores. The cell monolayer is then treated for 24h with EYESERYL[®] 1, 0.1 and 0.01mg/mL, or with Interleukin-1 β (100ng/mL), which due to its permeabilising properties is used as a positive control. Non treated monolayers are used as controls. After treatment, FITC-Dextran is added on top of the cells, allowing it to permeate through the cell monolayer. The extent of permeability can be determined by measuring the fluorescence of the plate well solution at 485 nm and 530 nm for subsequent time point readings (5, 10, 30 and 60 minutes).

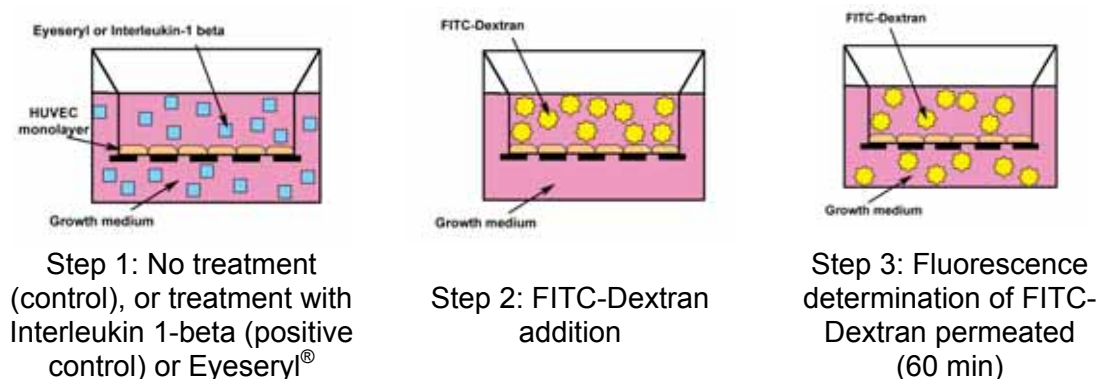


Fig. 2. Steps for the *in vitro* Vascular permeability Assay

Results

Results prove that EYESERYL[®] is able to inhibit vascular permeability in a dose-dependent manner, reaching a 50% inhibition respect to controls with 1mg/mL (0.1%) EYESERYL[®]. This activity *in vitro* would be translated *in vivo* in a decrease of water accumulation in eyebags, since EYESERYL[®] avoids water leakage from blood vessels.

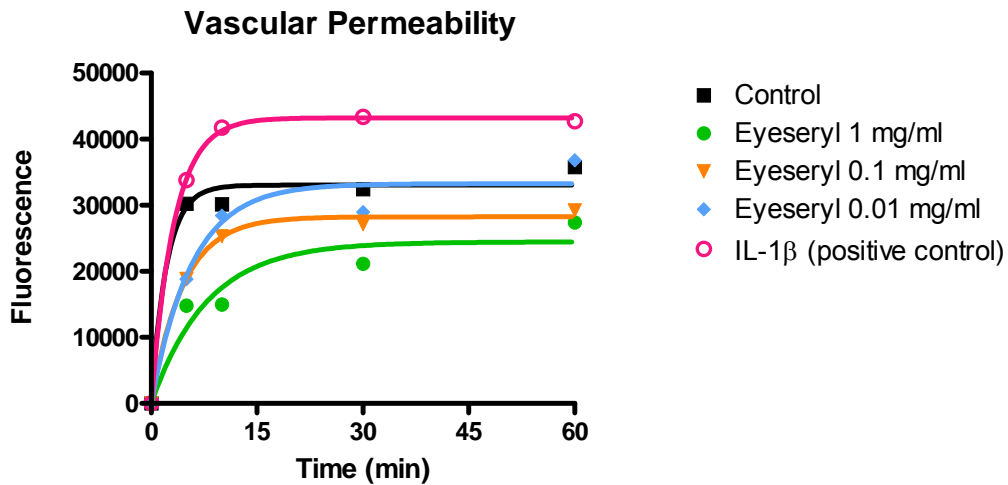


Fig. 3. FITC-Dextran across the endothelial cell monolayers

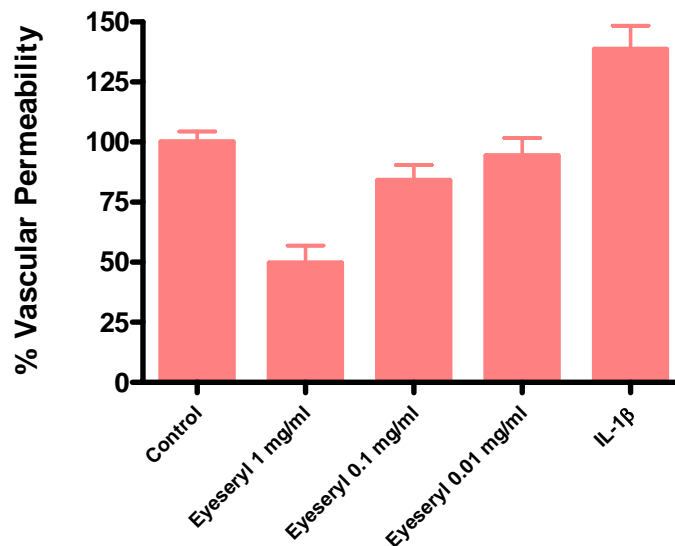


Fig. 4. Inhibition percentage of the vascular permeability 10 minutes after adding FITC-Dextran on the endothelial cell monolayers treated with EYESERYL[®] (1, 0.1 and 0.01mg/mL).

IN VIVO - Cream containing 10% EYESERYL[®] Solution

EYESERYL[®] has been tested in vivo on a group of 20 female volunteers, aged 18 to 65. A cream containing 0.01% EYESERYL[®] was applied twice a day during 60 days. Pictures were taken at 0, 15, 30, 45 and 60 days. Several parameters were measured instrumentally or visually and physically evaluated by a dermatologist.

Variation in the puffiness

A dermatologist assigned a score to the decrease in eye bag puffiness by compared to the picture taken at time 0:

- 1 – no reduction,
- 2 – slight reduction,
- 3 – fairly good reduction,
- 4 – good reduction

Volunteer A



Day 0



Day 15



Day 30

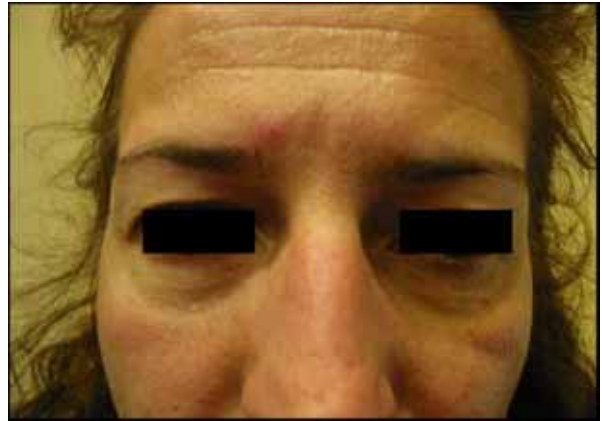


Day 45

Volunteer B



Day 0



Day 15



Day 30



Day 45

Volunteer C



Day 0



Day 15

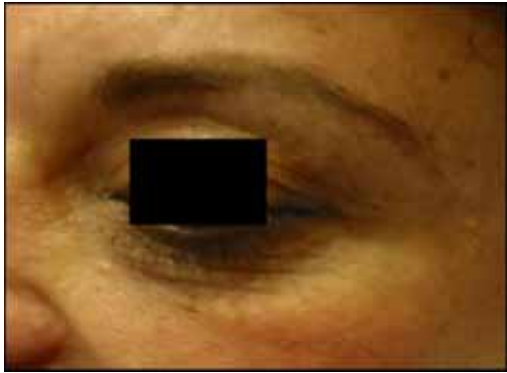


Day 30

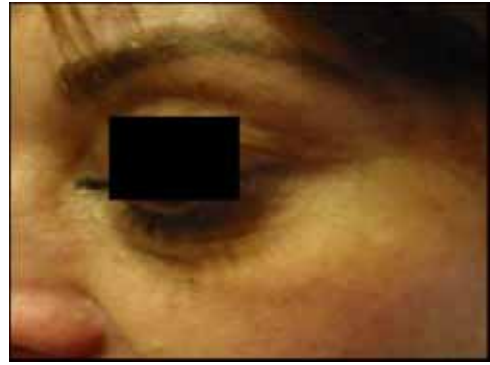


Day 45

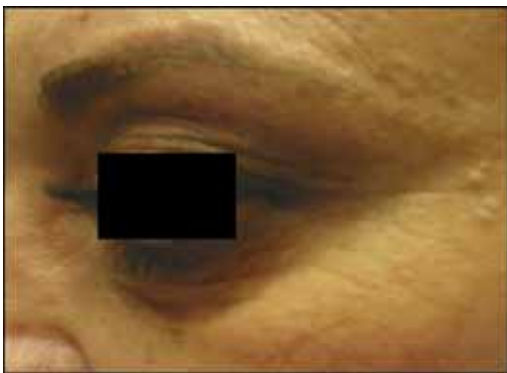
Volunteer D



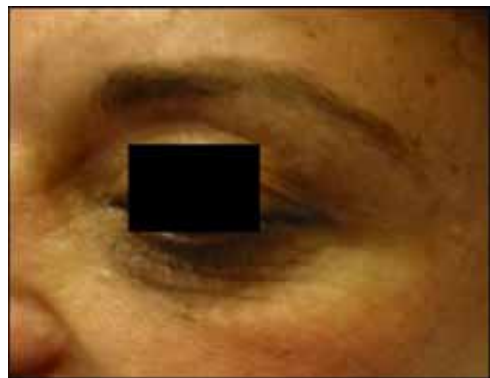
Day 0



Day 15



Day 30



Day 45



Day 60

Volunteer E



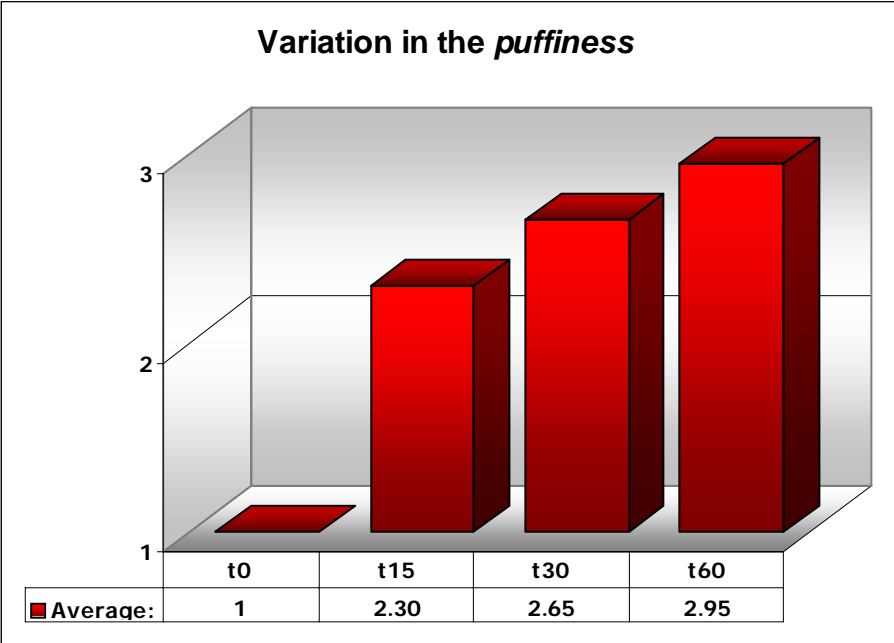
Day 0



Day 15

The scoring table of the dermatologist is enclosed, as well as the statistical analysis performed on the data. Since the measurements are non parametric (which means we don't know the underlying distribution of the variable measured), they are analysed using Friedman's Test.

Vol. Ref	Variation in the puffiness			
	t0	t15	t30	t60
01DV	Grade 1	Grade 1	Grade 2	Grade 2
02AZ	Grade 1	Grade 4	Grade 4	Grade 4
03GV	Grade 1	Grade 4	Grade 4	Grade 4
04VG	Grade 1	Grade 4	Grade 4	Grade 4
05MC	Grade 1	Grade 2	Grade 3	Grade 3
06GM	Grade 1	Grade 2	Grade 2	Grade 2
07RP	Grade 1	Grade 3	Grade 3	Grade 3
08AF	Grade 1	Grade 3	Grade 3	Grade 3
09GC	Grade 1	Grade 3	Grade 3	Grade 4
10VA	Grade 1	Grade 1	Grade 2	Grade 2
11LS	Grade 1	Grade 2	Grade 2	Grade 3
12MC	Grade 1	Grade 3	Grade 3	Grade 4
13LD	Grade 1	Grade 3	Grade 4	Grade 4
14RS	Grade 1	Grade 1	Grade 2	Grade 3
15CS	Grade 1	Grade 3	Grade 4	Grade 4
16EP	Grade 1	Grade 2	Grade 2	Grade 3
17AS	Grade 1	Grade 1	Grade 1	Grade 2
18MA	Grade 1	Grade 2	Grade 2	Grade 2
19OC	Grade 1	Grade 1	Grade 2	Grade 2
20TD	Grade 1	Grade 1	Grade 1	Grade 1



Puffiness under the eyes is greatly reduced, even after only 15 days: 70% of the volunteers had improved at Day 15.

Applying Friedman's test, the data for every timepoint is determined to be significant, according to the following table:

Friedman Test	t15-t0	t30-t0	t60-t0
sign. 95% val > 1.076959	1.30	1.65	1.95
	yes	yes	yes

Conclusions

Puffiness under the eyes is greatly reduced, even after only 15 days! - 70% of the volunteers had improved at Day 15.

At the end of the test, 95% of the volunteers had improved:

- ✓ 30% showed a slight improvement (grade 2)
- ✓ 30% showed a fairly good improvement (grade 3)
- ✓ 35% showed a good improvement (grade 4)

Skin elasticity

The elasticity measurements are performed with a Cutometer®. In elastometric measurement, the skin surface is aspirated from the depression induced by the machine into the aperture of the elastometer's measuring probe. The depth of the skin penetration inside the probe is measured by an optic sensor. Cutaneous elasticity reflects the skin's potential capacity (measured in mm) for retraction.

Cutaneous elasticity is defined as the ratio:

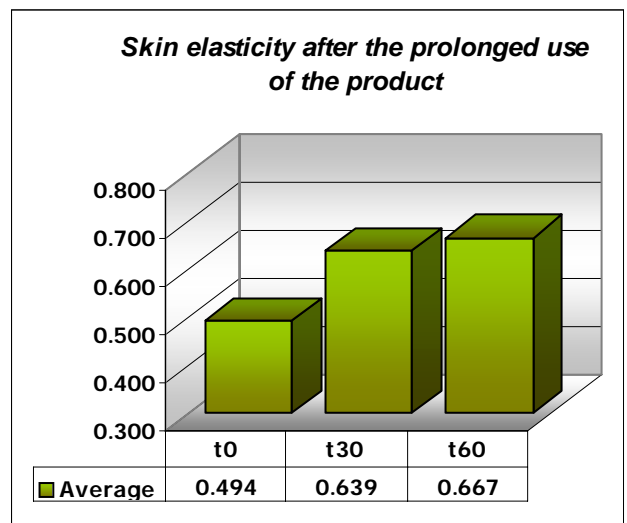
$$\text{Elasticity} = \frac{U_f - U_a}{U_f}$$

U_f = skin extensibility

U_a = residual deformation

Elasticity represents the recovery degree of the maximum deformation reached, whose values range between 0 and 1 (maximum elasticity). The experimental measurements, as well as the statistical analysis performed on the data, are shown below.

Vol. Ref	Skin elasticity after the prolonged use of the product		
	t0	t30	t60
01DVD	0.451	0.504	0.518
02AZ	0.529	0.721	0.729
03GV	0.543	0.667	0.681
04VG	0.513	0.740	0.766
05MC	0.418	0.667	0.679
06GM	0.576	0.731	0.739
07RP	0.538	0.687	0.704
08AF	0.455	0.727	0.734
09GC	0.667	0.722	0.734
10VA	0.444	0.564	0.574
11LS	0.444	0.618	0.645
12MC	0.452	0.538	0.604
13LD	0.533	0.724	0.745
14RS	0.423	0.625	0.645
15CS	0.524	0.600	0.687
16EP	0.462	0.545	0.574
17AS	0.501	0.530	0.578
18MA	0.324	0.401	0.521
19OC	0.500	0.714	0.728
20TD	0.576	0.750	0.763
Average	0.494	0.639	0.667
Dev. STD	0.0796725	0.0962711	0.0751718
Test t student	sign 95% (val. est. + -2.09)	5.1423637	7.0593276
		yes	yes



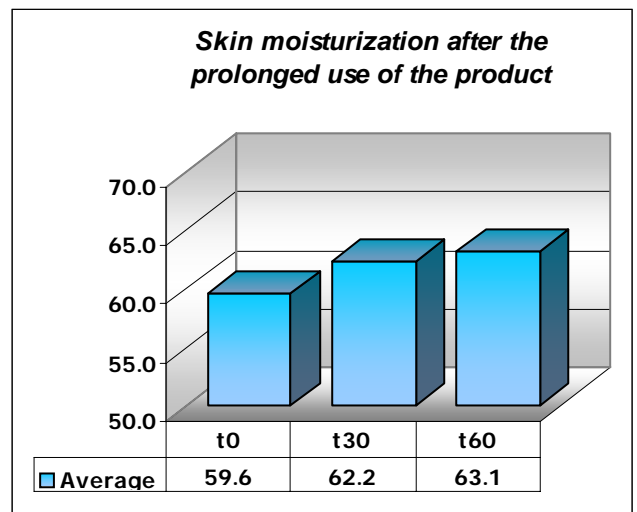
Skin elasticity, as measured with a Cutometer®, had a 30% increase after 30 days.

Skin moisturization

The moisturization measurements are performed with a corneometer (SKINLAB®). Corneometry measures how skin moisturization changes. The higher the water content of skin, the lower the skin dryness. Corneometry is an indirect method to measure skin moisturization. It is based on the electric properties of skin surface. Indeed both electric capacity and the conductance of biological tissue change according to the water content, i.e. they increase if the water content increases.

The experimental measurements, as well as the statistical analysis performed on the data, are shown below.

Vol. Ref	Skin moisturization after the prolonged use of the product		
	t0	t30	t60
01DVD	54.3	55.1	55.4
02AZ	56.2	57.1	59.1
03GV	56.8	62.4	63.1
04VG	61.4	63.4	63.6
05MC	58.7	62.4	63.4
06GM	64.3	64.8	65.3
07RP	58.4	62.3	64.0
08AF	59.8	61.5	62.1
09GC	60.2	61.8	62.2
10VA	63.5	64.1	65.3
11LS	65.1	65.8	66.2
12MC	54.4	56.1	57.3
13LD	57.4	64.1	65.1
14RS	56.4	63.4	63.8
15CS	61.0	62.9	64.1
16EP	59.6	62.1	62.8
17AS	57.4	59.1	60.9
18MA	64.3	66.1	67.2
19OC	56.4	62.3	62.8
20TD	65.4	68.0	68.4
Average	59.6	62.2	63.1
Dev. STD	3.368331	2.6273573	2.468203
Test t student	sign 95% (val. est. + -2.09)	2.7448182	3.2230678
		yes	yes



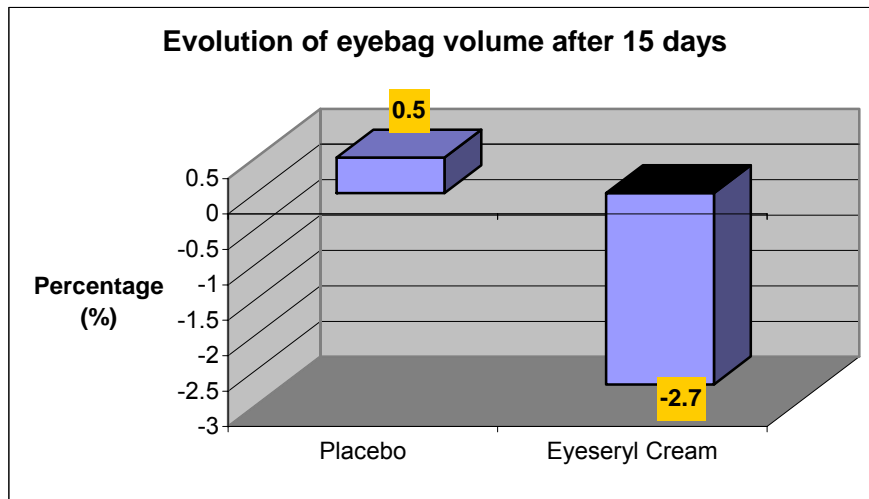
Skin moisturization, as measured with a corneometer (SKINLAB®), is significantly increased after 60 days.

IN VIVO – Cream containing 1% EYESERYL® Solution

Test was performed on 17 people, aged 34 to 54, using a cream containing 0.001% EYESERYL®, applied twice daily during 28 days.

Variation in the puffiness

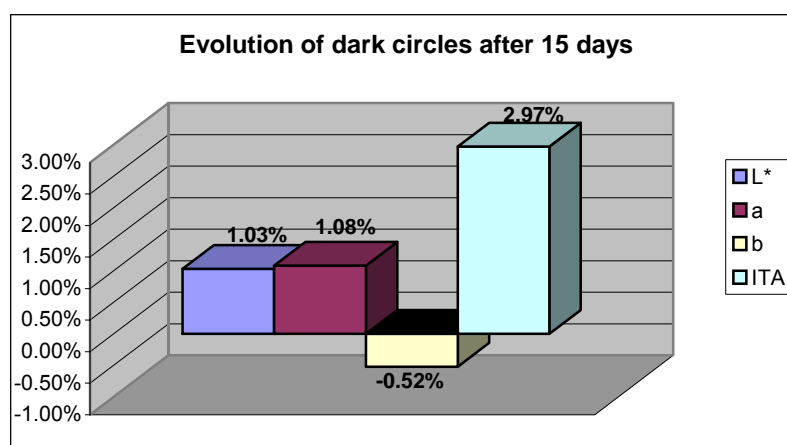
Eyebag volume was measured using a technique called Fringe Projection where 3D images of the study areas are obtained with a FaceScanner and processed with the software Optocat (Breuckman, Germany – EoTech, France).



After 14 days, 63% volunteers had reduced their eyebags
After 28 days, 70% volunteers had reduced their eyebags
Eyebags were significantly reduced by almost 3% in only 15 days!

Anti dark-circle effect

High resolution photographs of the dark circles were taken under polarised light and chromametry studies were performed



The ITA° (Individual Typological Angle) categorises skin colour :
Brown < 10° < tanned < 28° < normal < 41° < light < 55° < very light

ITA significantly increased (+3%) showing a slight lightening effect (at T14 and T28)

L* is luminance which represents relative brightness from total darkness ($L^*=0$) to absolute white ($L^*=100$), while a* is red/green colour axis and b* is yellow-blue colour axis.

L* was significantly increased at T14 (+1%)

Conclusion

Both results (eyebag volume and dark circles) are moderate but significant. These tests were done at a very low concentration (1% EYESERYL[®]) in order to define a minimum concentration for which an effect is measured.

GENERAL PRODUCT INFORMATION

Trade name	EYESERYL [®]
Product code	PD070

INGREDIENTS

INCI name	CAS No	EINECS No
WATER (AQUA)	7732-18-5	231-791-2
ACETYL TETRAPEPTIDE-5	820959-17-9	N.L. ^a
PHENOXYETHANOL	122-99-6	204-589-7
METHYLPARABEN	99-76-3	202-785-7
BUTYLPARABEN	94-26-8	202-318-7
ETHYLPARABEN	120-47-8	204-399-4
PROPYLPARABEN	94-13-3	202-307-7
ISOBUTYLPARABEN	4247-02-3	224-208-8

^a Not Listed

Note: Graphs and photographs are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lipotec. Customers are responsible for compliance with local and international advertising regulations.

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