[F-EYE CONTOUR]

Product identity

Eye contour is the ultimate treatment to achieve the best results in a shortest time. Each vial contains only the pure active ingredients, without any preservatives or other chemicals. The eye contour respond and prevent all concerns for this sensitive area in one product: eye bags, dark circles, wrinkles, flaccidity and dryness. No need to look further for the ultimate solution. Pal-GHK, Pal-GQPR and low molecular weight hyaluronic acid reinforce firmness, moisture and reduce wrinkles. Chrysin and N-hydroxysuccinimide activate the elimination of blood originated pigments responsible for dark circle colour and local inflammation. Organic silicon, cynara & ginkgo extract helps to eliminate the accumulated liquids & fat forming the eye bags. A unique synergy to achieve a beautiful, enlightened eye contour.

Benefits

- Reduces the appearance of dark circles.
- Eliminates accumulated liquids & fat to reduce eye bags.
- Reduces deshydratation wrinkles around the eyes.
- Firms the eye contour.
- Blocks free radicals.
- Fortifies the blood vessels & microcirculation.
- Moisturizes.

Active ingredients

- Palmitoyl Oligopeptide/Palmitoyl Tetrapeptide-7.
- N-hydroxysuccinimide (NHS).
- Chrysin (flavonoïd).
- Hyaluronic acid low molecular weight.
- Organic silicon.
- Ginkgo biloba & cynara extract.

Formulation specificities

- Sterilized.
- No paraben, alcohol, fragrance, animal origin ingredients, colouring and silicone.
- Non-animal tested.

User indications

- Topical application.
- Skin needling.
- Needle-free mesotherapy.
- Iontophoresis.
- Electroporation.

Can be mixed with:

10% to 50% of F-HA+ (for more viscosity and moisture).

Injections remain under the full responsibility of the practitioner. The manufacturer or distributor can not be held liable for any kind and in any cases of damages caused to third parties, or adverse effects. The products are dully registered as topical use only.

About EYE CONTOUR system

Presentation:
Fragile and sensitive, the zone around the eyes is a visible reflection of our tiredness, worries, stress and sleepless nights. The eye contour system is an exclusive synergy to globally rejuvenate the eye contours. It has a multi-action on all the main concerns of this delicate and sensitive area. It avoids eye bags & dark circles formation, the loss of firmness, wrinkles formation and dryness.

Focus on eye bags & dark circles:
The fineness of the epidermis of the inner canthus of the eye reveals a zone that is strongly perfused by blood micro vessels. Everyone agrees that dark circles under the eyes are directly associated with the status of the underlying vascular network: slow microcirculation results in less oxygenated blood and hence a bluish appearance. This contributes to formation of the unsightly dark circles under the eye and dulls the regard. Dark circles are considered different since they are darker than the complexion of the remainder of the face. They are mainly due to *local cutaneous deposit of pigments and iron* resulting from the degradation of hemoglobin. Less elastic and less impermeable vessel walls, locally create dilatation and blood stasis with local vascular excess pressure promoting leakages.

- Leakage of *plasma* produce *under eyes bags* if lymphatic drainage is unable to evacuate the local excess fluid.

- Leakage of *erythrocytes* produce *under eyes dark circles.* Outside of the vascular media, the cells burst releasing hemoglobin. The degradation process of hemoglobin is called «hemolysis» and generates pigments that are only sparingly soluble (need to be paired to UGRs enzymes to be eliminated) and which accumulate in the dermis and epidermis. We are familiar with these degradation products also observed after a bruise, into the surrounding tissues. The bruise or hematoma passes through several different colours: red (hemoglobin), violet-green (biliverdin), brownish-red-orange (bilirubin & inorganic ion), violet (hemosiderin) before disappearing after a few days eliminated by UGTs enzymes **(Figure 1: degradation pathway of hemoglobin).**

Over time, the dark circles become permanent and are very difficult to attenuate even after reactivation of the microcirculation. Vessel fragility is thus strongly involved in the formation of dark circles (blood cell leakage) and bags (plasma leakage). However, some subjects present with bags but no dark circles, and others with dark circles but no bags.

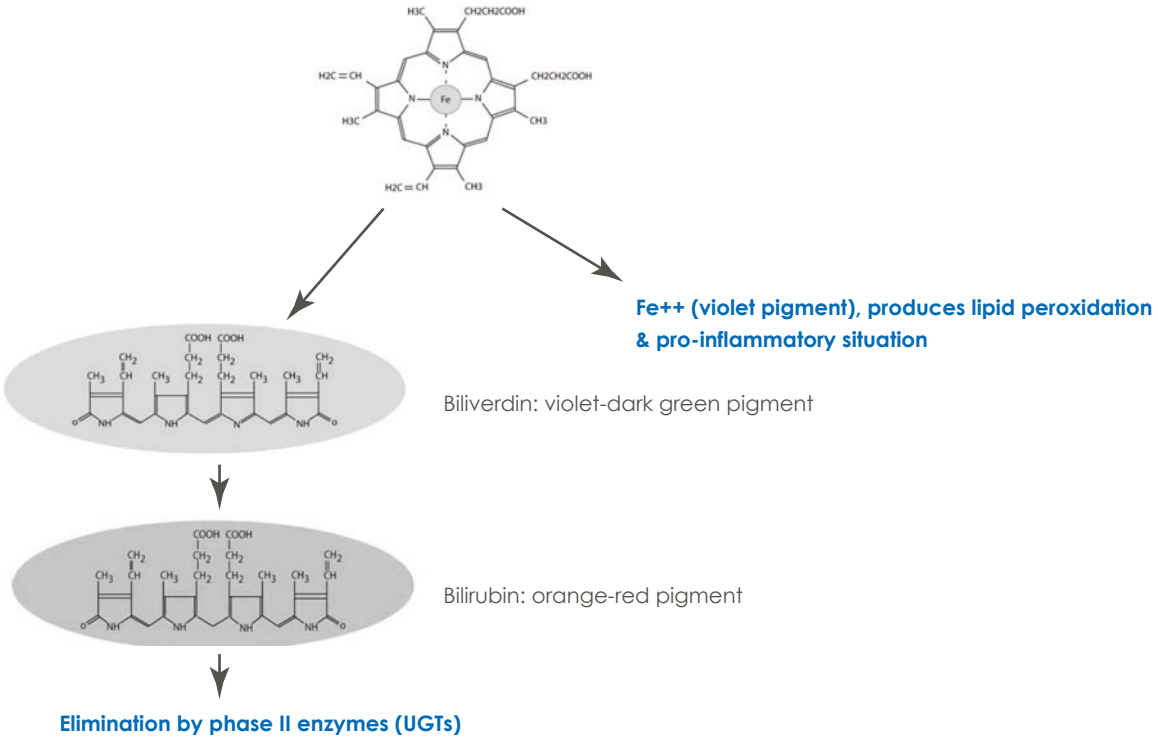


Figure 1: degradation pathway of hemoglobin.

Chrysin (Chrysin):
It is a flavonoid, relatively common in the plant kingdom, that will activate the elimination of locally accumulated bilirubin via cutaneous UGT_1A_1. UGT are enzymes expressed by both keratinocytes and fibroblasts. These enzymes are key detoxification enzymes (known as phase II enzymes) for a large number of exogenous compounds. The enzymes bind the compound, which is generally lipophilic, with glucuronic acid, thus rendering it hydrophilic, transportable and excretable by the urinary route. This process is known as "glucuroconjugation".

N-hydroxysuccinimide (N-hydroxysuccinimide):
It is a chelating agent bounding the local iron load to avoid the formation of toxic inorganic deposit or fixation to hemosiderin, which is very insoluble. Iron has a well-known pro-oxidant characteristic with respect to lipid peroxidation and pro-inflammatory situation inducing vasodilatation and a supplementary blood leakage from the vessels that turns brown over time.

In order to obtain the best results for both these indications we used in synergy: Chrysin and N.hydroxysuccinimide.

Focus on around the eyes loss of firmness, dryness and wrinkle formation:
Another main concern regarding eye contour area is the loss of firmness, dryness and wrinkle formation. But it´s closely linked to eye bags and dark circles. The degradation of the extra cellular matrix accentuates the local vascular leakages, and vice versa, the inflammation produced by the vascular leakage and release of iron accentuates the degradation of collagen. In order to complement and offer a global eye rejuvenation solution, it´s necessary to fortify the dermal matrix that maintains the micro vascular network. This is one reason we added in our exclusive eye contour system 2 lipopeptides Pal-GHK, Pal-GQPR & low-molecular-weight hyaluronic acid and organic silicon.

Lipopeptides Pal-GHK & Pal-GQPR (Palmitoyl oligopeptide, Palmitoyl tetrapeptide-7):
They act in synergy to repair the cutaneous damages of age as messengers of cutaneous restructuration and repair. They activate the neosynthesis of extracellular matrix macromolecules providing a visible anti-wrinkle efficacy. They protect the collagen and other proteins from glycation and degradation. The dermal matrix supporting the microvascular network is regenerated.

Low-molecular-weight hyaluronic acid (Sodium hyaluronate):
Provides a strong moisturizing effect for the eye contour area. Low-molecular-weight hyaluronic acid enables the skin to maintain skin homeostasis. It reinforces tight junctions between keratinocytes, thus limiting transepidermic water loss, thanks to its natural hygroscopic properties; it forms a "water reservoir" in the dermis.

Organic silicon (Methylsilanol mannuronate):
It will protect the cellular membrane from lipid peroxidation (cf Time Exception system), reduce inflammation and as detailled further act on the dermal matrix.

Levels of activity:
- Eliminates peri-orbital coloration by enhancing the efficacy of the natural elimination systems for biliverdin/bilirubin and iron.
- Strengthens skin density in order to better support the microvascular network perfusing the zone.
- Improves the skin tonicity and elasticity by restructuring and protecting the extracellular matrix.
- Reduces wrinkles.
- Moisturizes.
- Cytostimulation and oxygenation (the products contain oxygenation factor).

Clinicals:

Study of the induction of UGTs activity by chrysin.

Cultured cells express a basal level of UGT activity that can be indirectly measured by quantifying the mRNA coding for those enzymes. When cultured for a few days with an inductor, in this case chrysin, the cells increase mRNA synthesis and the increase may be quantified by the now classic quantitative RT-PCR method. Human hepG2 cells, a line expressing all the isoforms of UGT, were selected for the study and the quantification of UGT1A1 mRNA was performed after 3 days incubation.
A very marked increase in mRNA was observed in the presence of chrysin with a very clear dose effect: while the effect was not yet significant at 3.9 µM, a 2.5-fold increase was obtained at 7.8 µM (+347%) and a 6-fold increase was obtained at 11.8 µ M (+600%). Chrysin is thus an excellent inducer of UGT1A1, the enzyme catalyzing bilirubin conjugation.

Demonstration of the competitive effect of N-hydroxysuccinimide vs ferrospectral on iron.

To start with, we selected an iron-chelating agent with sufficient affinity not to be involved in the chelation of other cations. Thus, N-hydroxysuccinimide was selected. This compound is able to compete with Ferrospectral, a specific substrate for iron, developed by MERCK for the assay of iron traces in water.
In the presence of iron, Ferrospectral forms a Ferrospectral-iron complex with a violet color (cell T). N-hydroxysuccinimide acts as a competitor while the Ferrospectral and the Ferrospectral-iron complex, the violet colour is less pronounced (cell E1). N-hydroxysuccinimide completely antagonizes the reaction (cell E2). 0.1% of N-hydroxysuccinimide formed complexes with the blood iron equivalent to a volume of 100 mL.

Blank **T** **E1** **E2**

Figure 2: demonstration of the competitive effect of N-hydroxysuccinimide vs. ferrospectral on iron.

Study of the anti-inflammatory activity.

If iron complexion and elimination of excess bilirubin occurs, a positive contribution of the product to the known pro-inflammatory effects of iron and bilirubin is to be expected. Anti-inflammatory activity reduces micro vessels vasodilatation and decreases the local leakage of erythrocytes. Prostaglandins PGE2 are conventionally considered as mediators of inflammatory stress, particularly following UV-radiation exposure.
Human keratinocytes and fibroblasts were cultured in an appropriate medium in the presence of various concentrations of Eye Contour system for 24 hours. The cells were then transferred to a product-free medium and exposed to a pro-inflammatory dose of UVB radiation at a dosage of 35 mJ/cm2 for fibroblasts and 30mJ/cm2 for keratinocytes. Following irradiation, the cells were then post-incubated either in medium alone or in medium containing various concentrations of Eye Contour system, vs. a positive control (aspirin) for 24 hours. PGE2 release into the culture medium after 24 hours was determined using an ELISA method. **(Figure 3: Study of the variation of PGE2 release 24 hours after UVB irradiation of keratinocytes and fibroblasts).**

	KERATINOCYTES	**FIBROBLASTS**
Aspirin (acetylsalicylic acid)	**-92%**	**-95%**
Eye Contour system 3%	**-86%**	**-92%**

Figure 3: study of the variation of PGE2 release 24 hours after UVB irradiation of keratinocytes and fibroblasts.

As expected, aspirin was an excellent anti-inflammatory inducing almost total inhibition of PGE2, greater than 90% for both cell types. Eye Contour system exerted a marked effect up to 85% inhibition of keratinocytic PGE2, with the fibroblasts, the efficacy regularly increased, reaching 92% at the highest concentration (3%). The anti-inflammatory efficacy of Eye Contour system is thus very similar to that of aspirin.

Clinical study of the decrease in pigmentation of the rings under the eyes.
The efficacy study of Eye Contour system, vs. placebo, was conducted in a group of female volunteers presenting with violet rings but no bags under their eyes. The method used was image analysis, conducted on photographs taken under standardized conditions. The photographs were taken with a digital camera. The analysis of image color was conducted using an image processing program, which determined the parameters R (red), G (green) and B (blue). These parameters were converted to L, a* and b* parameters, using an analysis program.

The volunteers applied a mild cream-gel to each half of the face. A placebo cream-gel was applied to the left side and 2% Eye Contour system to the right side. Applications were conducted twice daily for 56 days. Each subject acted as her own control. Each subject's ring was characterized by the differential of parameters ΔL, Δa* (red) and Δb* (blue), an anti dark circle product should increase parameters ΔL and Δb* and decrease Δa*.

A significant difference was obtained for the improvement in parameters Δa* and Δb* on the treated side with -12.5% and +10% of mean change (19,5% and 19% of maximum change), in the expected direction. The changes on the placebo side, pre- vs. post-treatment, were non- significant. ΔL showed very little variation and the changes were not significant for either side. This marked change in Δa* reflected a less red circles and the marked change in Δb* reflected a less blue circles. Overall, the violet (red + blue) appearance was significantly alleviated. This was the expected effect required of a product treating or reducing the appearance of rings. The following example illustrates the effect obtained after 2 months of use of Eye Contour system **(Figure 4: dark circle reduction in vivo).**

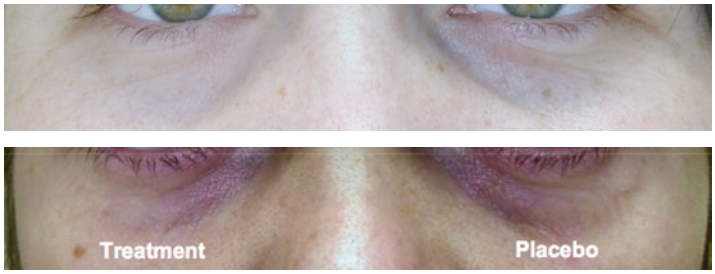


Figure 4: dark circle reduction in vivo.

Stimulation of the synthesis of matrix macromolecules (Collagen I, fibronectin, hyaluronic acid) by lipopeptides.
Study of the stimulation of synthesis of extracellular matrix components by fibroblasts incubated for 72 hours with *lipopeptides* (1, 3, 5%). As we can see on the below figure, the lipopeptide have a strong stimulating effect on the 3 tested matrix macromolecules with up to 65% improvement for fibronectin.

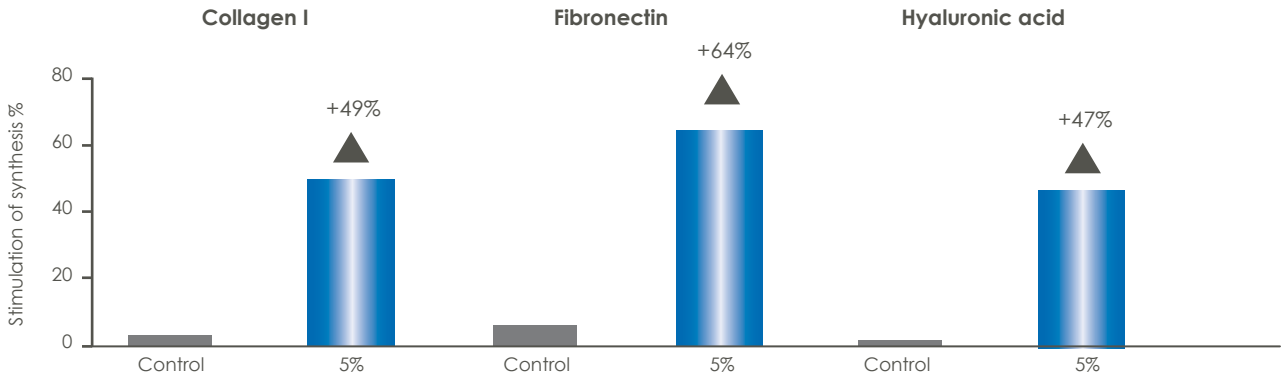


Figure 5: synthesis of ECM macromolecules.

Improvement of the skin tonicity and elasticity by lipopeptides.

2 groups of 23 volunteers aged between 39 and 74 years / Twice daily application to one half of the face a cream containing 3% of lipopeptides against placebo, for 2 months. Assessement of elasticity and tone by cutometry on the same group as above. With lipopeptides, skin tone and elasticity displayed a major and significant improvement up to 20% in the case of the skin tonicity and 10% for skin elasticity **(Figure 6: improvement of skin tonicity and elasticity)**.

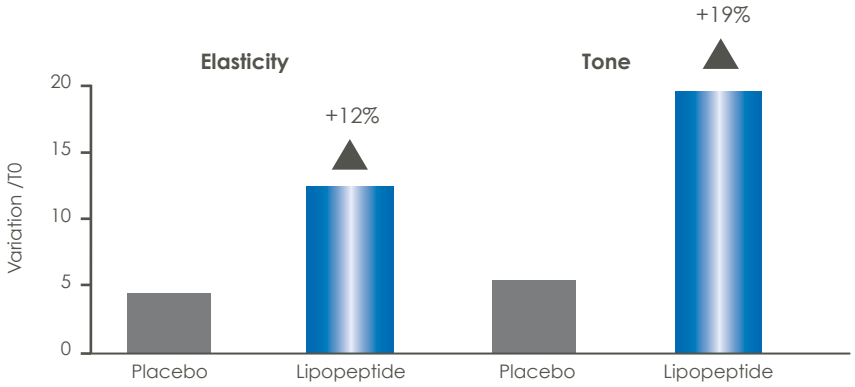


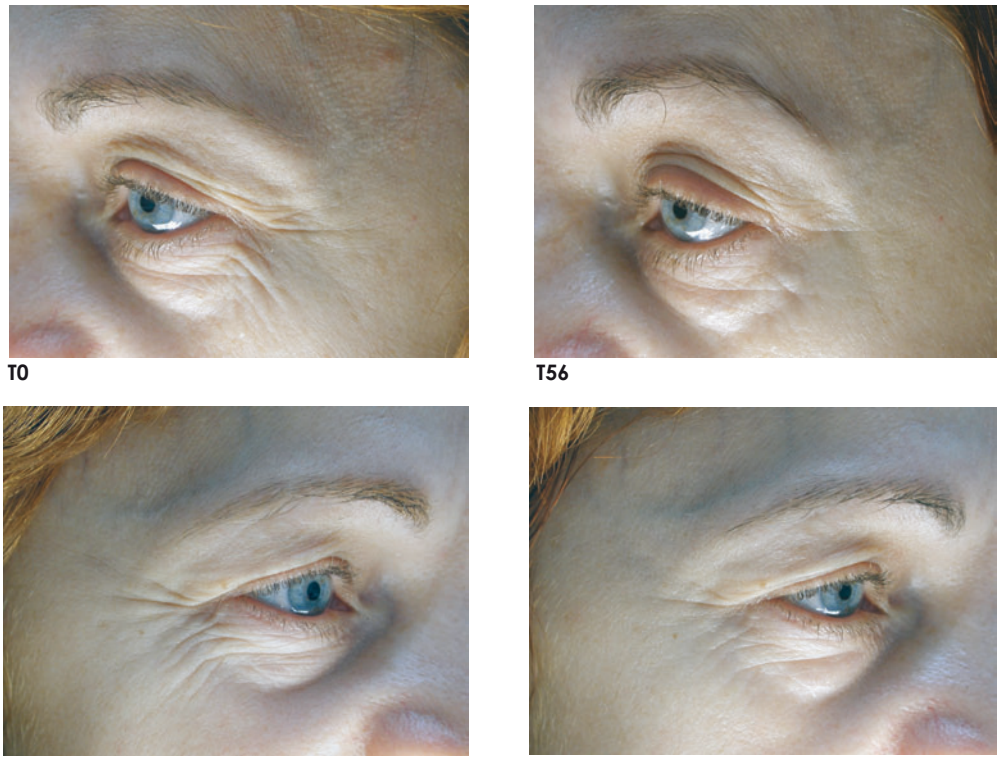
Figure 6: improvement of skin tonicity and elasticity.

Anti-wrinkle efficacy around the eyes by lipopeptides.

2 groups of 23 volunteers aged between 39 and 74 / Twice daily application to one half of the face a cream containing 3% lipopeptides against placebo, for 2 months. Assessment of the anti-wrinkle efficacy by profilometry and photography compared to T0 **(Figure 7: anti-wrinkle efficacy).**

Variation of parameters compared to T0 (%)	Lipopeptides	Placebo
Surface occupied by deep wrinkles (>200µm)	- 44.9**	4.3ns
Main wrinkle density	- 37.0**	-9.6ns
Main wrinkle average depth	- 15.1**	-3.2ns
Main wrinkle average volume	- 18.5**	-8.7*
Roughness	- 14.4**	1.4ns
Complexity (Lifting effect)	- 16.6**	4.2ns

ns : non significant *significant / T0 ($p<0.05$) **significant/ T0 ($p<0.01$)



T0 **T56**

Figure 7: anti-wrinkle efficacy.