dm™
SKINCARE

Science Booklet

SKINCARE

Table of Contents

AE Advanced BTX

A blend of medical grade Perfluorocarbons acting as gas carriers

INCI: Perfluorohexane, Perfluoroperhydrophenantrene, Perfluorodecalin

"Liquid Surgery without scars, a successful fight against time"

- **Anti-Wrinkle**
- **Increase the volume of the dermis by 10-15 %**
- **Induce strong muscular relexion**
- **Purge carbon dioxide from the skin**
- **Refresh's the skin**
- **Fast penetration of the dermis**
- **Long half life**

Effective Use Level for Leave-on products:

Up to 10%

Mode of Action

Concerning the different half life and variable molecular weight of the combined PFC's in *AE Advance BTX* maximizes the acting time in the dermis. Low Molecular weight PFC's penetrate the dermis readily while the higher molecular weight PFC's penetrate slower maintaining the initial results and further moisturizing the dermis

***AE Advanced BTX* is omni phobic (not oil or water soluble). It positions itself between intercellular lipids and moisture in the skin. It volumizes the dermis by creating a 3 dimensional hexagonal micelle structure in the dermis.**

***AE Advanced BTX* is a dielectric, when applied topically to the skin, it allows the glabellar muscles to relax and find its natural position.**

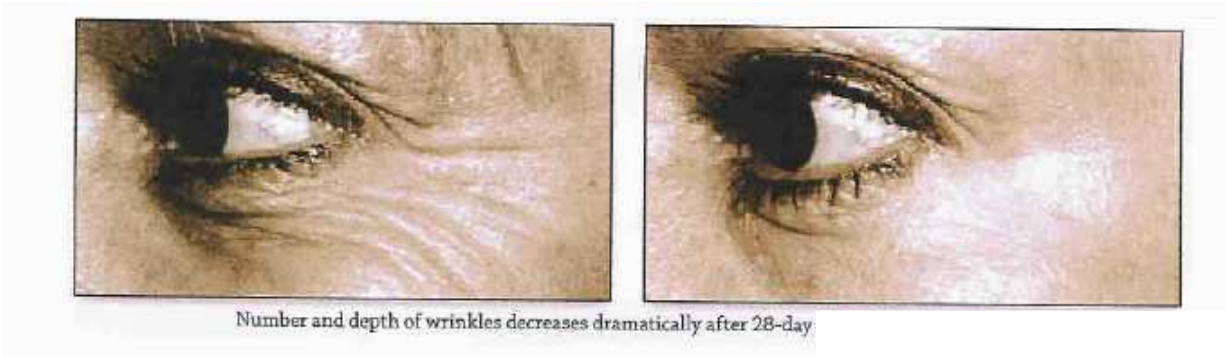
***AE Advanced BTX* is purges Carbon Dioxide from the skin cell allowing the skin to breath and be refreshed.**

Anti-Wrinkle Test:

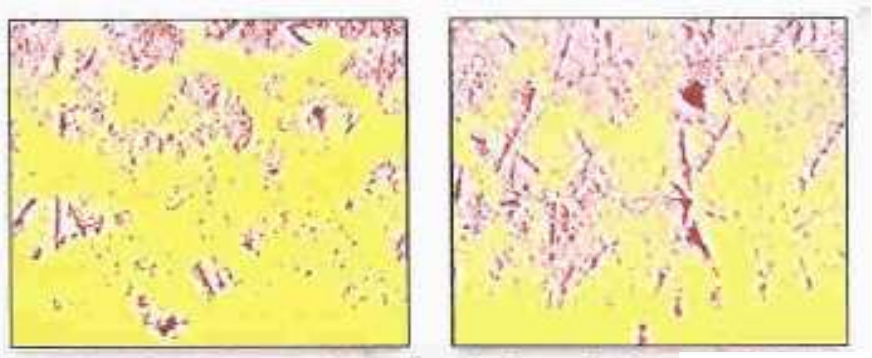
* **14 day panel test consisting of 30 women age 35 to 40 with visible wrinkle around the eye area.**
* **10% Advanced® emulsion was applied twice a day around the eye area for 7 days.**

Result :

* **The moisture content of the skin increased gradually during the period, reaching 52% increase**
* **The number, depth, and length of wrinkles decreased by 10 to 50%.**



Number and depth of wrinkles decreases dramatically after 28-day



Images of skin topography analyses before and after treatment. Skin volumising effect 10-15% after 7 days treatment.

Used in:

Lotion/Creams
Serum

Physical and Chemical Data

Appearance:	**Clear to colorless liquid**
Odor:	**Odorless**
Solubility in water:	**Insoluble**
Solubility in organic solvent:	**Sparingly soluble in most common solvents. Miscible with CRFC's**
Boiling point:	**> 54 C**
Stability:	**stable under ordinary conditions of use and storage**
Shelf life:	**1 year**

Worldwide Approval

Approved for use in Japan, Canada, Australia, Europe and USA

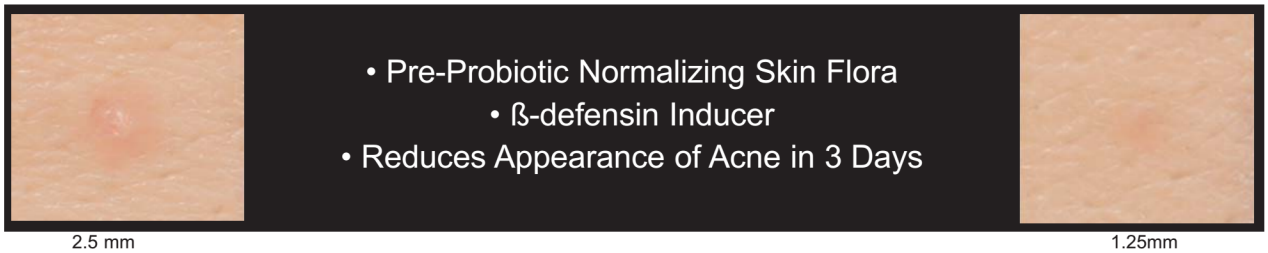
INCI: Perfluorohexane, Perfluoroperhydrophenantrene, Perfluorodecalin

Supplier:
AE Chemie, Incorporated
555 E. Airline Way•Gardena, California 90248,USA
310.523.2888 tel · 310.523.2882 fax

BARNET

Barnet Products Corporation 140 Sylvan Avenue Englewood Cliffs NJ 07632
Tel 201 346 4620 Fax 201 346 4333 Web barnetproducts.com

BioFense *- preliminary report*

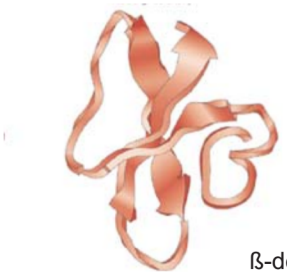


2.5 mm

1.25mm
1% Biofense after 3 days

DESCRIPTION

BioFense is a solution of fragments (Peptidoglycan [PTG] and Lipotechoic Acid [LTA]) of fermented Lactobacillus. On the skin, the pre-Probiotics PTG and LTA will activate toll-like receptors 2, which modifies NF-kB/IkB and release NF-kB that will translocate in the nucleus. There it will bind to the gene coding for an anti-microbial peptide called ß-defensin 2. ß-defensin 2 binds to the bacteria cell and disrupts the membrane by generating holes in it and emptying out the bacterias. Killing harmful bacteria creates a healthy balanced microflora.



ß-defensin: helical cell lytic peptide

PROPERTIES

BioFense was tested on keratinocytes at 0.0004% and 0.018% and was found to increase ß-Defensin synthesis by 33% and 50%, respectively.

BioFense was tested on 6 subjects with 2 acne lesions and 10 subjects with 8 acne lesions. The size and erythema of the lesions were measured. It normally takes 4 days without treatment to see a 50% reduction in the size of lesions. Using 1% BioFense or Salicylic Acid it takes 3 days. However, using 5% BioFense it takes 2 days.
In addition, erythema is decreased by 50% in 2 days with 5% treatments of BioFense.

BioFense was tested on 29 subjects and compared to Triclosan as a bactericide: the skin got used to Triclosan, but not to BioFense.

FORMULATION

The suggested use level is 1-5% which is to be added to the water phase.

LEGISLATION

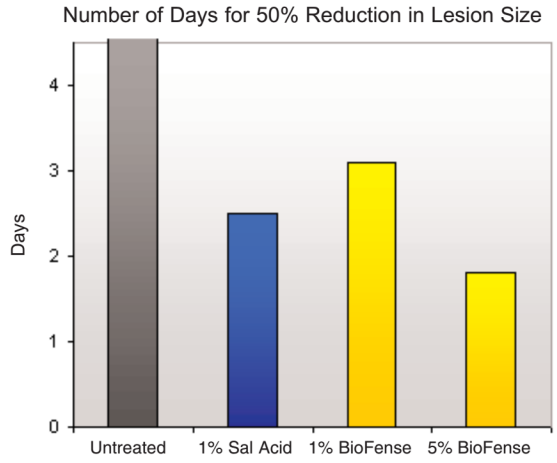
INCI Name: Lactobacillus ferment
EINECS: 295-777-8
Chinese INCI:

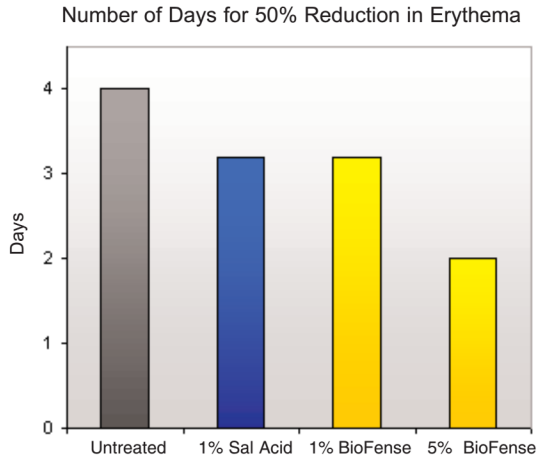
CAS: 92128-79-5
Chinese Chemical Inventory: Listed
Patented Technology

BioFense

ACNE CLINICAL

BioFense was evaluated for its benefits in an acne product to reduce erythema and the size of lesions. Results show BioFense was more effective than Salycylic Acid at improving erythema and lesion size (number of days for 50% improvement).

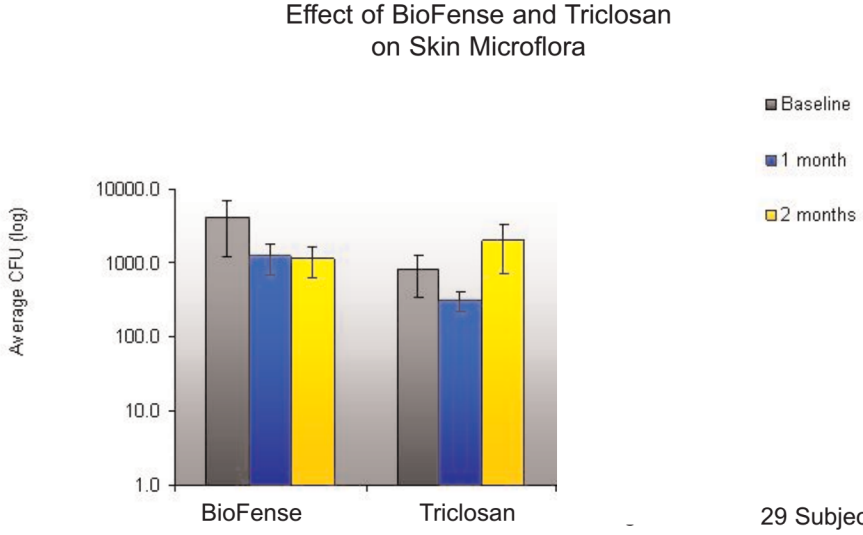




The pH of the Salicylic Acid formula was 3.0.
The pH of the BioFense formula was 4.4 - 4.7.

REDUCTION IN SKIN MICROFLORA

BioFense was evaluated against Trichlosan for its bactericide properties. As seen in the graph below, the skin gets used to Triclosan, but not to ß-Defensin induced by BioFense.



29 Subjects

1 & 2 month use of BioFense
40% reduction in bacterial load

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Dipotassium Glycyrrhizate

Product Data Sheet

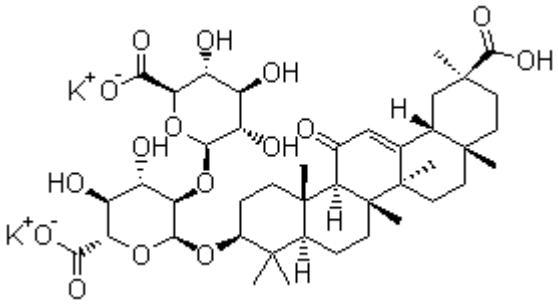
GENERAL INFORMATION

Licorice species are herbs native to the Mediterranean area.The root of licorice is a traditional medicine used mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases. Clinical and experimental studies have shown it to contain pharmacological properties such as anti-inflammatory ,antiviral, antimicrobial, anti-oxidative, anti-cancer activities, immune-modulatory , hepato-protective and cardio-protective effects. The licorice is made up of active constituents such as saponins , flavonoids , chalcones, isoflavones, coumarins, stilbenoids, as well as other compounds such as asparagines, glucose,sucrose, starch, and polysaccharides. It has been used for treating skin eruptions, including dermatitis, eczema , pruritus and cysts**. Dipotassium glycyrrhizinate is a compound obtained by extraction with water from liquorice root. Many clinical reports deal with the application of this product to medicines for external use in the field of dermatology; it is apparently effective in treating acute and chronic dermatitis . Moreover, they are used it in cosmetics product. It's main function as anti-inflammatory ,anti-oxidative and anti-irritant.**

SPECIFICATION

Chemical Name : Dipotassium Glycyrrhizinate

Chemical structure :



Empirical formula	:	$C_{42}H_{60}K_2O_{16}$	**Molecular weight** :	899.12
Appearance	:	White to slightly yellow powder	**CAS No.**	: 68797-35-3
Odor	:	Practically odorless		

Analytical Specifications

Assay % (Dry basis) Glycyrrhizic Acid	:	96.0% minimum
Loss on Drying	:	8.0 % maximum
pH	:	5.0-6.0
Ash	:	21.0% maximum
Application	:	Anti-inflammatory,
Recommended Dosage	:	0.1 to 1.0%

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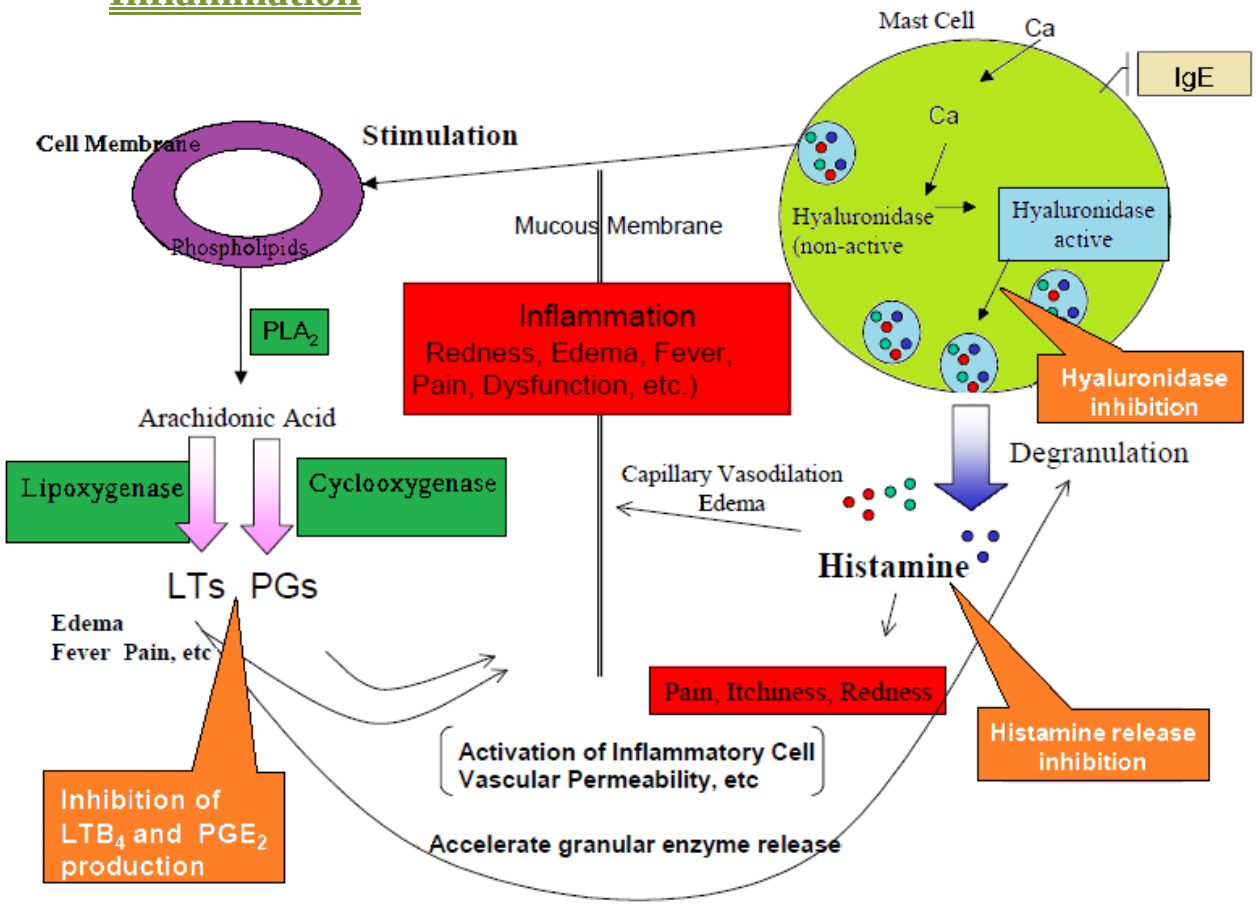
Mode of Action

Inflammation



Anti-inflammation and Hyaluronidase

Inhibition of hyaluronidase plays an important role not only in maintaining the hyaluronic acid level in the body but also in anti-inflammatory and antiallergic activities.

This enzyme is activated during inflammation, plays a role in the destruction of the connective tissue matrix, and increases the permeability of inflammatory cells and blood vessels. Hyaluronidase presents in mast cells in activated by the binding of IgE-antigen complex to receptors, and is involved in the release of histamine granules.

So far anti-inflammatory agents such as indonethacin and antiallergic agents such assodium cromoglicate have been reported as inhibitiors of hyaluronidase.

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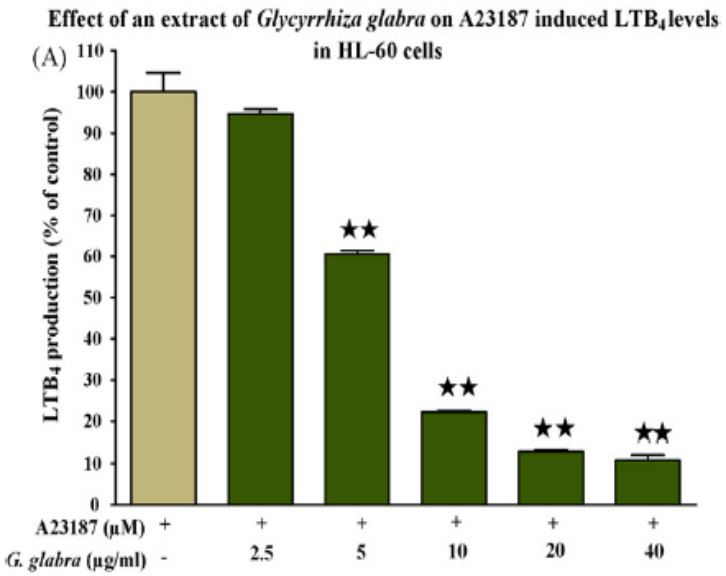
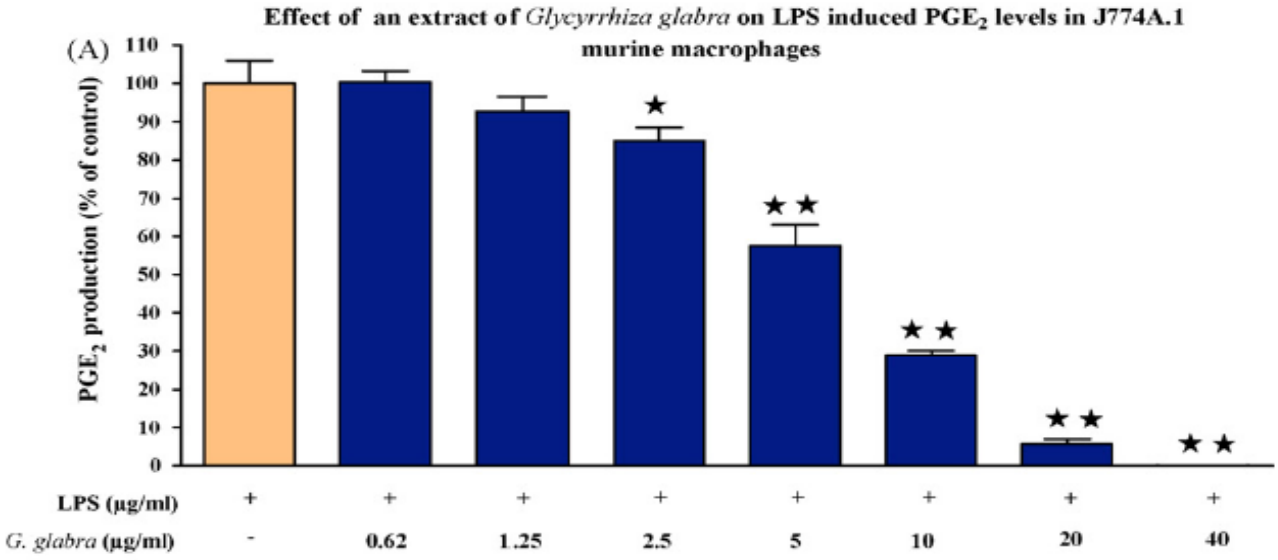
In-vitro Tests

Effects on Arachidonic Cascade



Effect of G. glabra on LTB4 production in A23187 stimulated HL-60 cells. Differentiated HL-60 cells were pretreated with indicated concentrations of G. glabra for 1 h. After stimulation with A23187 (5_M) for 15 min, the levels of LTB4 in the medium were quantified. G. glabra dose-dependently decreased the LTB4 productionand the values are expressed as a percentage of the control (A23187 alone).
Data are represented as mean±S.E.M. **P < 0.01 compared with the A23187 alone.

*** HL-60 cells = human neutrophil cells
A23187 = Antibiotic A23187 ,Calcimycin ,it is a mobile ion-carrier that forms stable complexes with divalent cations



Effect of G. glabra on PGE2 production in LPS stimulated J774A.1 murine macrophages. Cells were pretreated with indicated concentrations of G. glabra for 1 h, and then stimulated with LPS (0.1μg/ml) for 24 h. The PGE2 levels were dose-dependently decreased by G. glabra and the values are expressed as a percentage of the control (LPS alone). Data are represented as mean±S.E.M. **P < 0.01 and *P < 0.05 compared with the LPS alone.

*** J774A.1 = murine macrophages
LPS = lipopolysaccharide

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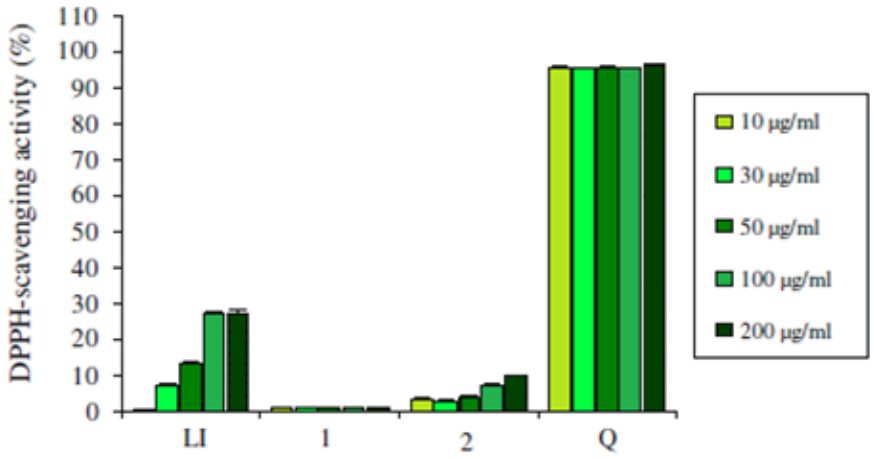
Antioxidant of Dipotassium Glycyrrhizinate

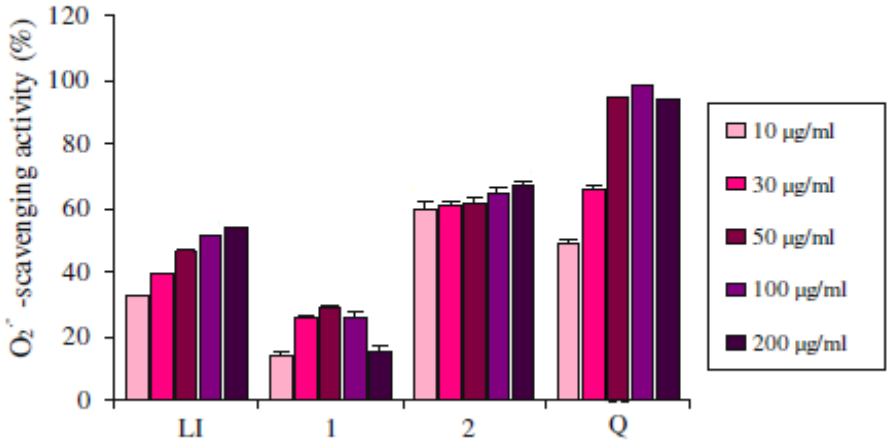
- **DPPH radical Test**



This assay is based on the ability of DPPH to react with H-donors.The change in absorbance produced by reduced DPPH is used to evaluate the antiradical ability of the samples. The DPPH – scavenging activities of LI, 1, 2 and Q .

*** LI = liquiritin
1 = glycyrrhizin
2 = Quercetin
Q = Rreference compound

- **Superoxide radical Test**



The superoxide anion radical is the most common reactive oxygen species formed in vivo. It is known to be very harmful to cellular components as a precursor of more reactive oxygen species,contributing to tissue damage and various diseases. The O2 scavenging activities of LI, 1, 2 and Q .

*** LI = liquiritin
1 = glycyrrhizin
2 = Quercetin
Q = Rreference compound

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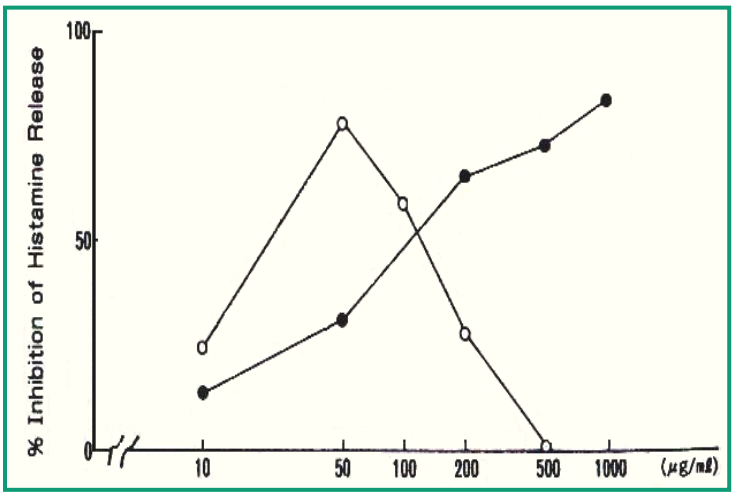
In-vivo Tests

Inhibitory Effects on Histamine Release

- **Effects of glycyrrhizin and glycyrrhetinic acid on histamine release from rat mast cells by antigen IgE antibody reaction**



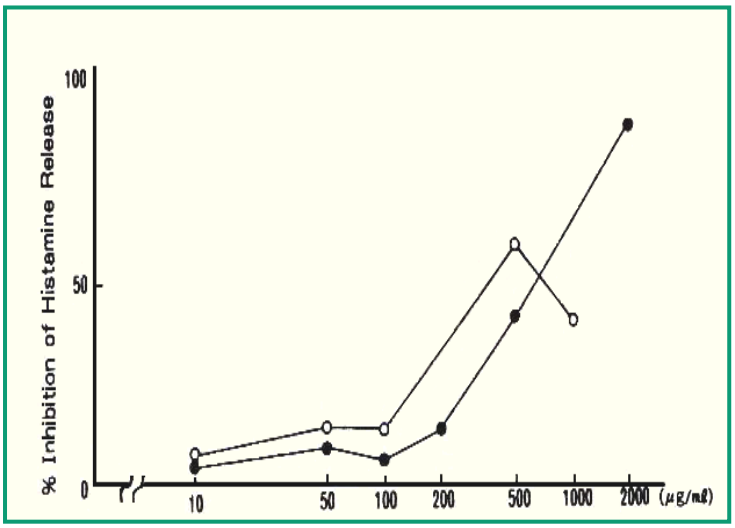
Effects of glycyrrhizin and glycyrrhetinic acid on histamine release from rat mast cells by antigen IgE antibody reaction

Concentration of antigen (DNP-As) was 100 μg/ml (%HR:33.3) . Each point represents the mean of duplicate.

***●-● = glycyrrhizin
○-○ = glycyrrhetinic acid

DPG:Inhibition 83.4% (Conc. at 1mg/mL)

- **Effects of glycyrrhizin and glycyrrhetinic acid on histamine release from rat mast cells by compound 48/80**



Effects of glycyrrhizin and glycyrrhetinic acid on histamine release from rat mast cells by compound 48/80.

Concentration of compound 48/80 was 1 μg/ml (%HR;74.0) .Each point represents the mean of duplicate.

***●-● = glycyrrhizin
○-○ = glycyrrhetinic acid

DPG:Inhibition 86.4% (Conc. at 2mg/mL)

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Acute and Chronic Toxicity

The LD50 of various Glycyrrhizin Salts administered to mice has been determined by Klosa (1957) and Fujimura, with results as shown in Table I

TABLE I

Acute Toxicity of Glycyrrhizin Salts in Mice

Route	Glycyrrhizin salt	LD_{50} (mg / kg)
Oral	ammonium (crude)	12,700
	diammonium	9,600
	potassium (crude)	12,400
	monopotassium	1,220
	dipotassium	8,100
Intraperitoneal	ammonium (crude)	1,050
	monoammonium	1,070
	diammonium	1,250
	potassium (crude)	1,260
	dipotassium	1,400

References :

1. Cheel J.,Antwerpen P.V.,Tůmová L.,Onofre G., et al.(2010). Free radical-scavenging, antioxidant and immunostimulating effectsof a licorice infusion (Glycyrrhiza glabra L.).Journal of Food Chemistry .122,508–517.
2. Chandrasekaran C.V., Deepak H.B., Thiyagarajan P., Kathiresan S., Sangli G.K., Deepak M., and Agarwal A.(2011). Dual inhibitory effect of Glycyrrhiza glabra (GutGardTM) on COX and LOX products. Journal of Phytomedicine ,18,278-284.
3. Armanini, D., Fiore, C., Bielenberg, J., Ragazzi, E., (2005). Licorice (Glycyrrhiza glabra). In Encyclopedia of Dietary Supplements, 371–399.
4. Noriaki I., Hiroshi K., Yasuhiro H., Kimio Y., and Atsushi I.(1989). Effects of glycyrrhizin and glycyrrhetinic acid on dexamethasone-induced changes in histamine synthesis of mouse mastocytoma P-815 cells and in histamine release from rat peritoneal mast cells. Journal of Biochemical Pharmacology . 38, 2521-2526

ALPAFLOR® EDELWEISS

Discover a new level of protection for an ultimate skin sensation

NEW *IN VIVO* RESULTS

HEALTH · NUTRITION · MATERIALS

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Exclusive N.A. Distributor
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Ph: 203-822-9800 Fax: 203-822-9820
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Alpine beauty to protect skin

Its beauty and rareness have made the Edelweiss flower a symbol for pure and balanced nature. But the elegant Alpine plant also has surprising inner strength: growing at high altitudes and exposed to strong UV radiation and extreme climate conditions, this seemingly delicate flower has become an expert in self-protection. It's this very resilience that is at the heart of the powerful protection of ALPAFLOR® EDELWEISS from DSM.

With many years of experience combining botanical and phyto-chemical know-how, DSM research has harnessed the ability of this legendary flower to protect the skin and rebalance environmentally induced stress. This provides vital support for the skin's natural protection processes. As a result ALPAFLOR® EDELWEISS is the perfect solution for formulations that meet consumers' needs – a whole new level of protection for an ultimate skin sensation.

Key facts

Unique product features

- Unique Edelweiss variety *Leontopodium alpinum* 'Helvetia', sustainably and organically cultivated according to Bio Suisse organic standards and fair trade principles
- Contains high levels of leontopodic acid
- *In vitro* stimulates several key genes and proteins responsible for epidermal protection (including transglutaminase 1, involucrin, loricrin, and keratins)
- Strong antioxidant, radical scavenging, and DNA protection properties
- Rebalances UV-induced oxidative and pro-inflammatory stress markers on the gene level

Benefits

- *In vitro* and *in vivo* supports skin's crucial protective barrier, enhancing its resistance to external stress factors
- Helps the skin preserve its natural balance, perceptibly reducing skin sensitivity
- Provides a new level of protection for an ultimate skin sensation
- 3 out of 4 consumers report a more comfortable skin feel

Cosmetic application

- High-performing, organic and natural skin care products
- Day-care protecting formulations for stressed and sensitive skin
- Sun care and after-sun formulations

Suggested concentration

- 1–5% ALPAFLOR® EDELWEISS

INCI name (active)

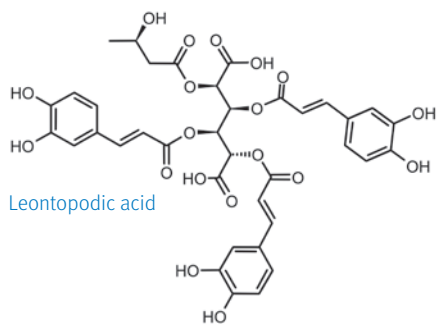
Leontopodium Alpinum Extract

Botanics

Edelweiss (*Leontopodium alpinum*) is an Alpine "survival star" that grows at altitudes of up to 3,000 m, where it is exposed to high levels of UV radiation. There it has to contend with extremes of temperature, making Edelweiss an expert in self-protection.

ALPAFLOR® EDELWEISS extracts are produced from a variety of *Leontopodium alpinum* carefully developed to meet the specifications of DSM research teams. *Leontopodium alpinum* 'Helvetia' is cultivated at high altitude in the Swiss Alps using a unique process designed to ensure a maximum content of active compounds.

The variety 'Helvetia' contains large amounts of leontopodic acid, chlorogenic acid and luteolin derivatives which fuel the powerful ability of ALPAFLOR® EDELWEISS to protect and regenerate the skin's barrier. Leontopodic acid especially, discovered for the first time in *Leontopodium alpinum*, has extraordinary antioxidant, radical scavenging, and DNA protection properties (Lit.: Tetrahedron 61 (2005) 4621-4630).



Leontopodic acid

Mechanism

The stratum corneum is the outermost layer of the epidermis. It forms the protective barrier between the body and the environment. This barrier is responsible for retaining moisture in the skin and protecting it against external stress factors such as UV light and harmful substances. The stratum corneum is mainly composed of cornified cells, the so-called corneocytes. Each of these cells is surrounded by a cornified envelope consisting primarily of proteins such as loricrin, involucrin and small proline-rich proteins (SPRRs), which are cross-linked by the calcium-dependent transglutaminase 1 (TG1). The resulting surface forms an effective shell. Additional skin protection is offered by keratin 1 and keratin 10, two major structural proteins within the corneocytes which give the stratum corneum its mechanical strength. Ensuring best possible protection for the skin is a vital factor in creating and maintaining a pleasant skin sensation.

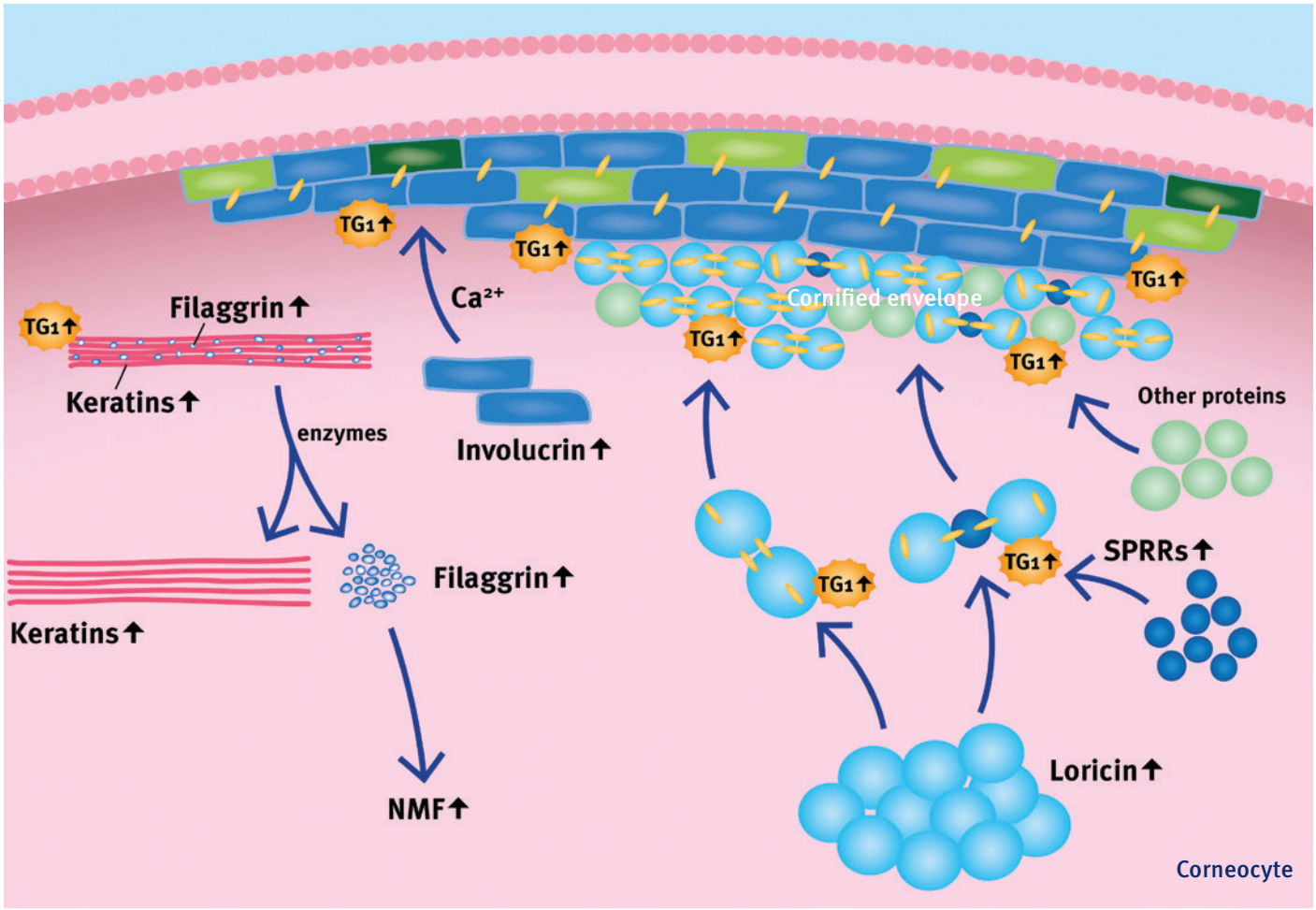


Figure 1: Model of a corneocyte indicating the key proteins up-regulated by ALPAFLOR® EDELWEISS

Extended *in vitro* data demonstrate that ALPAFLOR® EDELWEISS stimulates key proteins responsible for providing maximum protection and moisturization of the skin. Particularly, levels of transglutaminase 1 and involucrin are increased to reinforce the cornified envelopes. Gene expression studies have demonstrated the multi-target activity of ALPAFLOR® EDELWEISS on several levels. In particular loricrin, involucrin, filaggrin, small proline-rich proteins (SPRRs), keratin 1, and keratin 10 gene expression are significantly up-regulated (Figure 1).

Latest *in vivo* results confirm these ultimate barrier protection activities: ALPAFLOR® EDELWEISS significantly improves skin barrier integrity and stratum corneum cohesion, leading to better protected, more resistant skin. As a consequence, the skin is more relaxed, less sensitive, giving an ultimate sensation of skin comfort.

Supply forms:

EDELWEISS EP: Extract from the organically cultivated Swiss Alpine plant *Leontopodium alpinum* 'Helvetia' based on vegetal Glycerin and preserved with Sodium Benzoate and Potassium Sorbate.

EDELWEISS B: Extract from the organically cultivated Swiss Alpine plant *Leontopodium alpinum* 'Helvetia' based on vegetal Glycerin and organic Ethanol, preservative-free.

ECO CERT®

ALPAFLOR® EDELWEISS EP		ALPAFLOR® EDELWEISS B	
99.55%	Natural origin	100%	Natural origin
27.85%	Organic origin	27.30%	Organic origin

Certified to ECOCERT Standard for Natural and Organic Cosmetics
available at http://cosmetics.ecocert.com

ECO CERT® COSMOS CERTIFIED

ALPAFLOR® EDELWEISS EP		ALPAFLOR® EDELWEISS B	
27.85%	Organic origin	26.98%	Organic origin

Certified to COSMOS Standard
available at http://cosmos.ecocert.com

WWW.NATRUE.ORG

ALPAFLOR® EDELWEISS EP	
8%	Natural (95% Organic)
30%	Water
62%	Derived natural
0.45%	Nature-identical

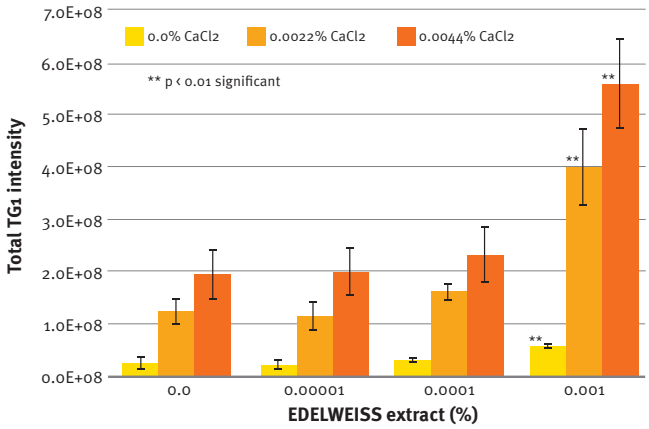
ALPAFLOR® EDELWEISS B	
16%	Natural (100% Organic)
22%	Water
63%	Derived natural
0%	Nature-identical

Complies with the NATRUE Criteria.

Efficacy *in vitro*

Transglutaminase 1 stimulation in human keratinocytes

Transglutaminase 1 (TG1) is a key element in the cross-linking of involucrin, loricrin, and SPRRs, and thus ensures proper functionality of the cornified envelope. TG1 is calcium-dependent.

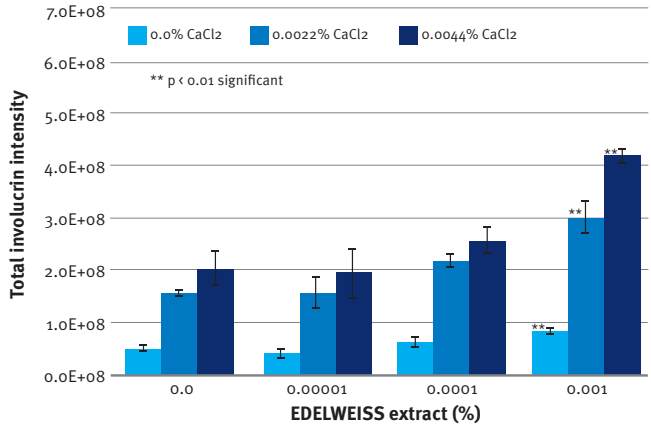


Result:

ALPAFLOR® EDELWEISS increases transglutaminase 1 (TG 1) protein level dose-dependently by up to 300%.

Involucrin protein level in cornified envelopes

Involucrin is a key protein that makes the cornified envelope robust enough to resist harmful environmental substances. Involucrin is calcium-dependent.



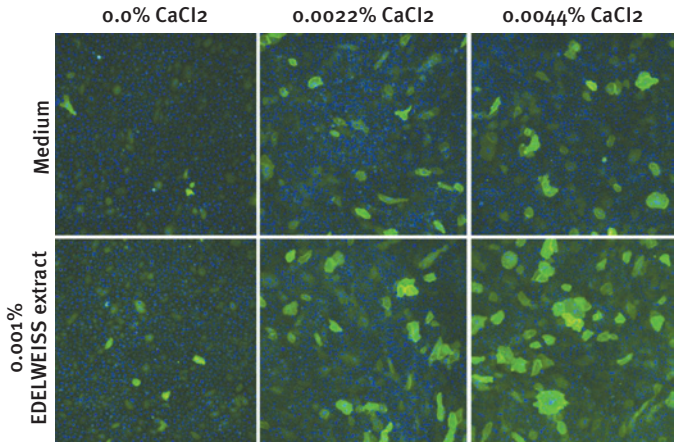
Result:

ALPAFLOR® EDELWEISS increases involucrin protein level dose-dependently by up to 300%.

Additional test results are available on request (gene expression, *in vitro*)

Immunofluorescent staining of transglutaminase 1 in human keratinocytes

The presence of functional transglutaminase 1 has been determined by microscopic immunofluorescence imaging technology.



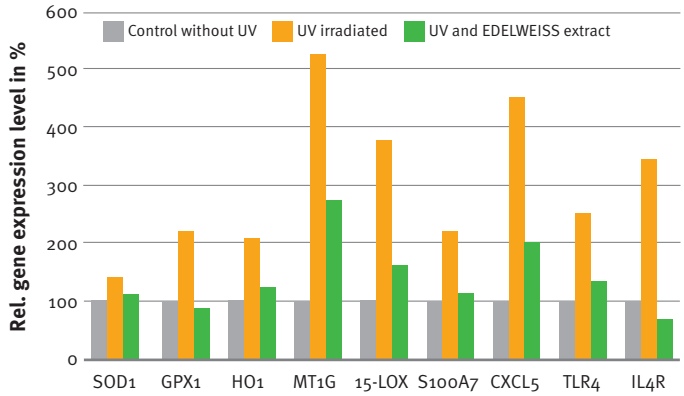
Result:

ALPAFLOR® EDELWEISS leads to a substantial increase in transglutaminase 1 (green fluorescence) even at low concentrations.

Gene expression of UV-induced oxidative and pro-inflammatory stress markers

Skin tends to react to UV exposure with the release of oxidative and pro-inflammatory stress markers as its natural defense mechanism.

Reduced activity of these molecules is a clear indication of a well protected skin status. Epidermal skin equivalents were treated topically with Edelweiss extract (0.02%) 24 hours before UVB/UVA irradiation (UVB 250mJ/cm²; UVA 3.5 J/cm²). Gene expression was measured 6h after UV irradiation.



(SOD1 = superoxide dismutase 1; GPX1= glutathione peroxidase; HO1= heme oxygenase 1; MT1G = metallothionein 1G; 15-LOX=15-lipooxygenase; S100A7 = psoriasin; CXCL5 = chemokine (C-X-C motif) ligand 5; TLR4 = toll-like receptor 4; IL4R = interleukin 4 receptor)

Result:

ALPAFLOR® EDELWEISS has a positive effect on several genes recorded as oxidative and pro-inflammatory stress markers. It rebalances the skin stress level until it is comparable to a situation with no UV exposure. This provides vital support for the skin's natural powers of protection.

Efficacy *in vivo*

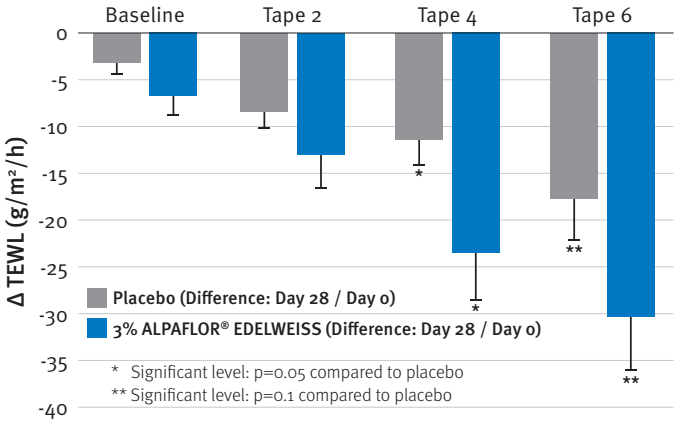
Forty-eight female volunteers (30 – 52 years old) participated in this 1-month, randomized, double-blind study. They were recruited on the basis of self-assessed and expert-graded skin sensitivity.

Half of the volunteers applied a 3% ALPAFLOR® EDELWEISS emulsion to the full face twice daily, while the other half applied the placebo form.

Skin barrier integrity

Transepidermal water loss (TEWL) is a measure for the quantity of water evaporating through the stratum corneum to the surrounding atmosphere. It is used to study the water barrier function of human skin.

The integrity of the stratum corneum is an indicator for the strength of the barrier or the barrier reserve. It was evaluated by measuring the TEWL after impairment of the stratum corneum by six consecutive tape strippings. A low TEWL after the stratum corneum has been damaged in this way is evidence of good barrier integrity.



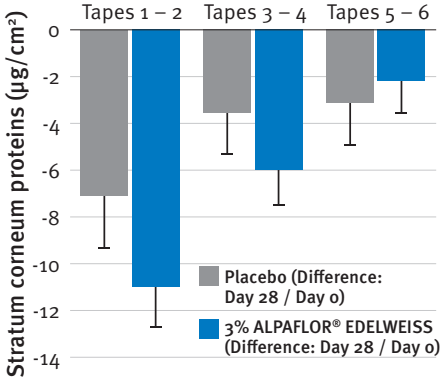
Result:

Measurements after consecutive tape stripping showed TEWL to be statistically significantly reduced, indicating that ALPAFLOR® EDELWEISS improves barrier strength.

Stratum Corneum Cohesion

The quantity of stratum corneum protein content adhering to tape strippings was evaluated as a measure of stratum corneum cohesion, using infrared densitometry.

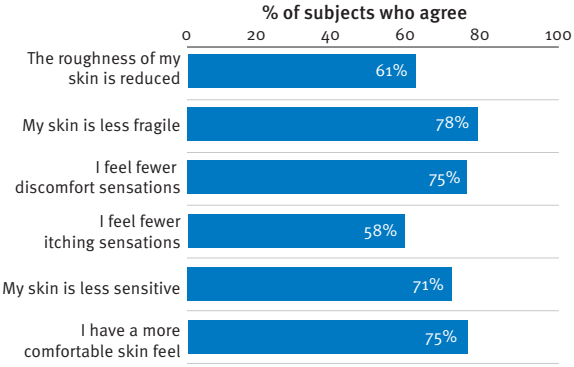
A reduction in the quantity of stratum corneum proteins present on the tape indicates increased cohesion of the stratum corneum.



Result:

ALPAFLOR® EDELWEISS stimulates stronger adhesion of stratum corneum proteins, reflecting a strengthening of the epidermal barrier.

Self-evaluating questionnaire



Result:

ALPAFLOR® EDELWEISS received an excellent rating from the volunteers. This demonstrates its outstanding ability to visibly and perceptibly improve skin structure, leading to an ultimate skin sensation.

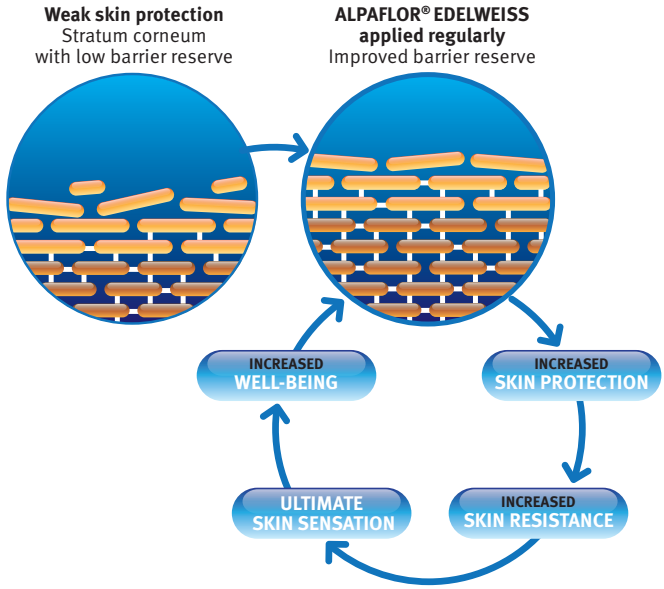
Capsaicin Stinging Test

This test enables subjective evaluation of skin sensitivity based on the ability to feel a stinging sensation after application of capsaicin to the permeable stratum corneum of the nasolabial folds. Successively stronger concentrations of capsaicin solution were applied until the subject reported a sensation. The product is shown to have a soothing effect if associated with a significant increase in the detection threshold.

Result:

50% of the subjects showed an improvement in skin sensitivity after 1-month application of an emulsion containing 3% ALPAFLOR® EDELWEISS.

Overview of ALPAFLOR® EDELWEISS efficacy



Regular application of cosmetic products containing ALPAFLOR® EDELWEISS improves skin barrier protection, making skin more resistant to environmental influences. Skin is more relaxed and less sensitive, giving an ultimate sensation of skin comfort.

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Email: info.pc-latam@dsm.com

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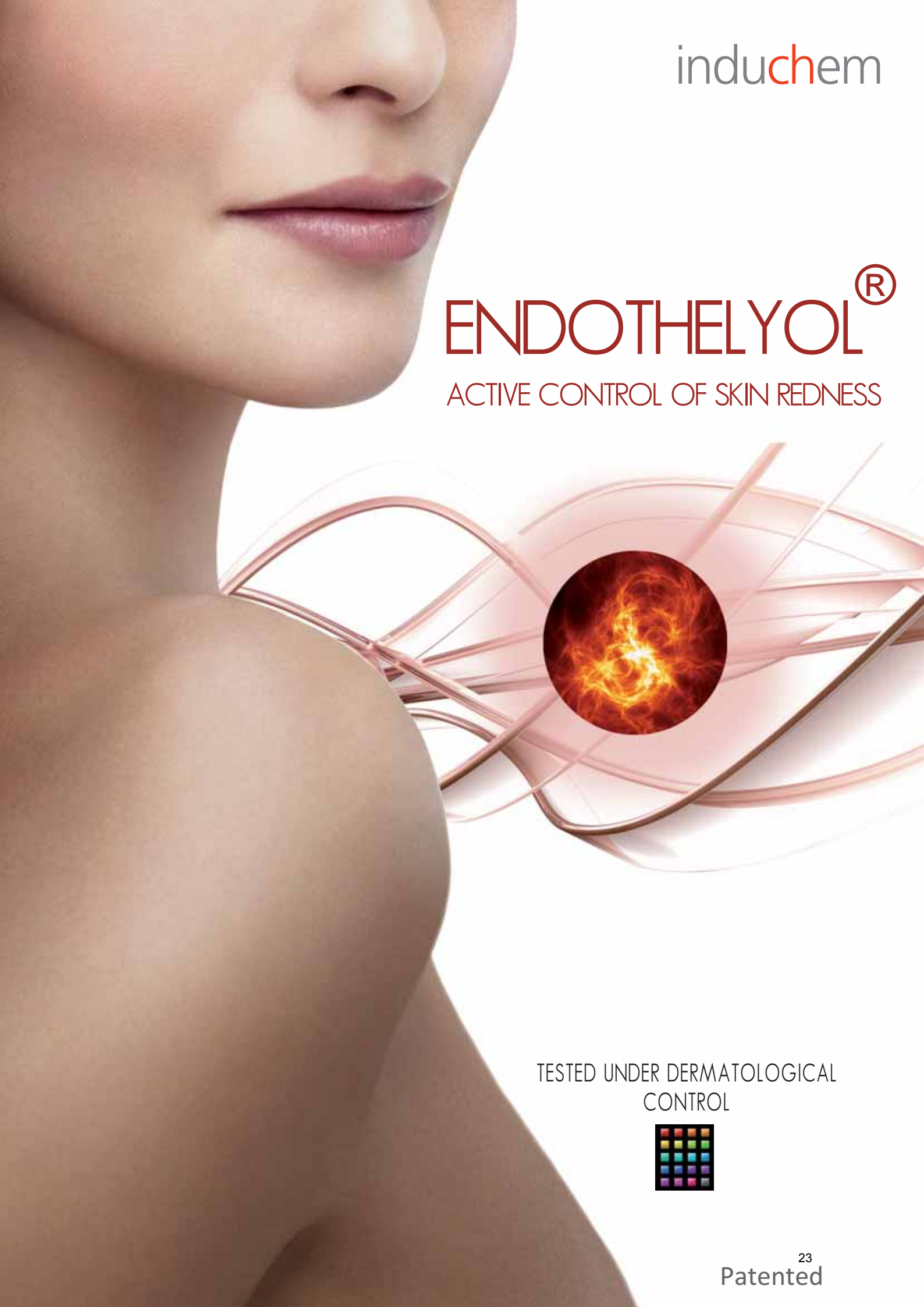
induchem

ENDOTHELYOL®

ACTIVE CONTROL OF SKIN REDNESS

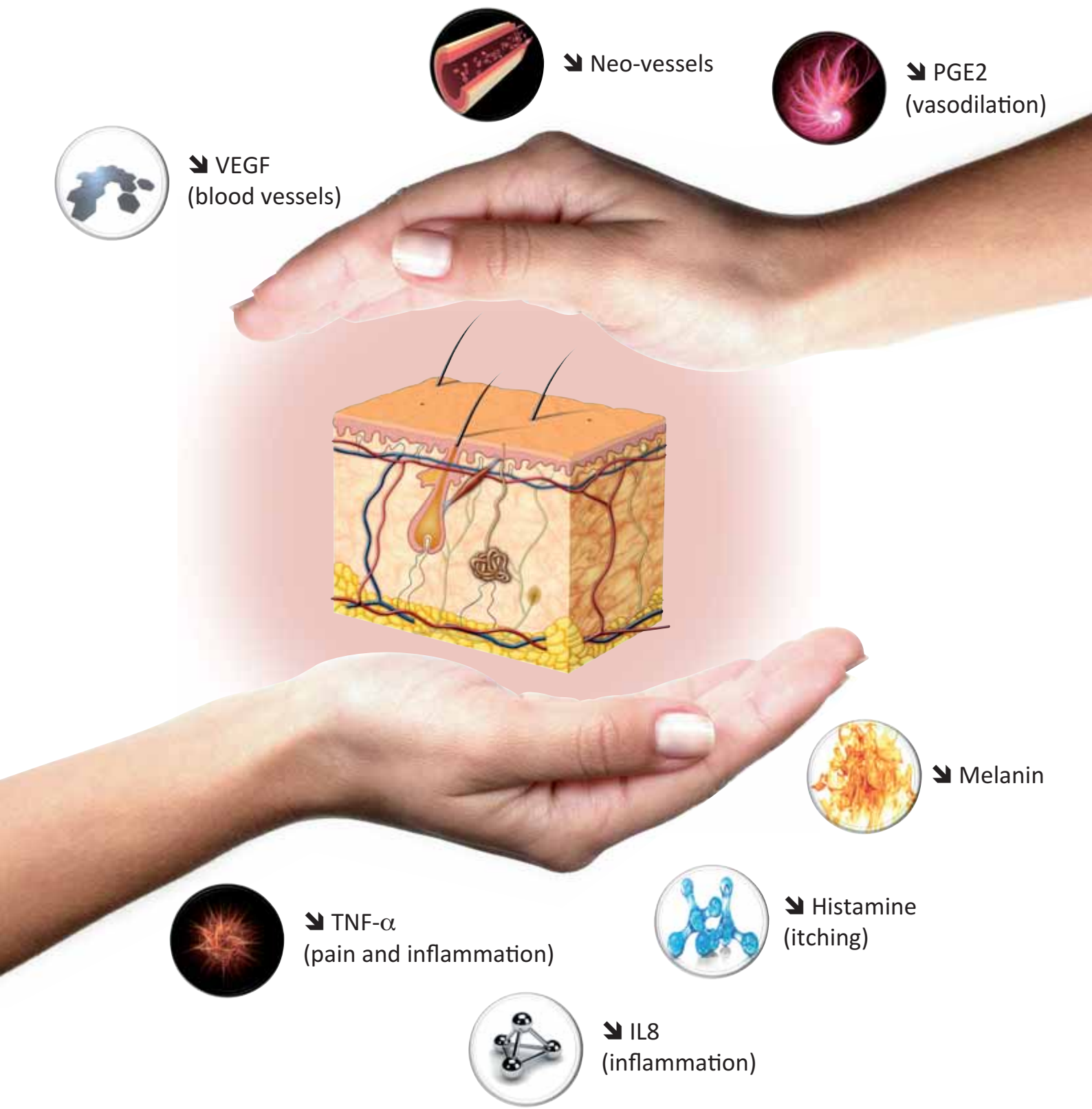


TESTED UNDER DERMATOLOGICAL CONTROL

Patented

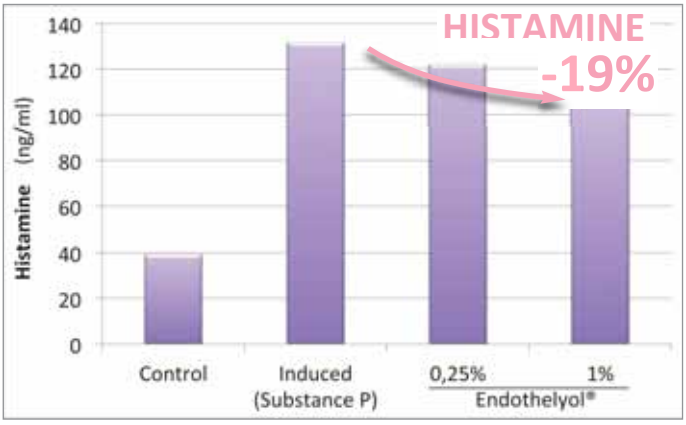
ENDOTHELYOL®

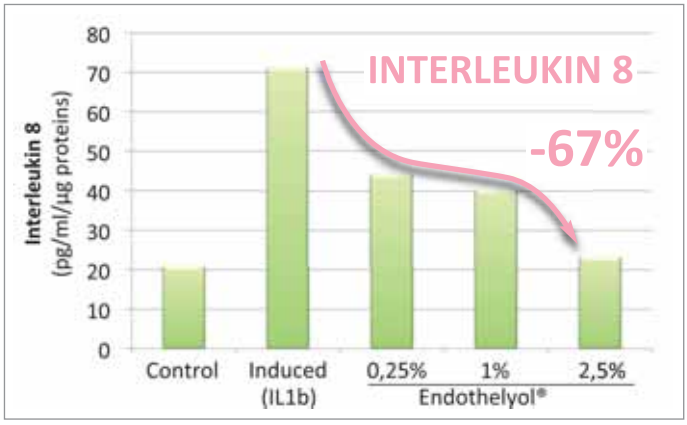
ENDOTHELYOL® is a unique 5 in 1 active ingredient to prevent skin redness and improve rosacea. It controls all the major skin inflammation factors, reduces neovascularisation mechanisms while brightening skin tone. Tested under dermatological control, it is perfectly compatible for all types of sensitive, red and irritated skins.

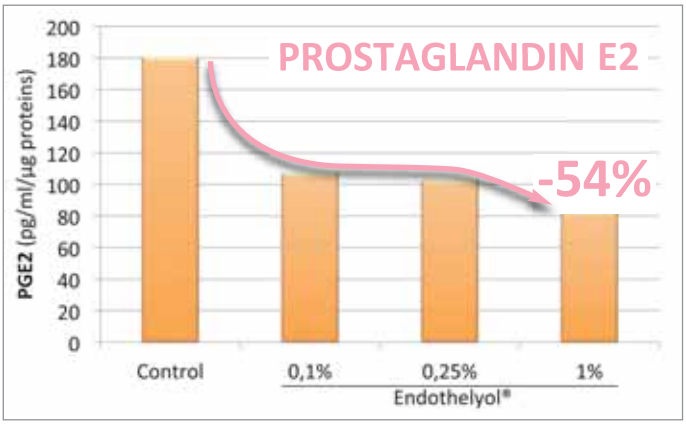


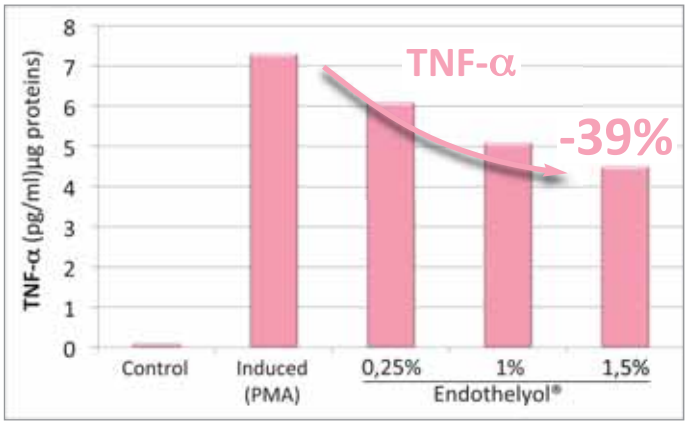
ENDOTHELYOL® est un ingrédient actif 5 en 1 pour prévenir les rougeurs de la peau et améliorer la rosacée. Il contrôle les principaux facteurs de l'inflammation de la peau, réduit les mécanismes de la néovascularisation, et améliore la couleur de la peau. Testé sous contrôle dermatologique, il est parfaitement compatible pour tous les types de peaux sensibles, couperosées et irritées.

TIGHT CONTROL OF REDNESS FACTORS









IL8 – PGE2 – TNF-α : tests on keratinocytes extracted from a **clinical human skin sample**, and incubated with ENDOTHELYOL® during 48h00.

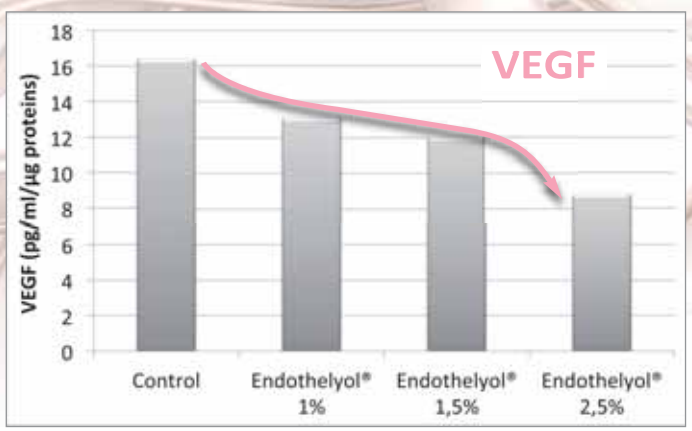
Histamine : Mastocytes incubated with ENDOTHELYOL® during 30 minutes.

IL8 – PGE2 – TNF-α : tests sur kératinocytes *extraits d'un* **échantillon clinique de peau humaine** *et cultivés en présence d'ENDOTHELYOL® pendant 48h00.*

Histamine : *Mastocytes incubés pendant 30 minutes avec ENDOTHELYOL®.*

REDUCTION OF NEOVASCULARISATION

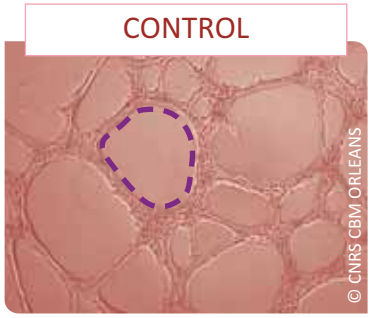
ENDOTHELYOL® CONTROLS Vascular Endothelial Growth Factor

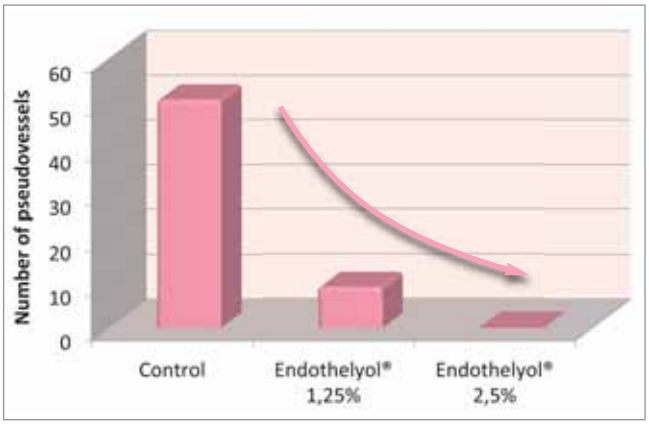


Keratinocytes extracted from a clinical human skin sample, and incubated with ENDOTHELYOL® during 48h00.

Kératinocytes *extraits d'un échantillon clinique de peau humaine et cultivés en présence d'ENDOTHELYOL® pendant 48h00.*

ENDOTHELYOL® BLOCKS NEO-VESSELS GROWTH



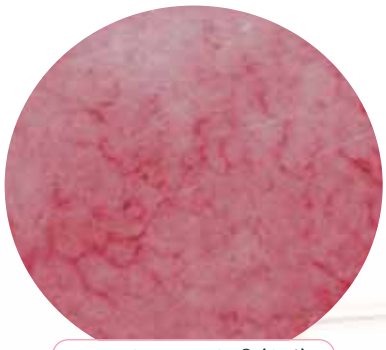


Microvascular endothelial human skin cells (HskMEC) cultured on MATRIGEL™ with or without ENDOTHELYOL® during 17 hours. Quantification of the number of neo-blood vessels created upon time. (dashed line : contour of a neo-vessel).

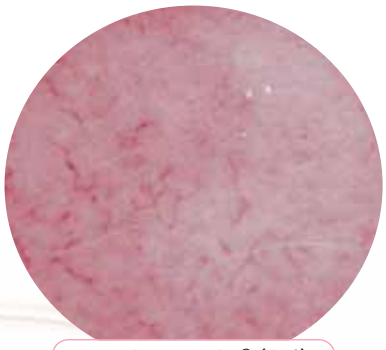
Cellules endothéliales microvasculaires de peau humaine *(HskMEC) cultivées sur MATRIGEL™ avec ou sans ENDOTHELYOL® pendant 17h. Quantification du nombre de néo-vaisseaux sanguins formés au cours du temps. (ligne hachurée : contour d'un néo-vaisseau).*

CLINICAL EVALUATION ON SKIN

ENDOTHELYOL® REDUCES ROSACEA
7% to 12% improvement in 28 days*



ENDOTHELYOL® (2%)
Day 0



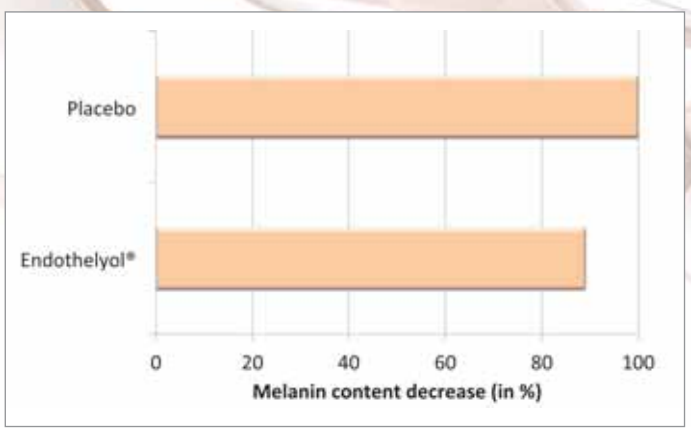
ENDOTHELYOL® (2%)
Day 28

Clinical evaluation versus placebo on a panel of women having rosacea.
* respectively on 50% and 30% of women who tested the product.

Evaluation clinique versus placebo sur un panel de femmes ayant de la couperose.
** respectivement sur 50% et 30% des femmes ayant testé le produit.*

Clinical tests under dermatological control.
Tests cliniques sous contrôle dermatologique.

ENDOTHELYOL® IMPROVES SKIN COLOR
11% of brightening in 28 days*



Same protocol as above. Quantification of skin melanin content by Siascopy™. * on 75% of women who tested the product.

Même protocole que ci-dessus. *Quantification de la teneur en mélanine par Siascopy™. * sur 75% des femmes ayant testé le produit.*

ENDOTHELYOL®

ACTIVE CONTROL OF SKIN REDNESS
CONTRÔLE ACTIF DES ROUGEURS CUTANEES

INCI NAME : GLYCERIN / ROSMARINYL GLUCOSIDE / CAFFEYL GLUCOSIDE / GALLYL GLUCOSIDE

MANUFACTURER

Induchem AG
Industriestrasse 8a
CH-8604 Volketswil, Switzerland
Phone: +41 44 908 43 33
Fax: +41 44 908 43 30

ACTIVE INGREDIENT

INNOVATIVE CALMING MECHANISM

- **Regulates major inflammation factors**
- **Controls neovascularisation**
- **Improves rosacea in 28 days**
- **Brightens up the skin**

ORIGIN

ENDOTHELYOL® is a pure active ingredient obtained by enzymatic modification of natural plants hydroxybenzoic acid and hydroxycinnamic acids, by means of a proprietary biotechnology process.

FORMULA

- Vegetal glycerin : 35-40% (w/v)
- Rosmarinic acid-α-D-glucoside : 1.1 to 1.6%
- Caffeic acid-α-D-glucoside : 0.85 to 1.15%
- Gallic acid-α-D-glucoside : 0.85 to 1.15%
- Water up to 100%

APPLICATIONS

- Sensitive and irritated skin
- Anti-aging products
- Eye contour
- Face contour
- Sun creams and lotions
- After sun care products
- Body lotions

PACKAGING – FORMULATION

- Liquid sterile water-glycerin solution
- Concentration : ~33g/L of active ingredients
- SOLVENT FREE – PRESERVATIVE FREE

SUGGESTED RANGE OF USE

From 0.25% to 2.5% of the commercial solution.

FABRICANT

Induchem AG
Industriestrasse 8a
CH-8604 Volketswil, Switzerland
Phone: +41 44 908 43 33
Fax: +41 44 908 43 30

INGREDIENT ACTIF

MODE D'ACTION INNOVANT

- **Régulation des facteurs inflammatoires**
- **Contrôle de la néovascularisation**
- **Amélioration de la couperose en 28 jours**
- **Illumination du teint**

ORIGINE

ENDOTHELYOL® est un actif pur obtenu par modification enzymatique d'acides hydroxybenzoique et hydroxy-cinnamiques naturels de plantes par un procédé biotechnologique breveté.

COMPOSITION

- Glycérine végétale : 35-40% (p/v)
- Acide rosmarinique-α-D-glucoside : 1,1-1,6%
- Acide caféique-α-D-glucoside : 0,85 - 1,15%
- Acide gallique-α-D-glucoside : 0,85 - 1,15%
- Eau qsp 100%

APPLICATIONS

- Soin des peaux sensibles et irritées
- Soin anti-âge
- Produit contour des yeux
- Produit contour du visage
- Soins et lotions solaires
- Soins après-solaires
- Soins et lotions pour le corps

CONDITIONNEMENT - FORMULATION

- Solution aqueuse glycérinée stérile
- Concentrée à ~33g/L d'actifs
- SANS SOLVANT – SANS CONSERVATEUR

DOSE RECOMMANDÉE

De 0,25% à 2,5% de la solution commerciale.

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companies

cosmetic engineering

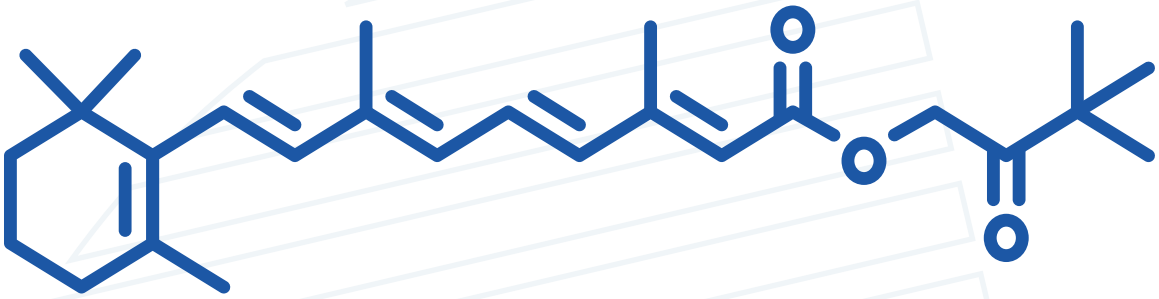
LEAFLET-ENDOTHELYOL-05.12-500

GRANT INDUSTRIES
Where Performance Matters

Granactive Retinoid

THE POWER OF RETINOL WITHOUT THE IRRITATION

GRANACTIVE RETINOID: THE NEXT STEP IN VITAMIN A CHEMISTRY



Granactive Retinoid is Hydroxypinacolone Retinoate, a cosmetic grade ester of all-trans retinoic acid. This skin care active ingredient belongs to a class of chemical compounds termed retinoids, which are natural and synthetic derivatives of Vitamin A capable of binding to retinoid receptors. The binding of retinoid receptors can enhance gene expression, which effectively turns key cellular functions on and off. When skin cell retinoid receptors are bound with retinoids, a cascade of mechanisms that benefit skin complexion are switched on. This can result in enhanced cell proliferation, biosynthesis of extracellular proteins and glycans, and improved cellular turnover. Stimulating these age defying processes in the skin is critical for fighting and reversing signs of aging.

BIOLOGICAL PATHWAYS TO YOUTHFUL SKIN WITH GRANACTIVE RETINOID

Stimulating cell proliferation and cell turnover are important for normalizing cell renewal and repair processes. As we age our skin becomes thinner and less elastic, leading to skin with a loose, sagging, and wrinkled appearance. Granactive Retinoid helps renew skin plumpness, elasticity, and hydration to provide a radiant and fresh appearance. Moreover, Granactive Retinoid stimulates skin cell proliferation; restoring thickness to skin that has become thinner over time. These processes help fill in lines and wrinkles to give a youthful appearance, while safeguarding skin from further wrinkle development. The effectiveness of Granactive Retinoid at filling lines and wrinkles can be seen in Image 1. Granactive Retinoid is highly recommended for promoting clear skin when used in conjunction with monographed anti-acne treatments. This cosmetic ingredient promotes cell turnover and renewal, which translates to improved skin clarity and the appearance of a healthier complexion.

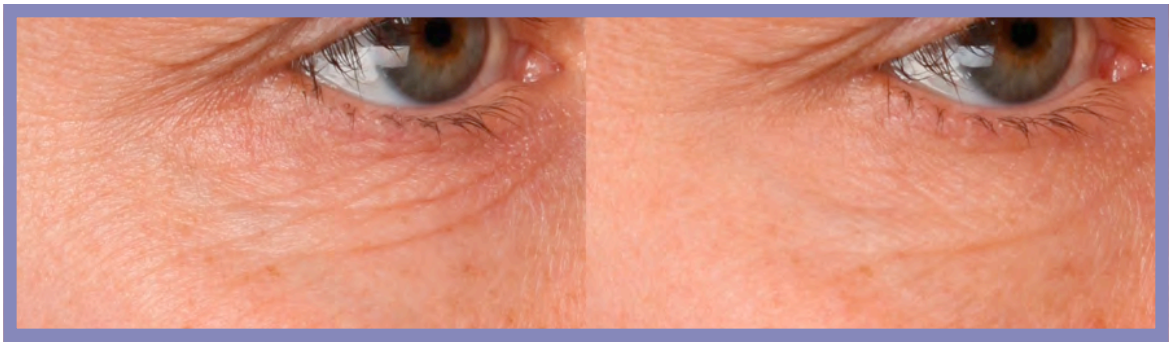


Image 1. Dramatic reduction of lines and wrinkles was observed after 14 day Granactive Retinoid application.

CHALLENGES WITH OLDER GENERATION VITAMIN A CHEMISTRIES

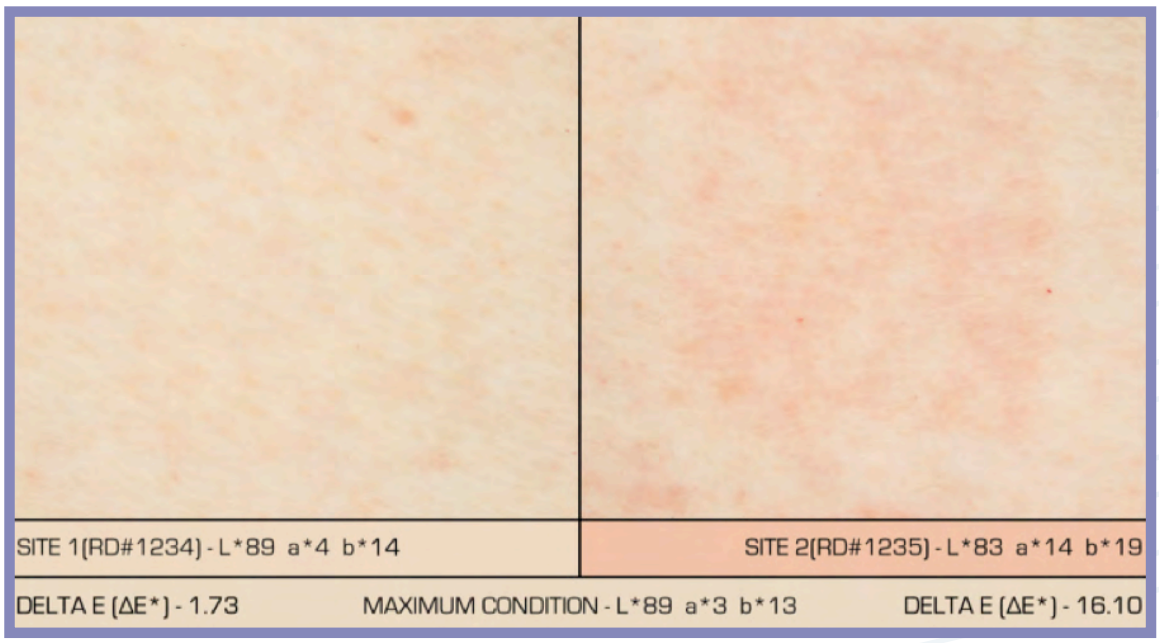
Although the benefits of retinoids have been known for decades, skin irritation, photochemical instability, and toxicity concerns have hindered their use. Retinoic acid is a topically applied ingredient recognized for its anti-aging benefits, however it can be irritating to skin and is sold as a prescription medication. Instead in recent years, milder synthetic and natural derivatives have become popular alternatives to Retinoic acid. These derivatives are metabolized to the active form by skin cells. Retinol (Vitamin A) is the most popular topical retinoid used to date, but its skin irritancy and instability to sunlight has limited its scope and appeal. Retinol esters are often used to lower the irritation potential and increase stability, but a tradeoff is decreased retinoid activity and benefits.

GRANACTIVE RETINOID ADVANCES OPPORTUNITIES IN VITAMIN A SKIN CARE

Granactive Retinoid is a next-generation anti-aging product, delivering the performance of retinol and retinoid derivatives with significantly lower irritation potential, thus supporting clear, visibly more youthful looking skin with better consumer acceptance. The mechanism of action of Granactive Retinoid is advanced compared to retinol derivatives. To interact with retinoid receptors, retinol must first be metabolized to more active forms, such as retinaldehyde and retinoic acid using several enzymatic steps. Granactive Retinoid is unique in that it processes innate retinoic activity, binding directly with retinoid receptors without the need for metabolic breakdown to more biologically active forms. Granactive Retinoid is dermatologically tested to offer less irritation potential than retinol, providing a gentle, safe and effective anti-aging retinoid.

GRANACTIVE RETINOID ACTIVE - CLINICAL RESULTS		
Assay	**Subject**	**Result**
Cumulative irritation patch	Human clinical panel	No irritation
Local irritation and sensitization potential assay	Human clinical panel	No adverse experiences
Skin roughness	Human clinical panel	50% improvement
Skin surface scaling	Human clinical panel	40% improvement
Skin irritation potential vs. retinol	Human skin cells	Lower irritation potential
Toleration under environmental stresses vs. retinol	Human skin cells	Better toleration
Retinoid gene expression modulation	Human skin cells	Typical retinoid expression
In vitro percutaneous penetration	Human skin	Better toleration
In vivo percutaneous absorption	Human skin	Passed – proven safe

These results add credence to the beneficial safety and irritation profile of Granactive Retinoid as a topical cosmetic. The low irritation profile of Granactive Retinoid versus retinol was demonstrated on a 24 hour occlusive patch test and can be seen in Image 2.



GRANACTIVE RETINOID **RETINOL**

Image 2. After application on a 24 hour occlusive patch test, Granactive Retinoid demonstrated a significantly lower irritation profile versus retinol. Test samples were 0.5% retinoid in Gransolve DMI (dimethyl isosorbide).

PERFORMANCE

Granactive Retinoid combats the appearance of fine lines and wrinkles for a younger appearance while increasing skin elasticity for firmer, smoother skin texture. Overall skin clarity and appearance is enhanced for a healthier complexion. Dark spots and skin imperfections are reduced for a more even skin tone to give a brighter and more luminous appearance to skin. Reduction in pore size is observed leading to overall skin clarity. Granactive Retinoid delivers high performance compared to retinol derivatives, but with a significantly lower irritation potential.

FORMULATION

Granactive Retinoid is intended to support anti-aging and anti-acne beauty treatments by providing low irritating, effective active delivery in daily maintenance applications. Granactive Retinoid is available as a concentrated and easily formulated anhydrous matrix containing 10% of the retinoid active in a premium delivery solvent. This offers easy formulation into oil-in-water emulsions, water-in-oil emulsions, and other formula types. Applications include (but are not limited to) skincare, color cosmetic, and body care formulations. For anti-acne claims, the retinoid must be co-formulated with an approved anti-acne active as subject to regional laws. Granactive Retinoid should be added to the oil phase of all emulsion types with a clinical use level of 1-2%, but higher levels are possible without irritation, depending on the formula. It is recommended to formulate this product in a manner that avoids infusing air/oxygen into the final cosmetic product. Like all retinoids, the highly specific set of conjugated double bonds are susceptible to oxidation; anti-oxidants like Vitamin- E and BHT/BHA are suggested.

Contact us today to discover the ways we help ensure your product's performance is flawless, or learn more at **www.grantinc.com**.

GRANT INDUSTRIES
Where Performance Matters

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Where Performance Matters
USA: 103 Main Ave., Elmwood Park, NJ 07407 | (201) 791-8700 | www.grantinc.com

Granactive Retinoid

THE POWER OF RETINOL WITHOUT THE IRRITATION

Presented by John Gormley
Director of Regulatory Affairs

Granactive Retinoid:

"A next generation anti-aging active for cosmetics"

High performing nonprescription retinoid ester

Anti-aging

Improved fine lines and wrinkles

Non-irritating

(as compared to retinol)

Improved skin clarity

Easy to formulate

Brighter, more youthful appearance for healthier complexion

Partner over Supplier

Balancing the Activity Profile of Retinoids

Growth
Vision
Reproduction
Immunity
Cancer prevention
Normal - Healthy skin

Skin irritation
Instabilities
- O_2
-Photochemical
-Heat
Toxicity –high oral IU's
Regulatory

Retinol: A highly recognized active for cosmetics and dermatology

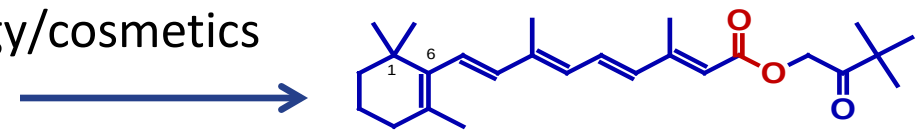
Use to smooth wrinkles, unclog pores, lighten superficial brown spots, improve the texture of the skin…

…BUT with a well documented irritation profile

Partner over Supplier

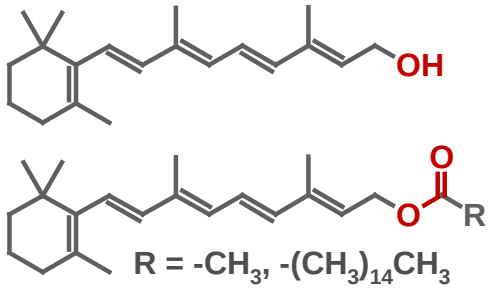
The "all trans" retinoid family

- **Retinoic Ester -** Dermatology/cosmetics
  - ***Granactive Retinoid***

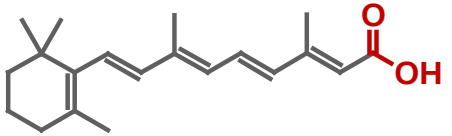


Hydroxypinacolone Retinoate (HPR)

- Retinol – Dermatology/cosmetics
- Retinol Esters –Cosmetics
- Retinal – Vision / cosmetics



- Retinoic acid (RA)
  - Rx – drug (standard for receptor activity)

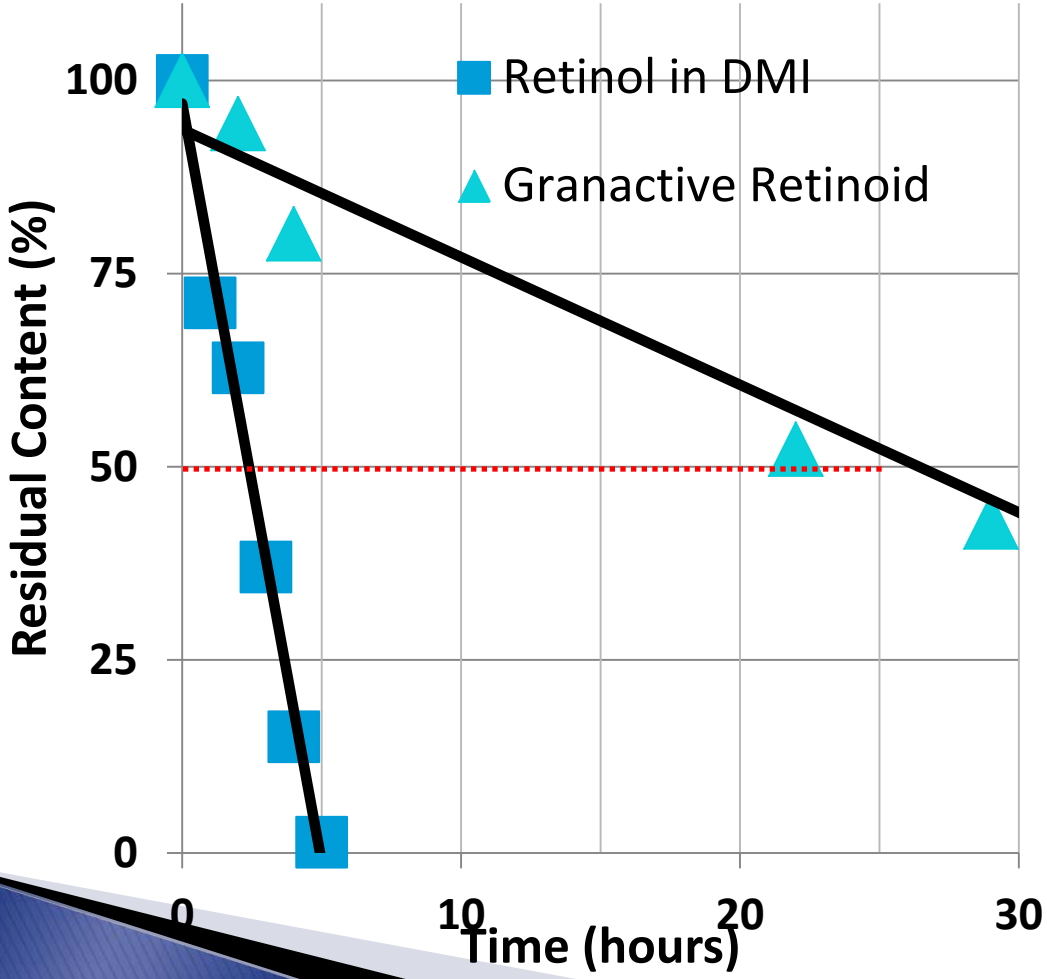


Partner over Supplier

Retinoids: Whats the difference?

	Benefits	Drawbacks
Granactive Retinoid (Hydroxypinacolone Retinoate)	Low irritation index Enhanced activity Correct oxidation state Improved stability	UV / oxygen
Retinol	Well characterized activity Well known in market	UV / oxygen Skin irritation Lowest stability Lowest oxidation state
Retinol Ester	Low irritation index Well known in market Improved Stability	UV / oxygen Lowest activity Lowest oxidation state
Retinal	Potentially more effective than retinol if enzymatic metabolism to retinoic acid is the target	UV / oxygen Poor shelf stability Not in final RA oxidation state
Retinoic Acid	Anti aging recognized by FDA	Prescription medication Irritation UV / oxygen

HPR 10x stability over retinol 50°C



0.1% by wt. retinoid in DMI, low Actinic glass, N2 atm, 50°C water bath , shaken samples HPLC results.

NO ANTIOXIDANTS INCLUDED

Partner over Supplier

Granactive Retinoid

- INCI: Dimethyl Isosorbide (and) Hydroxypinacolone Retinoate
  - 10% retinoid active (in **Gransolve DMI)**
    - **BHT/BHA free**

- **Gransolve DMI:**
  - Solvent for actives, including retinoids
    - Both water and oil compatibility
  - Safe /mild to use on skin and around the eyes
  - Enhances penetration of retinoid and actives to the epidermis

Granactive Retinoid:
Applications and Formulation

- Emulsions, serums and anhydrous systems
  - Add to the oil phase of all emulsions, 1-3% use level
  - Recommended in night time and treatment applications
- Co-formulated other active ingredients, photo-stabilizers, and even sunscreens for daily wear.
  - Formulas may be more stable that individual raw materials
- Use Conditions that favor stabilized retinoid compositions:
  - antioxidants, chelating agents, neutral pH, dark and air-tight containers

Granactive Retinoid:

Skin renewal

Image 1. Dramatic reduction of lines and wrinkles was observed after 14 days Granactive Retinoid (2%) application. Demo formula – Retinoid Serum. Applied twice daily.

Image 2. After application on a 24 hour occlusive patch test, hydroxypinacolone retinoate demonstrated a significantly lower irritation profile versus retinol. Test samples were 0.5% retinoid in Gransolve DMI .

Partner over Supplier

Granactive Retinoid:

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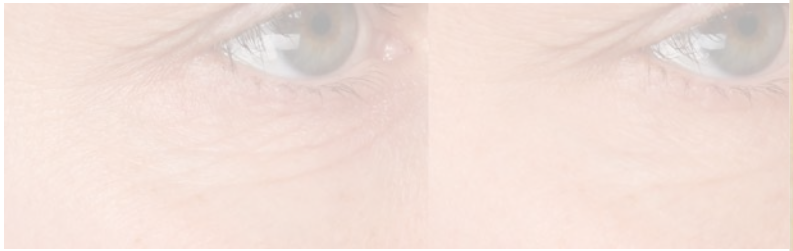
Partner over Supplier

Granactive Retinoid:

No irritation



Overall skin color →

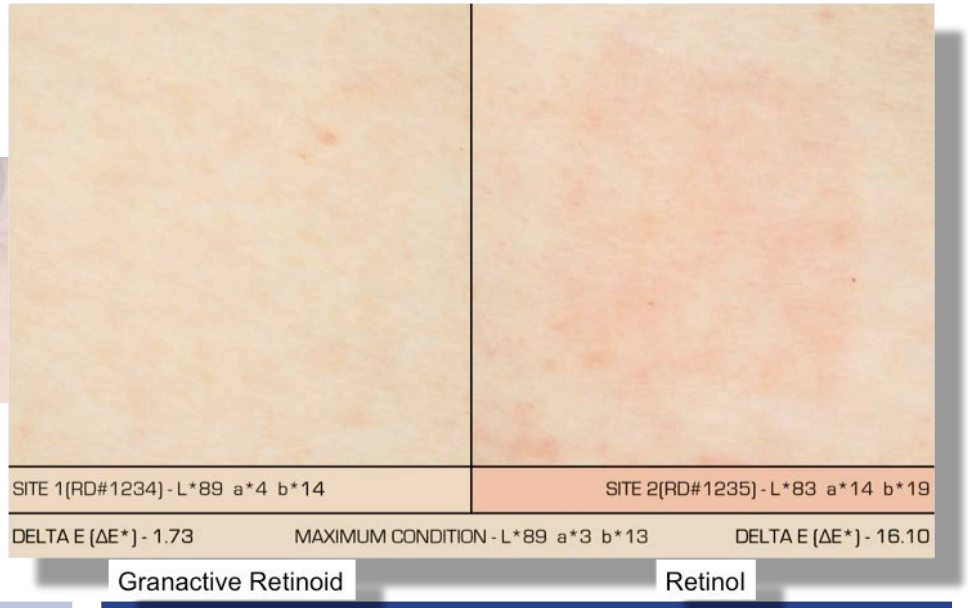


Image 1. Dramatic reduction of lines and wrinkles was observed after 14 day Granactive Retinoid application.

Image 2. After application on a 24 hour occlusive patch test, hydroxypinacolone retinoate demonstrated a significantly lower irritation profile versus retinol. Test samples were 0.5% retinoid in Gransolve DMI .

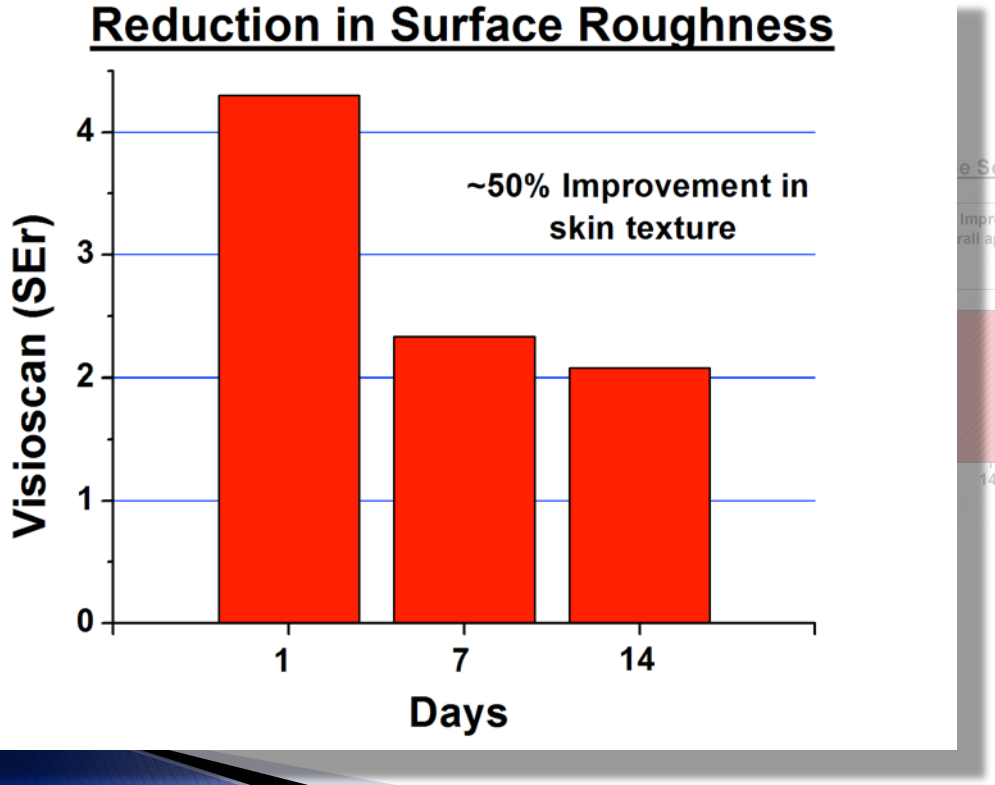
Partner over Supplier

Granactive Retinoid:
No irritation or sensitization found

- 24hr Patch Test 0.5% HPR in DMI (vs 0.5% Retinol)
  - See Image on prior slide
- Human Repeat Insult Patch Test (HRIPT)
  - 50 subjects, 9 Inductions
    - 1) Demo formula incl. **0.2% HPR**
    - 2) **0.5% HPR** in Silicone elastomer + petrolatum vehicle
      - **Both tests - No irritation after 21 days**
- Sponsored clinical -21 day cumulative irritation test
  - **0.1% HPR test emulsion– zero irritation**

Granactive Retinoid:

Reduction in surface roughness

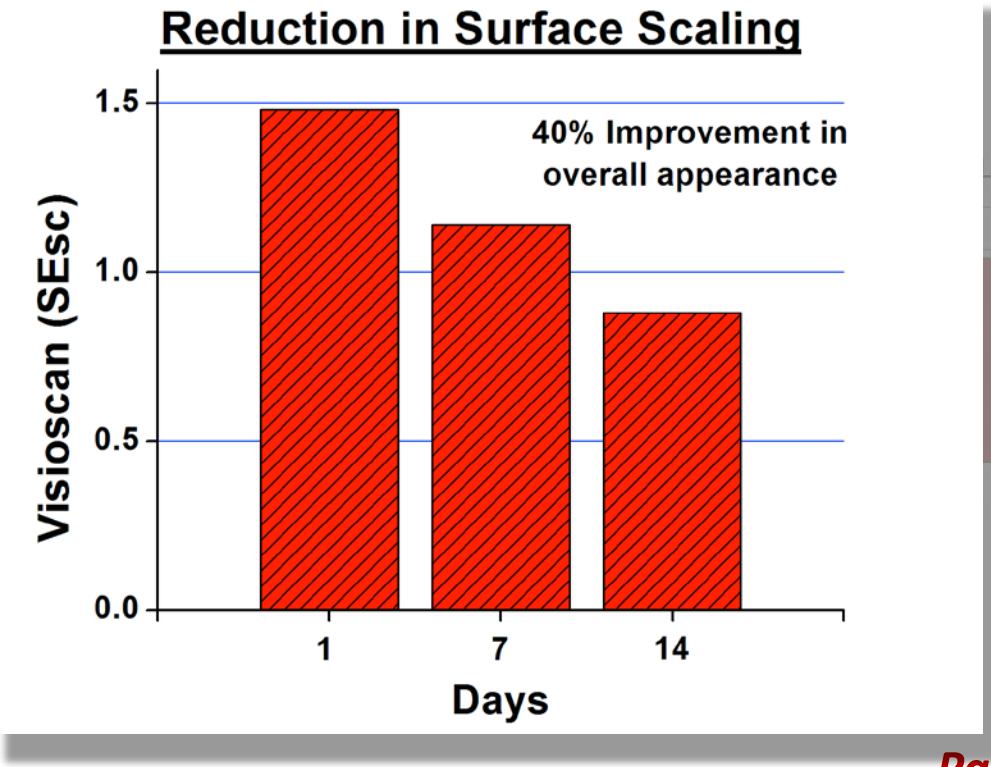


Test formula:
Skin Lightening Cream

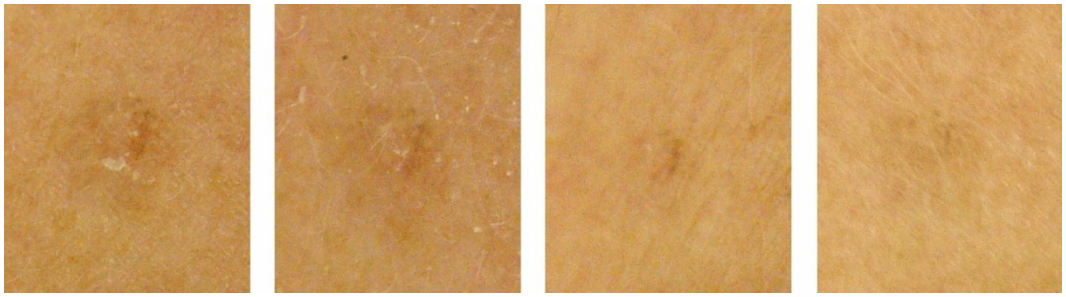
Partner over Supplier

Granactive Retinoid:

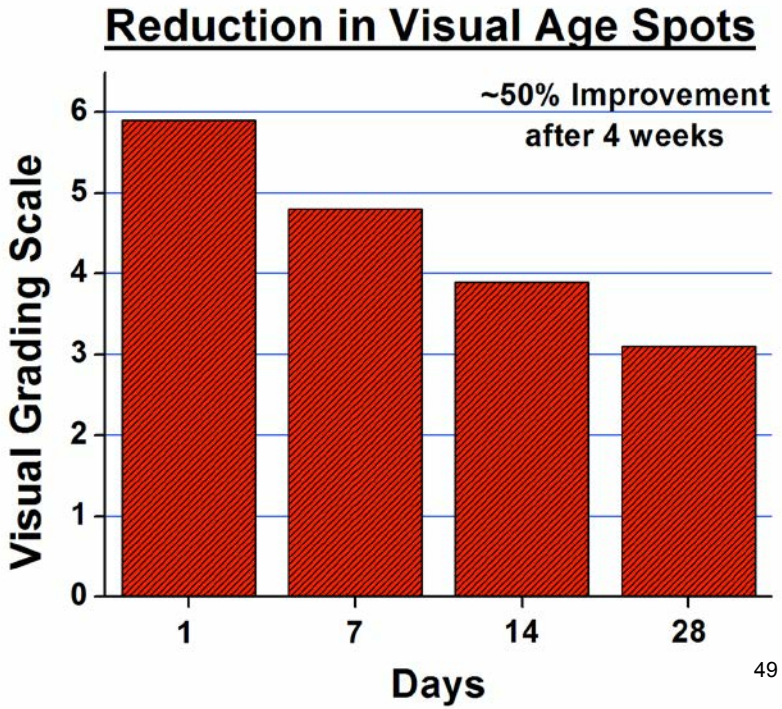
Reduction in skin scaling



Granactive Retinoid: Reduction in age spot



Contributes to
Skin Lightening / Brightening



Granactive Retinoid:

14 Day Questionaire Summary

- 100% panelist agreed significantly with:
  - Reducing roughness and dryness
  - Improving:
    - the skin's softness and smoothness
    - skin's radiance, tone and clarity
    - the skin's firmness, tightness and elasticity
    - the skin's overall appearance

Granactive Retinoid:

14 Day Questionaire Summary

- 80% of panelist agreed significantly with:
  - Reducing appearance of fine lines and wrinkles
  - Diminishing appearance of age spots and skin discolorations
  - Improving the texture of sun-damaged skin

Granactive Retinoid:

Assay	Subject	Result
Cytotoxicity vs. retinol palmitate	In-vitro Cells lines	Better toleration
Retinoid gene expression modulation	In-vitro Cells lines	Typical retinoid expression
In vitro percutaneous penetration Franz-Cell diffusion Radio-labeled HPR	Human skin	Passed – proven safe
In vivo percutaneous absorption Radio-labled HPR	Human Clinical	Passed – proven safe
Retinoid Receptor Assay (RAR, RXR)	In-vitro Cells line	Good Activity on two RAR receptors

THANK YOU
ANY QUESTIONS?

STAND
NUMBER: 6D60

Granactive Retinoid

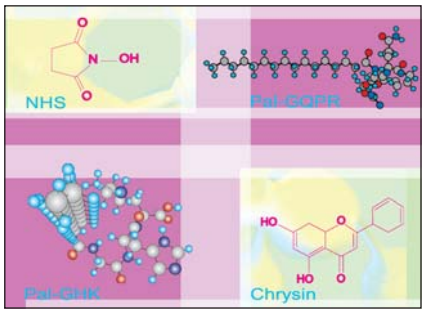
THE POWER OF RETINOL WITHOUT THE IRRITATION

- Contact John Gormley jgormley@grantinc.com

Key Contributions by: Dr. Ron Lerum ***Partner over Supplier***

Patent N° WO 2005/102266

HALOXYL™



Composition of HALOXYL™

Function:
Lessens under eye dark circles.

Definition:
Association of 2 matrikines: Pal-GHK and Pal-GQPR with N-hydroxysuccinimide (NHS) and a flavonoid: chrysin.

Properties:
Pal-GHK and Pal-GQPR reinforce firmness and tone of the eye area. Chrysin and N-hydroxysuccinimide activate the elimination of blood originated pigments responsible for dark circle color and local inflammation.

Characteristics:
Infra-orbital shadows are due to the accumulation of hemoglobin and its colored degradation products (biliverdin, bilirubin and iron) in the dermis and epidermis. Chrysin stimulates the enzyme (UGT_1A_1) leading to the clearance of bilirubin. N-hydroxysuccinimide makes the iron soluble for elimination.

INCI name:
(Check CTFA on-line dictionary for latest INCI name)

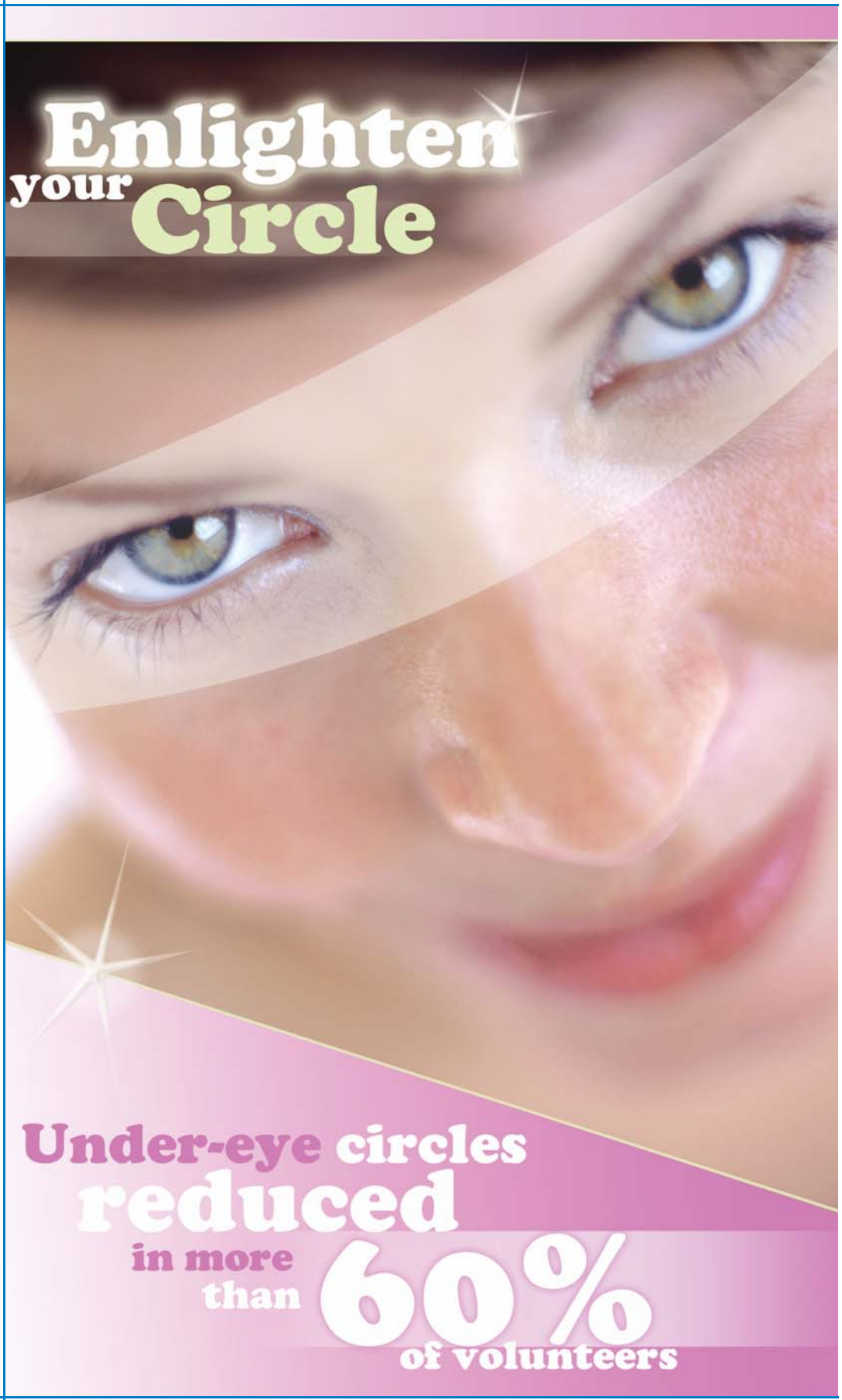
Water (Aqua) – Glycerin – Steareth-20 – N-Hydroxysuccinimide – Chrysin – Palmitoyl Oligopeptide – Palmitoyl Tetrapeptide-7*

* former INCI name: Palmitoyl Tetrapeptide-3

Applications:
Dark-circle treatments, eye contour care, concealers.

Formulation:
Water soluble.
Incorporate at 45°C in emulsions or at room temperature in gels.

Recommended use level:
2%



In vitro tests

- **Ability of NHS to bind iron**

  The decrease of color demonstrates the iron complexation by N-hydroxysuccinimide.

- **Anti-inflammatory effect**

  Measurement of the decrease of PGE2 release by keratinocytes and fibroblasts after UVB irradiation, with HALOXYL™.

  HALOXYL™ demonstrates anti-inflammatory properties similar to those of aspirin.

- **Stimulation of expression of UGT**

  Cells in culture are incubated for 3 days with chrysin. The gene expression for UGT_1A_1 is determined by RT-PCR.

  Chrysin strongly stimulates the expression of the enzyme involved in the clearance of bilirubin (end product of hemoglobin degradation).

In vitro

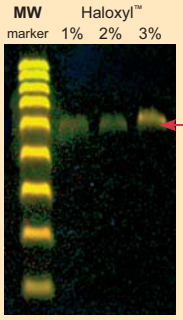
Iron complexation by NHS

N-hydroxysuccinimide binds iron to make it soluble for elimination

Increasing iron complexation by NHS

In vitro

Gene amplification



Product	Gene Amplification
Chrysin 7.8µM (eq. 2% Haloxyl™)	+247%
Chrysin 11.8 µM (eq. 3% Haloxyl™)	+600%

Clinical study: Anti-dark circle efficacy

22 female volunteers applied to the contour of one eye a gel containing 2% HALOXYL™ for 56 days against placebo on the other one. The anti-dark circle effect is assessed by image analysis and measurement of the color parameters (L,a,b system) by a specific software.

	Δa	Δb
Variation	-12.5%*	+10%**
Rate of volunteers with improvement	72%	63%
Variation for volunteers with improvement	**-19.5%**	**+19%**

*significant / T0 (p<0.01) **significant /T0 (p<0.05)

Red and blue colors of dark circles significantly decreased by 19%

Formulation

Anti-Dark Circle Gel with HALOXYL™

Tested formulation ref.: SED0308383 D1t

Part A	%
Deionized water	qs 100
Ultrez 10 *(Carbomer, Noveon)*	0.30
Part B	**%**
Glycerin	5.00
Preservatives	qs
Part C	**%**
Hydroxyethyl Cellulose	0.30
Part D	**%**
Pemulen TR2 *(Acrylates / C10-30 Alkyl Acrylate Crosspolymer, Noveon)*	0.20
Crodamol CAP *(Cetearyl Ethylhexanoate, Croda)*	6.00
Part E	**%**
Potassium sorbate	0.10
Part F	**%**
Deionized water	4.00
Sodium hydroxide 30%	0.46
Part G	**%**
Crillet 1 (Polysorbate 20, Croda)	0.50
Part H	**%**
HALOXYL™ (Sederma)	2.00

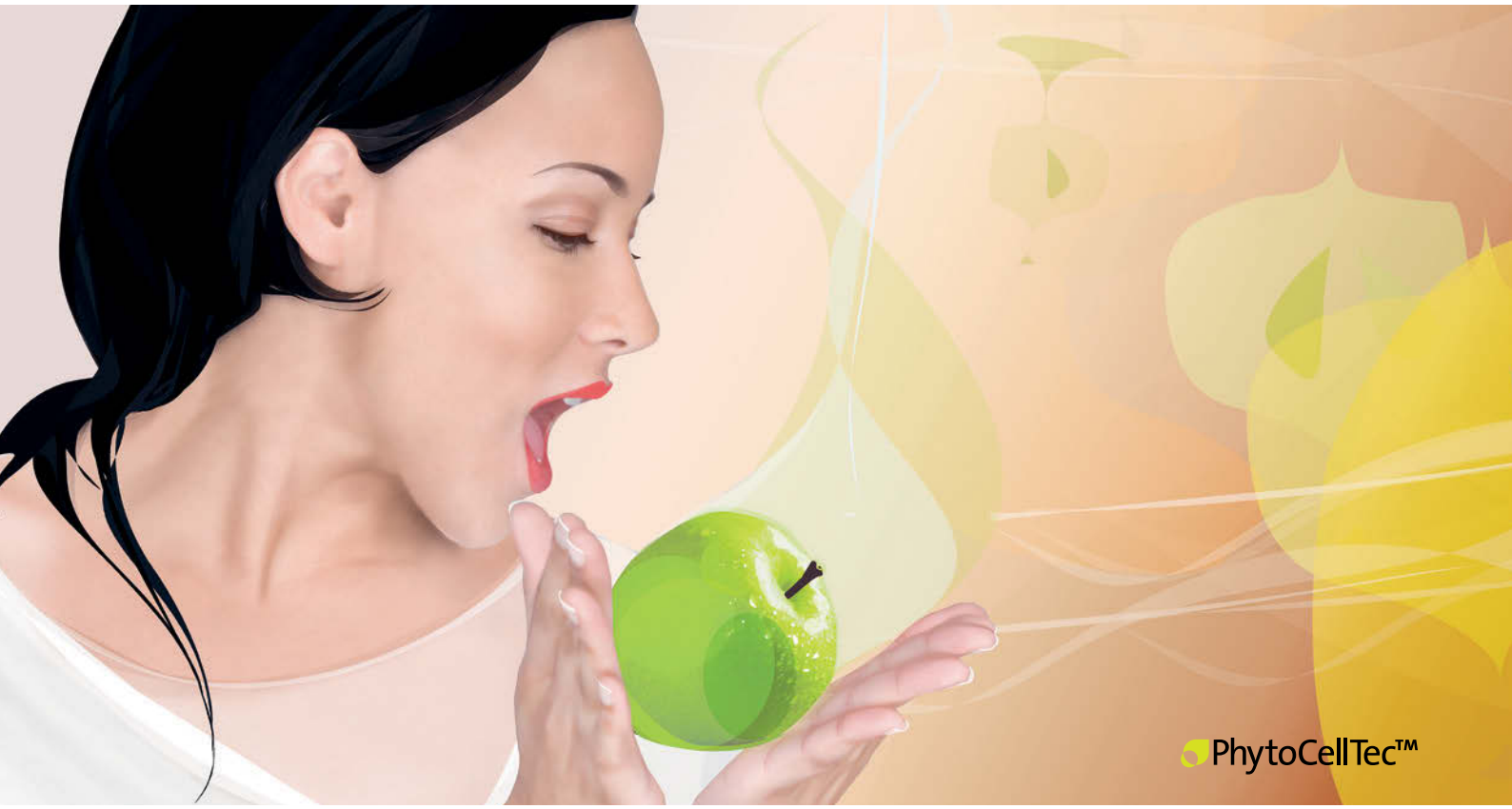
Protocol

Part A: Sprinkle Ultrez 10 in water and allow to swell for 15 minutes. Part B: heat the glycerin to 60°C, dissolve the preservatives. Cool to 40°C. Add Part C to Part B, homogenize, then add Part B+C to Part A with helix stirring. Allow to swell for 1 hour. Add Part D, then Part E to Part (A+B+C), homogenize. Neutralize with Part F. Let swell for 1 hour. Incorporate Part G, homogenize, then add Part H.

Non-warranty:
This formulation has been subjected to limited stability tests and has been shown to perform well. However formulators adopting this approach should ensure to their own satisfaction long term stability and functionality. It is good practice to conduct safety tests on all final formulations prior to marketing. Suggested uses should not be taken as an inducement to infringe any existing patents.

Sederma Inc E-mail: marketing@crodausa.com www.crodausa.com Tel 732-692-1652 Fax 732-417-0804

mibelle GROUP biochemistry



PLANT STEM CELLS
PhytoCellTec.ch™
SKIN CELL PROTECTION

PhytoCellTec™ Malus Domestica
Plant stem cells for skin stem cell protection

SWISS QUALITY PRODUCT

PhytoCellTec™ Malus Domestica
Plant stem cells for skin stem cell protection

A Revolutionary Technology to Protect Skin Stem Cells
PhytoCellTec™ Malus Domestica is a liposomal preparation based on the stem cells of a rare Swiss apple.

Uttwiler Spätlauber is an endangered apple variety that was well-known for its excellent storability and thus its longevity potential.

Mibelle Biochemistry has developed a novel technology enabling the cultivation of rare and endangered species like Uttwiler Spätlauber. Thanks to this technology called PhytoCellTec™, plant stem cells can be obtained and incorporated into cosmetic products to ensure the longevity of skin cells.

Studies showed the double activity of PhytoCellTec™ Malus Domestica:
- it helps skin stem cells to maintain their characteristics and their capacity to build new tissues
- it delays aging and has an anti-wrinkle effect.

PhytoCellTec™ Malus Domestica is the first plant stem cell active ingredient on the market whose effect was evaluated on human skin stem cells. This unique and revolutionary ingredient is able to protect the most precious skin cells, the skin stem cells, against premature aging.

Claim Ideas for PhytoCellTec™ Malus Domestica
- Protects longevity of skin stem cells
- Delays senescence of essential cells
- Increases the vitality of skin stem cells
- Combats chronological aging

Applications
- Advanced "stem cell cosmetic" formulas
- Real rejuvenation for face and body care
- Innovative skin care formulations

Formulating with PhytoCellTec™ Malus Domestica
- Recommended use level: 2–5%
- Incorporation: For cold processes, mix PhytoCellTec™ Malus Domestica into the aqueous phase. In cold/hot processes, add during the cooling phase below 40° C.
- Thermostability: Temperatures of up to 60° C for a short time do not affect the stability of PhytoCellTec™ Malus Domestica.

INCI/CTFA-Declaration
Malus Domestica Fruit Cell Culture Extract (and) Xanthan Gum (and) Glycerin (and) Lecithin (and) Phenoxyethanol (and) Aqua/Water

Additional Information
- PhytoCellTec™ Malus Domestica contains 9% of Malus Domestica stem cell extract
- Available in a phenoxyethanol-free version
- Available in powder form

Patent pending

RENOVAGE™

Geranylgeranone

Function:
Comprehensive anti-ageing.

Definition:
Geranylgeranone (GGA) in a lipophilic solvent.

Properties:
Fights against all signs of ageing:
functional: dehydration, age spots, ineffective barrier function.
structural: ptosis, wrinkles, pores, erythrosis.

Characteristics:
Anti-ageing and protective effect against stress, by telomere stabilization and DNA maintenance (cell division).
Improvement of tissue quality by optimal cell interactions (cell communication).
Rebalance of cell functions (metabolism).

Point of interest:
By delaying senescence, Renovage™ can extend cell lifespan by 1/3.

INCI name (proposed):
(Check CTFA on-line dictionary for latest INCI name)
Caprylic/Capric Triglyceride
Teprenone –

Applications:
Anti-ageing creams, gel-creams.

Formulation:
Oil soluble.
Incorporate to oil phase at 60°C prior to forming the emulsion.

Recommended use level:
3%

Warning:
Not to be sold or used in a cosmetic product in Japan.

www.sederma.fr E-mail: sederma@sederma.fr

Protocol: Simple blind study including 24 women, 58±6 years old, with wrinkles and not having used anti-age products for at least 1 month. No intentional UV exposure. Twice daily application of a cream formulated with 3% Renovage™, for 6 months. Evaluation of the functional and structural signs of ageing at 1 and 6 months.

In vivo tests: Efficacy on functional signs of ageing

- **Skin moisturisation** ····· **+30%* up to +58%**, improvement for 100% panellists**
  Measurement by corneometry of the remnant skin moisture 24h after the last application. (*significant/T0 p<<0.01)
- **Epidermal barrier integrity** ····· **+19%* up to +46%**, improvement for 75% panellists**
  Measurement by vapometer of the transepidermal water loss. (*significant/T0 p<0.05)
- **UV Sun spots** ····· **- 42%* up to -56%**, improvement for 100% panellists**
  Measurement of invisible UV spots by VISIA® system under UV light. (*significant/T0 p<<0.01)

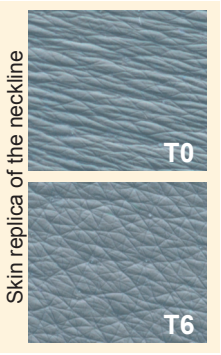
** = 1st quartile

In vivo tests: Efficacy on structural signs of ageing

- **Micro depressionnary network**

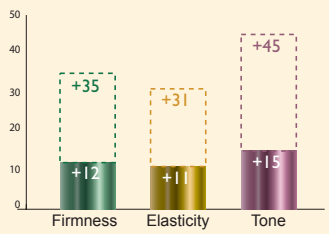
Measurement of roughness, lines and their homogeneity of the forearm (after 1 month) and the neckline (after 6 months), by skin replicas.

Renovage™ significantly improves the surface of the skin.
Skin is smoother, more uniform and looks younger.



% / T0	Forearm Mean	Forearm 1st quartile	Neckline Mean	Neckline 1st quartile
Line homogeneity	+31	+103	+28	+109
Roughness	-11	-26	-14	-36
25-50µm	-4	-22	-12	-38
50-75µm	-13.5	-54	-34.5	-66.5
>75µm	-29	-64	-47	-56

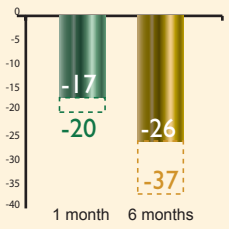
- **Firmness / Elasticity / Tone**
  Measurement by cutometry after 1 month



75% of the panellists had an improvement in skin firmness.

Top 25%
Mean

- **Dilated pores**
  Measurement of dilated pores by Visia® system



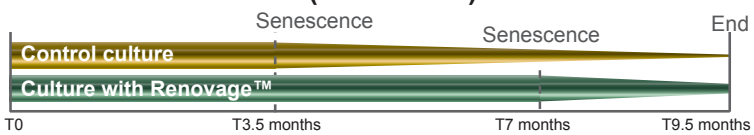
More than **90%** of the panellists had an improvement in pore size.

Top 25%
Mean

- **Erythrosis (redness)** ····· **- 30.5%***
  Measurement of skin redness by Doppler ultrasound after 1 month on 23 panellists. (*significant/Placebo p<0.05)

In vitro tests: Effect of Renovage™ on the functional and structural ageing markers

- **Function loss** ····· **- 60%**
  Apoptotic cells after UVB irradiation, skin explants.
- **Cell resistance** ····· **+75 and +100%**
  HSP 27 & 70 reduction after UVB irradiation, skin explants.
- **Self-defence** ····· **+100%**
  Catalase activity after UVB irradiation, skin explants
- **Wound healing capacity** ····· **+++**
  Cell re-colonization after lesion and irradiation, keratinocytes
- **Skin cell senescence (fibroblasts)**



For a total culture duration of 9.5 months, cell life span is significantly increased by 1/3. With Renovage™, cell senescence is delayed by 3.5 months.

Renovage™ works on the origin of youth and targets the cell actors that ensure lifespan and youth.
Renovage™ creates the optimal conditions in the epidermis and the dermis for visible effects on the skin surface.

Sederma Inc E-mail: marketing@crodausa.com www.crodausa.com Tel 732-692-1652 Fax 732-417-0804

Technical Information

July 2005
Supersedes issue dated May 2005

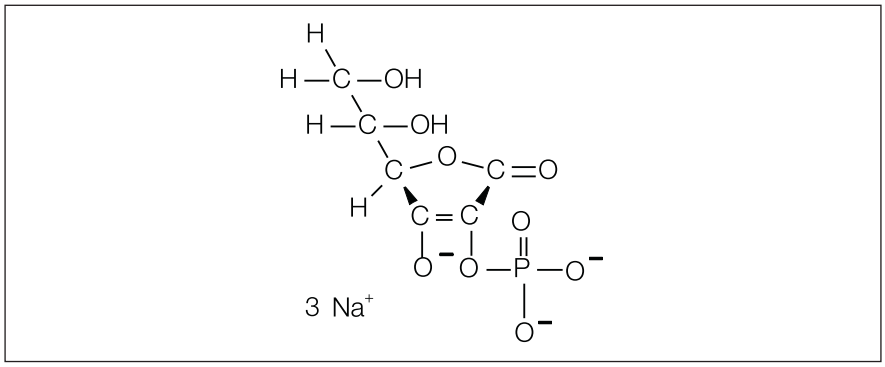
MEMC 050423e-00/Page 1 of 24

Sodium Ascorbyl Phosphate

® = Registered trademark
of BASF Aktiengesellschaft

Active ingredient for the cosmetics industry.
Acts as an in-vivo antioxidant, promotes collagen formation, lightens the skin.

Structural formula



Synonym

L-ascorbic acid-2-monophosphate, trisodium salt

Molecular formula

$C_6H_6O_9Na_3P$

Molar mass

322.05 g/mol

INCI name

Sodium Ascorbyl Phosphate

CAS-No.

66170-10-3

Description

Sodium Ascorbyl Phosphate is a stable vitamin C derivative. The product is a white to pale beige powder with practically no odor.

Specification

Parameter	Requirement	Method
Purity	min. 95%	05/0071.00
Vitamin C content	min. 45.0%	05/0072.00
Identity	Passes test	05/0072.00
Solubility (10% in water)	Solution is clear	-
Water	Max. 11%	05/0070.00
pH value (3% in water)	9-10	05/0073.00
Heavy metals	max. 20 ppm	05/0030.00
Arsenic	max. 2 ppm	05/0030.00

Application

Sodium Ascorbyl Phosphate is an active ingredient for sophisticated cosmetic skin-care products. It is a stable vitamin C derivative. It protects the skin, promotes its development and improves its appearance.
Sodium Ascorbyl Phosphate is cleaved enzymatically in the skin to release active vitamin C.
Sodium Ascorbyl Phosphate is therefore an effective antioxidant which protects the cells against damage caused by free radicals.
Sodium Ascorbyl Phosphate counteracts skin aging in promoting collagen formation.
Sodium Ascorbyl Phosphate also acts on the melanine formation process to prevent hyperpigmentation and senile keratosis. It therefore has skin lightening properties.
Because of its wide spectrum of action, Sodium Ascorbyl Phosphate is suitable for use in a wide range of skin care products.
As an effective watersoluble anti-oxidant which is stable in cosmetic formulation it is the perfect completion to Vitamin E Acetate, which is the common oil-soluble equivalent. The oilsoluble Vitamin E Acetate together with the water-soluble Sodium Ascorbyl Phosphate are the perfect anti-oxidant system in all skin-care formulations which are used against the daily environmental stress for the skin.
Other very important areas of use are sun protection formulations, antiwrinkle products, body lotions, day creams and night creams, and whitening products.

Recommended concentrations

	Sodium Ascorbyl Phosphate
Daily skin care	0.2-2%
Sun care products	0.2-1%
Lightening products	>3%

Solubility

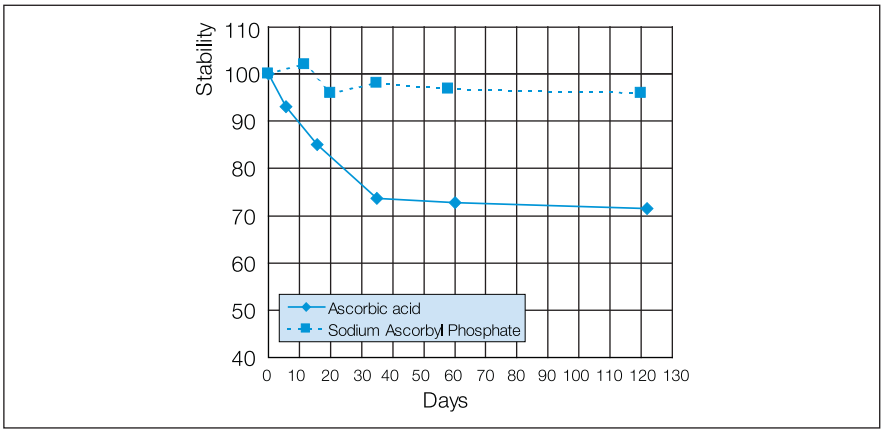
It is possible to prepare solutions of up to 64% in water, 13.2% in glycerol and 1.6% in propylene glycol with sodium ascorbyl phosphate. It is practically insoluble in ethanol, isopropyl myristate, cetostearyl octanoate, caprylic/capric triglyceride and C12-15 alkyl benzoate.

Stability/Storage

The product should be stored and transported in the original sealed containers, protected from light and moisture, at temperatures below 25°C. Contact with metals should be avoided. The product is stable for at least 24 months if stored in the original sealed containers at 25°C.

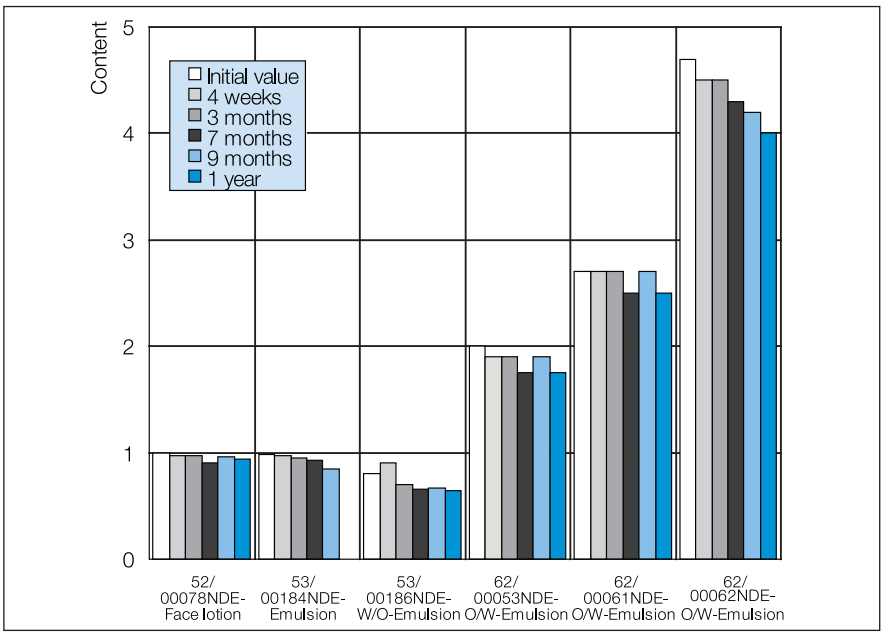
Stability in water and cosmetic formuations

Sodium Ascorbyl Phosphate is far more stable than Ascorbic Acid in water. Stability of Sodium Ascorbyl Phosphate and ascorbic acid in 3% solutions in water at 40°C and pH 6:



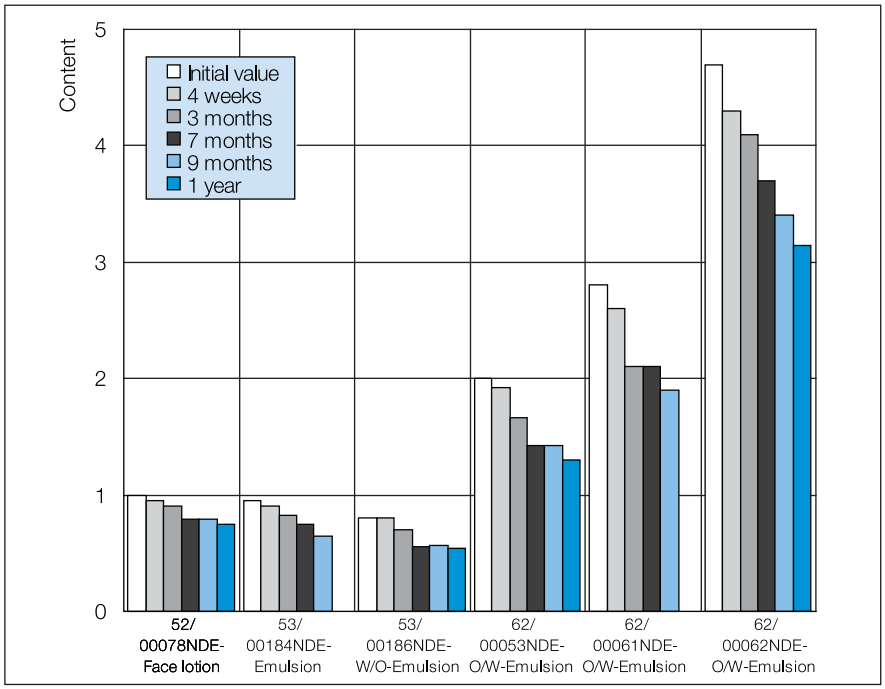
Sodium Ascorbyl Phosphate is stable in a wide range of formulations (see typical formulations).

Stability of Sodium Ascorbyl Phosphate in different formulations (see typical formulations) at 20°C, pH 6.5:



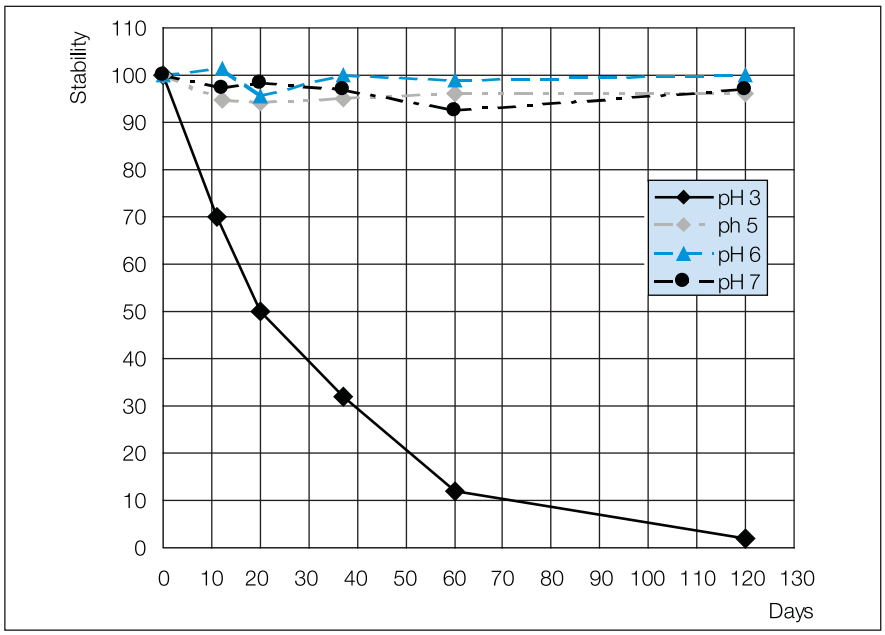
When the formulations were kept at 40°C, they became discoloured after about 2 months, and assumed a pale beige colour. Finished products should therefore be stored at temperatures below 25°C.

Stability of Sodium Ascorbyl Phosphate in different formulations at 40°C, pH 6.5:

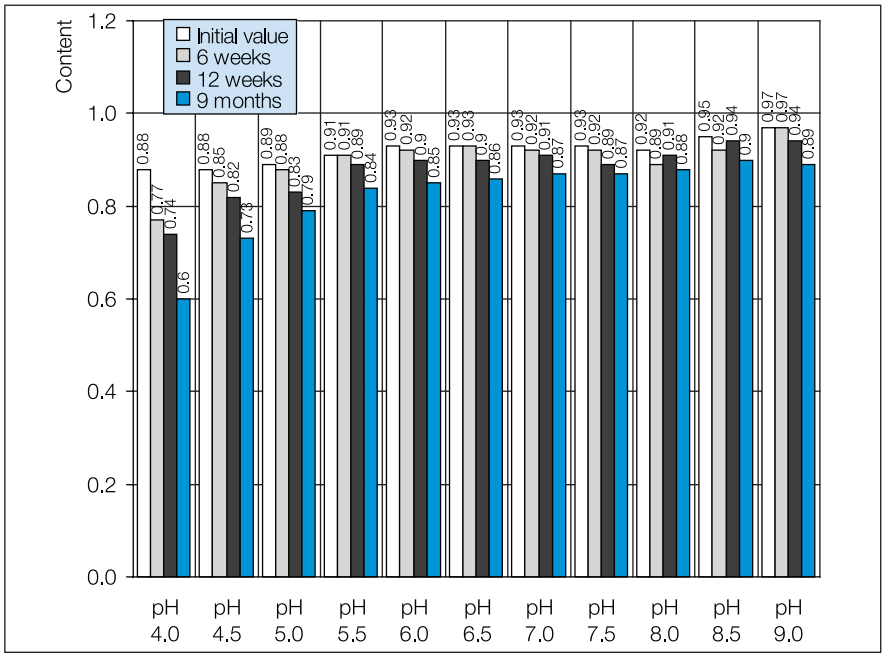


The stability of the product depends very much on the pH value of the formulation. The best stability is obtained at pH values above 6.5.

Stability of Sodium Ascorbyl Phosphate 3% in water at different pH values at 40°C:



Stability of Sodium Ascorbyl Phosphate approx. 1% in formulation 62/00082NDE at different pH values at 20°C:



Technical properties and handling

Sodium Ascorbyl Phosphate is a crystalline solid that is sensitive to heat, moisture, low pH values and heavy metals.
In the production of cosmetic care products, it is recommended to add Sodium Ascorbyl Phosphate to formulations at a low temperature (<40°C).
It can be exposed to higher temperatures up to 80°C, but only for a short time.
The product is most stable above pH 6.5. It is recommended to use a buffer system and to add a chelating agent.
Finished formulations should be stored at a temperature below 25°C.

Safety Data Sheet

A Safety Data Sheet is available for Sodium Ascorbyl Phosphate. It contains the main results of the toxicological studies.

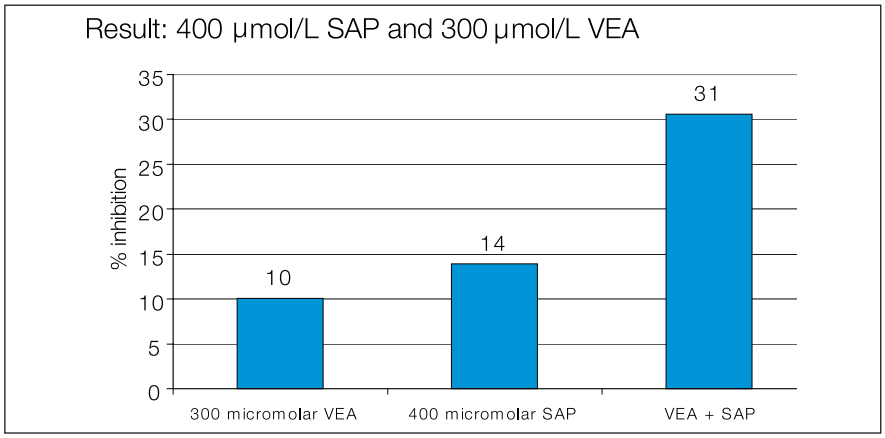
Antioxidant activity and Synergy with Vitamin E:

Results of in-vitro study

Introduction
This study is able to show the synergistic action of Vitamin E and Vitamin C working together as anti-oxidants in the human skin.
Living human keratinocytes (HaCaT cells) were chosen as an in-vitro model.
Due to the reduced stability of Tocopherol (Vitamin E) and Ascorbic Acid (Vitamin C) in cosmetic formulations pro-drugs are used, typically Vitamin E Acetate and Sodium Ascorbyl Phosphate, respectively. They were therefore used in this in-vitro cell test.
The HaCaT-cell system contains the esterases and phosphatases needed to convert the pro-drugs into the active form.
Sodium Ascorbyl Phosphate is water soluble and can be used as such in this aqueous cell system. Vitamin E Acetate is insoluble in water and has to be brought into solution with a vehicle. To keep the conditions as simple as possible, ethanol was used as vehicle. Vitamin E Acetate was dissolved in 0.1% ethanol. A control experiment ensured that the vehicle (0.1% ethanol solution in water) has no disturbing effect.
Due to different kinetics of the cleavage of the prodrug into the active form, the optimum reaction time had to be determined empirically in preliminary experiments. It could be shown that a reaction time of 48 hrs. for Sodium Ascorbyl Phosphate and 7 days for Vitamin E Acetate are the ideal conditions. (The compounds are stable in water during this time.) If a combination was tested, Vitamin E Acetate supplementation started 5 days before adding Sodium Ascorbyl Phosphate.

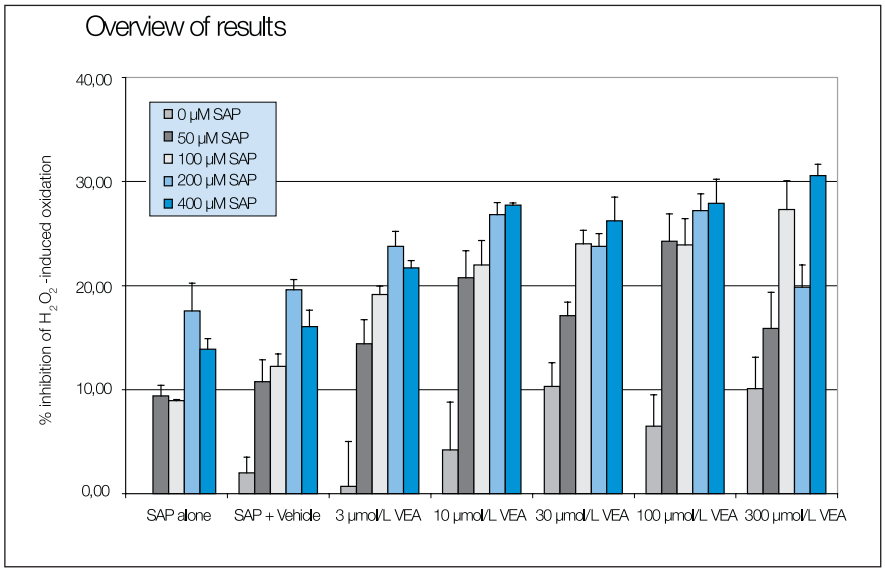
Results

Human keratinocytes (HaCaT-cells) were supplemented with Vitamin E Acetate (VEA) diss. In 0.1% ethanol for seven days and/or Sodium Ascorbyl Phosphate (SAP) for 48 hrs. The following concentrations were tested alone and in combination with the other active ingredient.
VEA: 3, 10, 30, 100, 300 micromolar
SAP: 50, 100, 200, 400 micromolar
The anti-oxidant effect was determined in measuring the ability to inhibit hydrogen-peroxide induced oxidation. The cells were incubated with the fluorescence label DCFH. The oxidative stress was induced with 200 micromolar hydrogen peroxide. (These a very harsh conditions.) The capability of VEA and SAP to inhibit oxidation was measured in determing the resulting fluorescence.



The chart above shows an example with both SAP and VEA at high concentrations.
With VEA alone in a concentration of 300 micromolar the inhibition of oxidation is 10%. With SAP alone in a concentration of 400 micromolar the inhibition of oxidation is 14%.
If VEA and SAP are used together in the above mentioned concentration, the inhibition of oxidation is over 30%. This is a synergistic effect, because the theoretical addition of the two ingredients results only 24%.
The effect is much higher than with the single compounds alone and even higher than the theoretical addition.

The following chart shows all the results together.



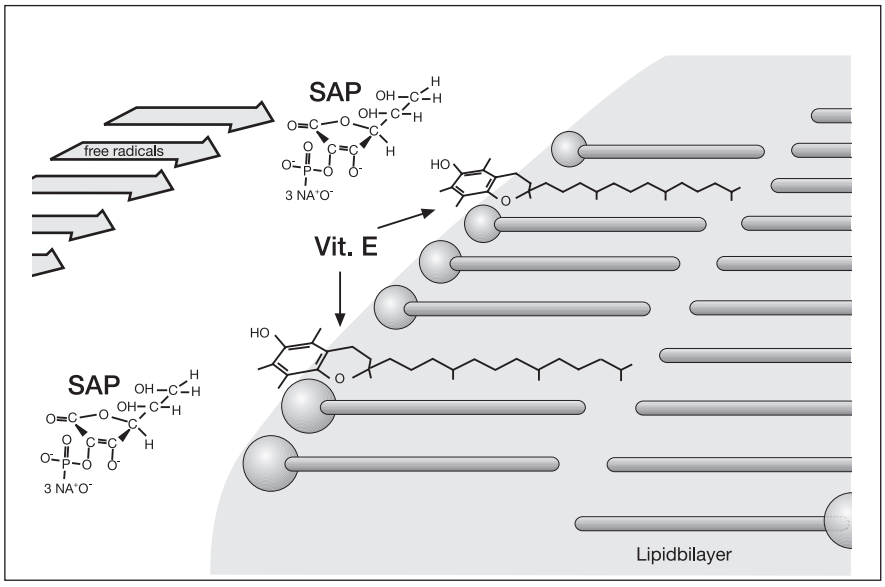
First column is SAP alone at different concentrations. The effect is dose dependant, because it increases with the concentration. However, a saturation occurs. From a certain concentration level on, an increase in concentration does not lead to a higher effect. The fact, that 200 µmol/L gives the highest effect with 18% while the concentration of 400 µmol/L is a bit lower should not be overrated. This will be due to margin of error.

The second column is the control experiment. The cells are in an aqueous environment. SAP is soluble in water, so not a problem. Vitamin E Acetate (VEA) is oil-soluble, so it has to be brought into solution with a vehicle. This vehicle is 0.1% Ethanol, so a simple dispersion. The results shown in this column are the same as in the first column without the vehicle (within margin of error). Therefore the vehicle does not have an effect. The results for VEA in this test-system are trustworthy.
The columns 3-7 show the results with VEA alone and the results of the experiments with the combination of VEA and SAP.
The results of VEA alone are the blue ones, always the first column in one group. The concentration increases to the right. The effect is similar to the one of SAP, however lower in value. Increase of concentration leads to a higher effect and again there is a saturation effect. At a certain point an increase of concentration does not lead to a higher effect. Maximum effect is 10% inhibition.
All the other columns represent combinations of SAP and VEA. It becomes clearly visible that much higher effects can be obtained if a combination of SAP and VEA is used compared to the values where only one ingredient is used.

Summary of in-vitro study and conclusions
The maximum inhibition of hydrogen peroxide-induced oxidation with Sodium Ascorbyl Phosphate (SAP) and Vitamin E Acetate (VEA) alone are 18% and 10%, respectively.

Higher values of inhibition can be obtained only if a combination of SAP and VEA is used.

Due to their different solubilities SAP protects the aqueous cytosol part of the system, while VEA is incorporated into the oil-soluble cell-membranes. The synergistic effect of SAP and VEA is therefore due to the fact that only a combination of a watersoluble with a fatsoluble anti-oxidant offers integral protection.

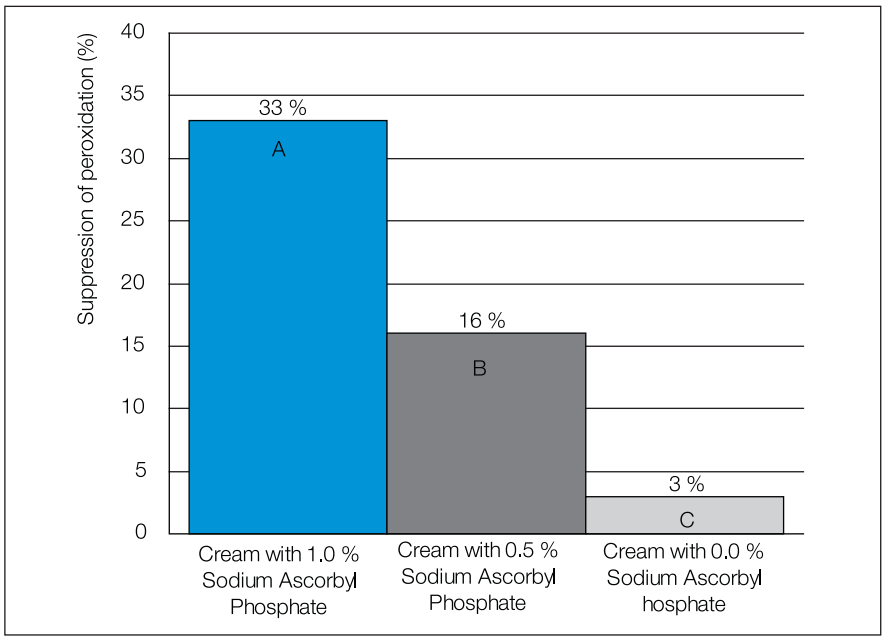


Further efficacy studies

The penetration of ascorbyl phosphates into the skin and their cleavage to release vitamin C have been demonstrated in studies by Sakamoto (T. Sakamoto, M. Egawa, M. Tanaka, 19th IFSCC Congress, 2, 5-17 (1996)) and Takashima (H. Takashima, H. Nomura, Y. Imai, H. Mima, Amer. Perfumer a. Cosmetics, 86, 29-36 (1971).
The action of vitamin C phosphate in promoting collagen formation has been demonstrated in an in-vitro study by Hata (R-I. Hata, H. Senoo, J. Cell. Physiol. 138, 8-16 (1989)).
Vitamin C phosphate is capable of supressing the formation of melanine. Sakamoto has demonstrated this in-vitro on melanocyte cultures. He found that melanine production was reduced by 80%. A study by Majmudar (G. Majmudar, G. Jacob, Y. Laboy, L. Fisher, J. Cosmet. Sci., 49, 361-367 (1998)) demonstrates the skin lightening effect of ascorbyl phosphate in another in-vitro model that used human epidermis. A reduction of tyrosinase activity by 35% was demonstrated.

The antioxidant effect of ascorbyl phosphates in protecting the skin against UV damage has also been demonstrated in a number of studies, e.g. by Kobayashi (S. Kobayashi, M. Takehana, S. Itoh; Photochem. Photobiol. 64, 224-228 (1996)). A reduction in lipid peroxidation and an anti-inflammatory effect have been demonstrated in hairless mice after treatment with ascorbyl phosphates. A study conducted by BASF on 20 test persons shows that a formulation containing 1% Sodium Acorbyl Phosphate applied to the skin can reduce UV-induced lipid peroxidation by 30%.

Suppression of lipid peroxidation by topically applied Sodium Ascorbyl Phosphate compared with an untreated area of skin, in 20 test persons:



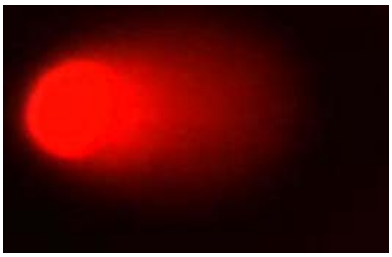
The efficacy of a combination of Sodium Ascorbyl Phosphate and a nucleophile as nitrosamine blockers for cosmetic formulations that contain secondary amines and potential nitrosation reagents has been demonstrated by Guthrie (W. Guthrie, Safety First, Soap Perfumery and Cosmetics, 17(2), 43-46 (1998)).

Barnet Products Corporation 140 Sylvan Avenue Englewood Cliffs NJ 07632
Tel 201 346 4620 Fax 201 346 4333 Web barnetproducts.com

Presents

BV-OSC

(Tetrahexyldecyl Ascorbate)



A Stable, Oil-Soluble Form of

Vitamin C

Anti-Oxidant

Whitening

Collagen Synthesis

UV Protection

MMP Inhibition

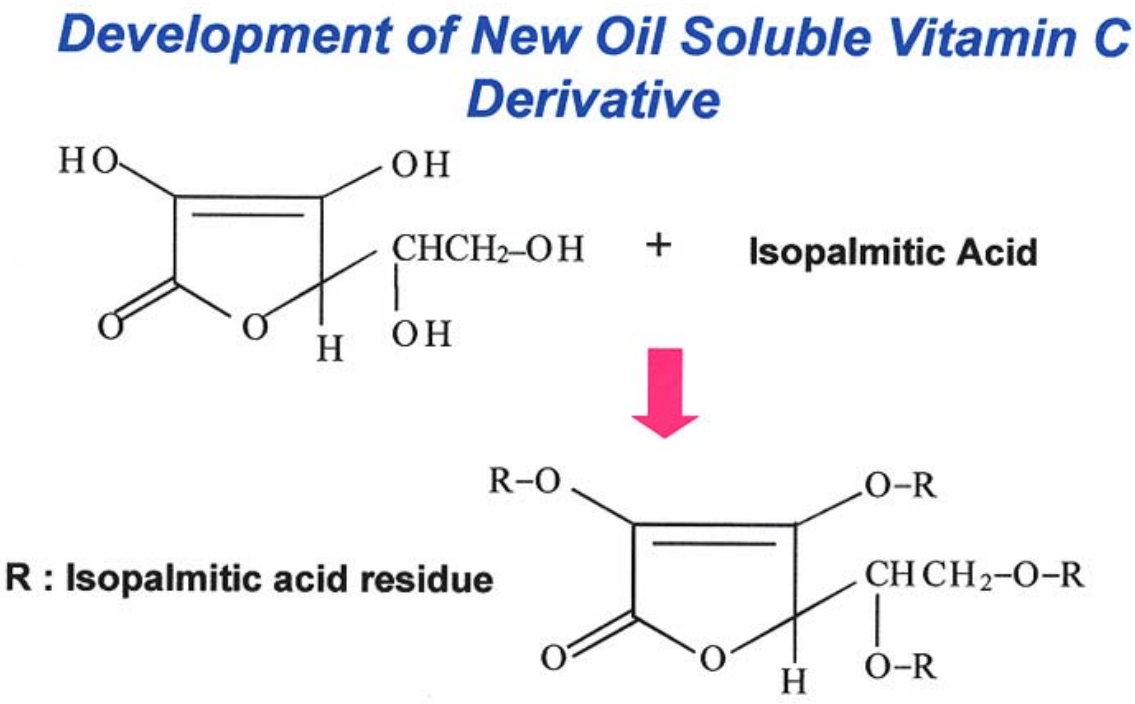
Collagen Protection

DNA protection

New Data: Comet Assay

BV-OSC

INTRODUCTION



INCI Name : Tetrahexyldecyl Ascorbate

(BV-OSC)

BV-OSC is a stable, oil-soluble Vitamin C ester.

The benefits of using Vitamin C in formulations include:

--- Anti-oxidant activity, inhibiting lipid peroxidation

--- UV-A and UV-B protection

--- Clarifying and brightening activity, inhibiting melanogenesis

--- Stimulation of collagen production

--- Inhibition of MMP's

Pure Vitamin C is very unstable. It is sensitive to oxidation and gives finished formulas a yellowish tint. Note also that pure vitamin C is not the most active form for collagen synthesis and anti-oxidation.

Barnet Products offers a stable, oil-soluble Vitamin C derivative:

---BV-OSC INCI NAME: Tetrahexyldecyl Ascorbate

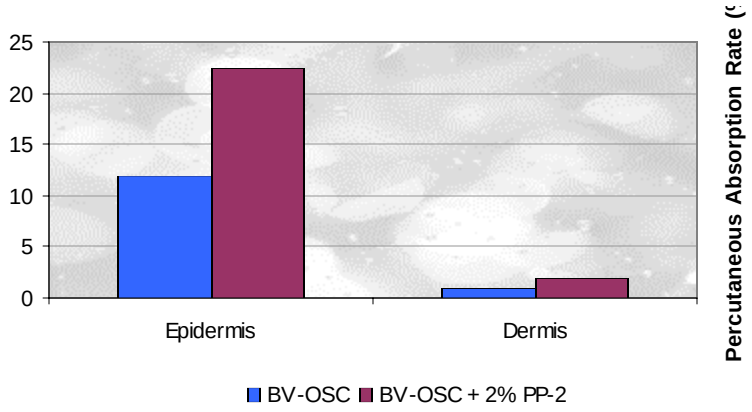
1. BV-OSC is very bio-available.

A. Percutaneous Absorption of BV-OSC and Delivery and Deposition with Polyolprepolymer-2 (PPG-12/SMDI Copolymer)

The first part of this presentation demonstrates that BV-OSC is retained in the epidermis and, to some extent, in the dermis. This retention can be doubled with the use of 2% PP-2 (Figure 1). A cream containing 5µM of BV-OSC was applied on the skin set on Franz. diffusion cells. BV-OSC concentrations in the epidermis and dermis were determined after 24 hours.

The second part of the presentation compares the penetration of "equivalent Ascorbic Acid" into the epidermis of BV-OSC and VC-PMG. Results show equivalent Ascorbic Acid penetration with 0.75% BV-OSC and 3% VC-PMG, suggesting that BV-OSC provides excellent penetration (Figure 2).

Figure 1: Excellent Percutaneous Absorption



Method of Measurement: Diffusion cell with human skin

Figure 2: Amount of Ascorbic Acid Penetrated Into the Epidermis

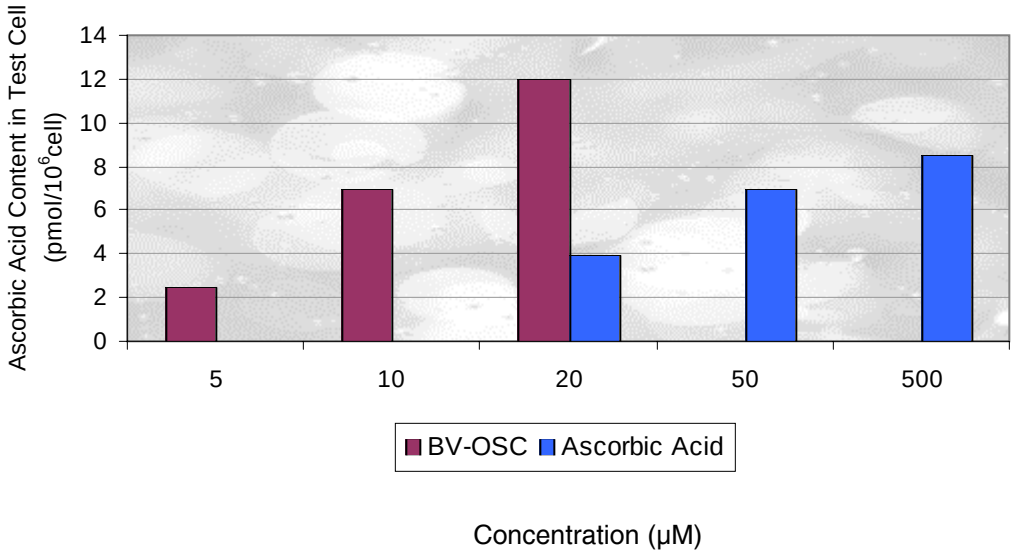
	VC-PMG	BV-OSC
Molecular Weight	289.5	1129.8
Ascorbic Acid Moiety in the Molecule	59.4%	15.2%
Skin Penetration (Amount in Epidermis)	0.7%	11.6%
Ascorbic Acid Penetrated into the Epidermis	**0.42%**	**1.76%**
Example In the Formulation	3% VC-PMG **0.0126%**	1% BV-OSC **0.0176%**

Tested on excised human skin with the addition of 3% Polyolprepolymer-2 from Bertek using radiolabelled samples on diffusion cells.

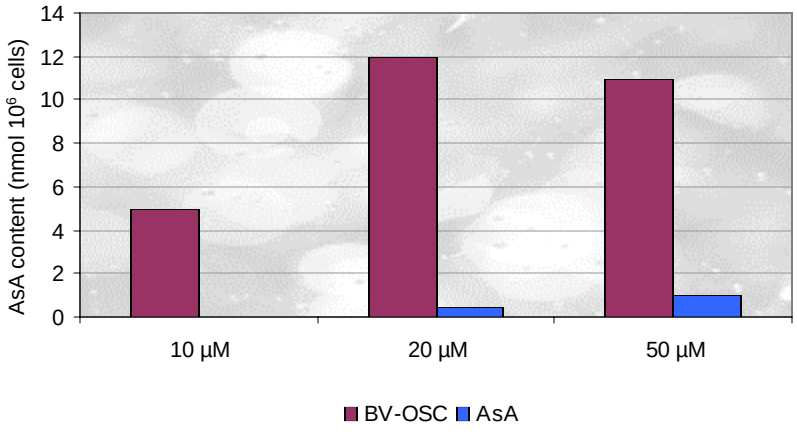
B. The penetration of Ascorbic Acid is very limited. The difference in penetration at levels between 50 µM and 500 µM is minimal.

The penetration of BV-OSC is dose-dependent, and surpasses that of Ascorbic Acid at the same concentration (20µM) by three-fold. BV-OSC maintains a higher penetration rate even when the Ascorbic acid is increased by 25 times that of BV-OSC.

Uptaken Content of Intracellular (Keratinocytes) Ascorbic Acid



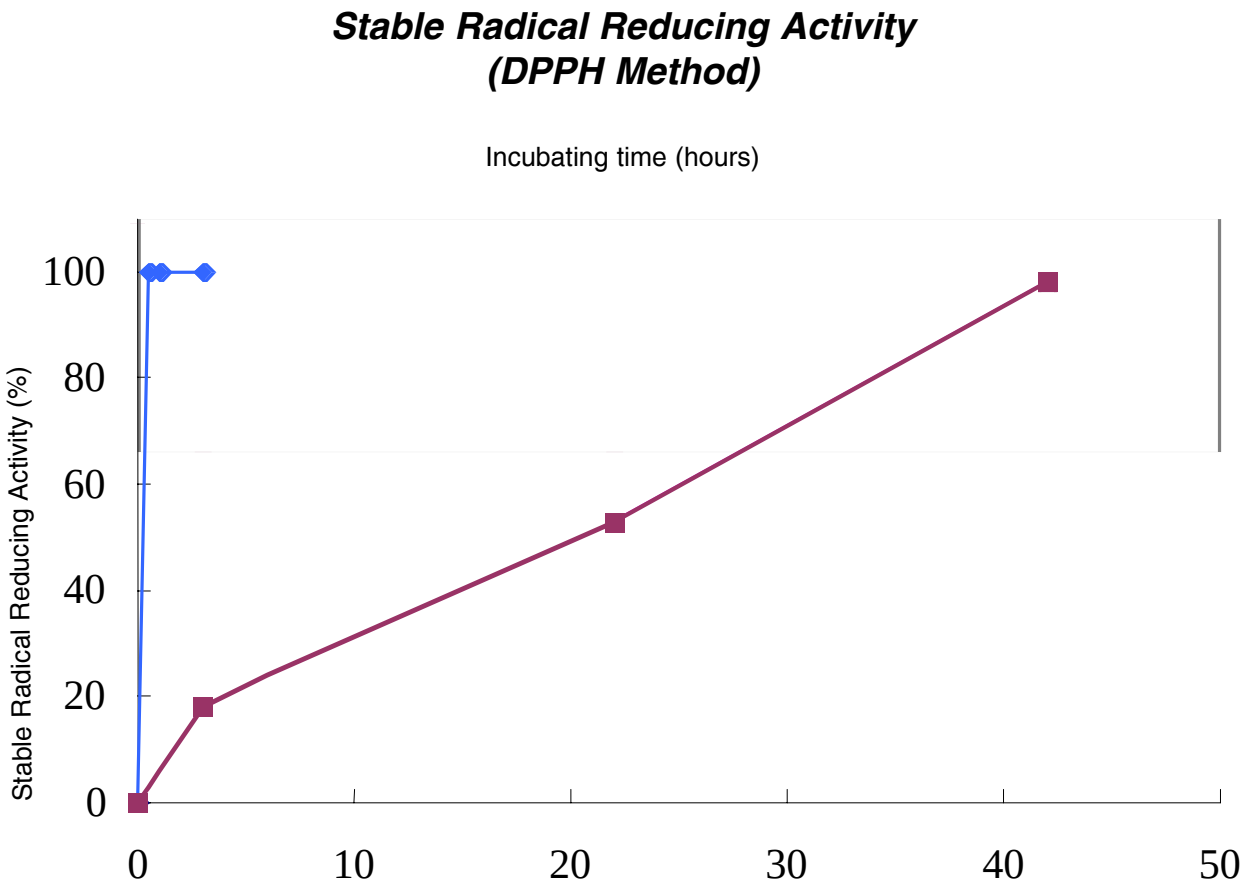
Fibroblast



Cells were treated with the medium containing various concentrations of BV-OSC or Ascorbic Acid (AsA). After 2 hours in incubation, cells were homogenized and the content of free Ascorbic Acid was determined using HPLC.

2. BV-OSC is very functional for stress protection.

A. Anti-Oxidant Activity of BV-OSC



The reducing activity of each 2.0mmol of BV-OSC (in red above) or Ascorbic Acid (blue) was measured by using a stable radical DPPH (0.01mmol) with phosphate buffer (pH 7.0) at 37° C for 48 hours.

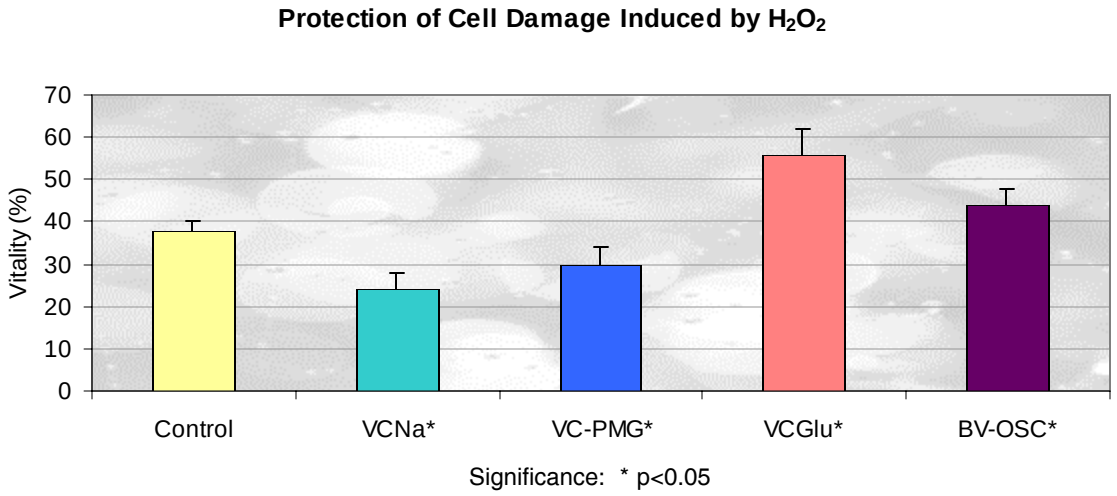
As shown, for BV-OSC, the reduction ratio (%) of DPPH after 3 hours, 24 hours and 42 hours from the reaction started was 18.7%, 52% and 98.1%, respectively.

On the other hand, for Ascorbic Acid, the reduction ratio (%) of DPPH reached almost 100% after 30 minutes. The difference of the reducing activity between BV-OSC and Ascorbic Acid seems to be related to the difference of activity of the 2-hydroxyl group in the structure which possesses the protom donating ability.

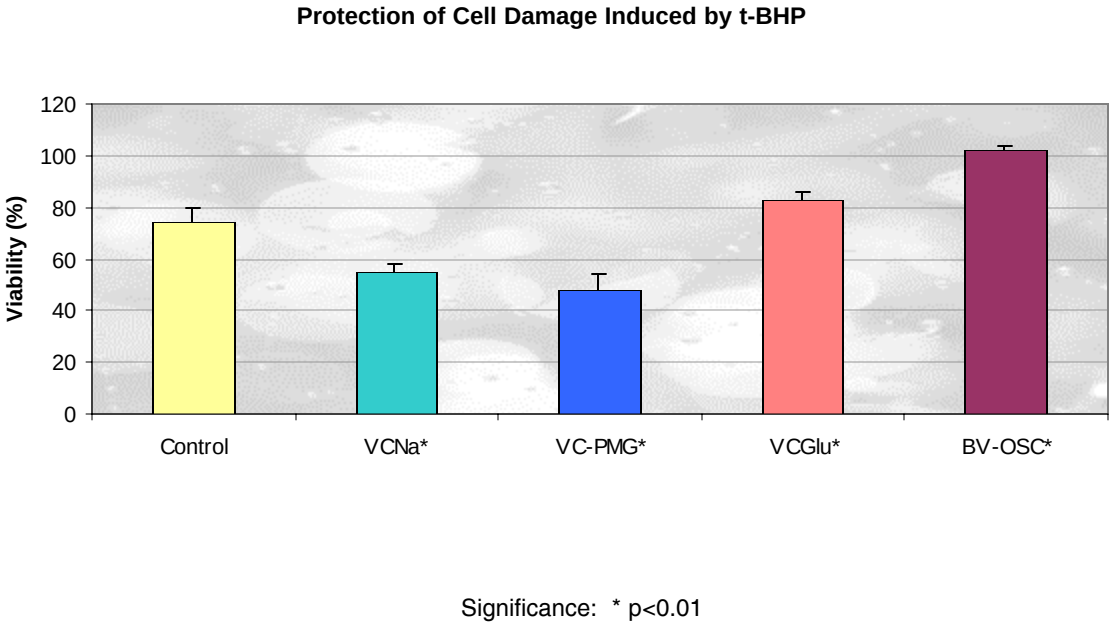
2-hydroxyl group in BV-OSC is blocked with 2-hexadecanoyl moiety. In order that BV-OSC possesses the reducing activity against DPPH, it is necessary to hydrolyze the 2-acyl moiety and liberate the 2-hydroxyl group.

Accordingly, BV-OSC seems to act as a radical scavenger more slowly than Ascorbic Acid.

B. BV-OSC Protects Against Cell Damage



HaCaT keratinocytes were treated with various 100 µM of various Vitamin C derivatives for 24 hours. After treatment of 20 µM for 2 hours, cell survival was estimated.

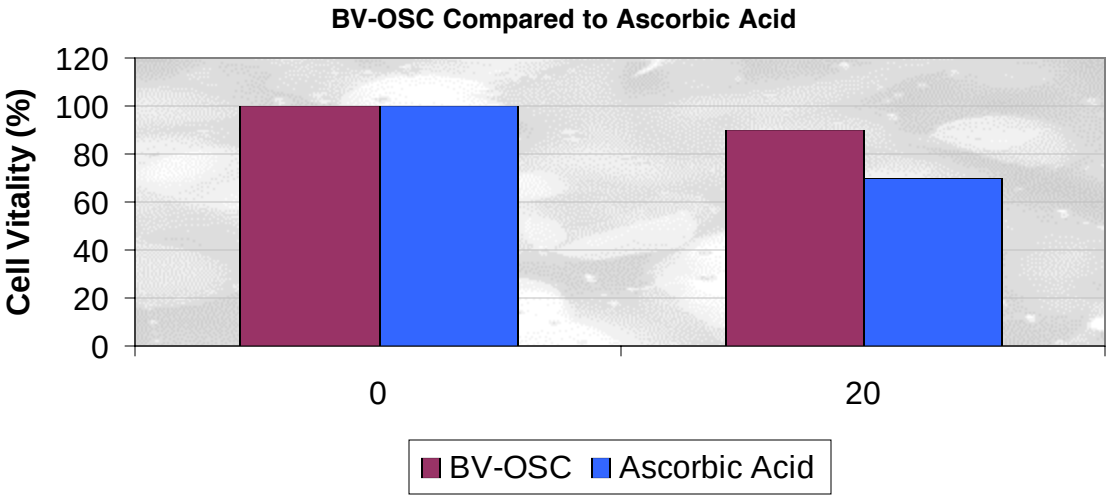


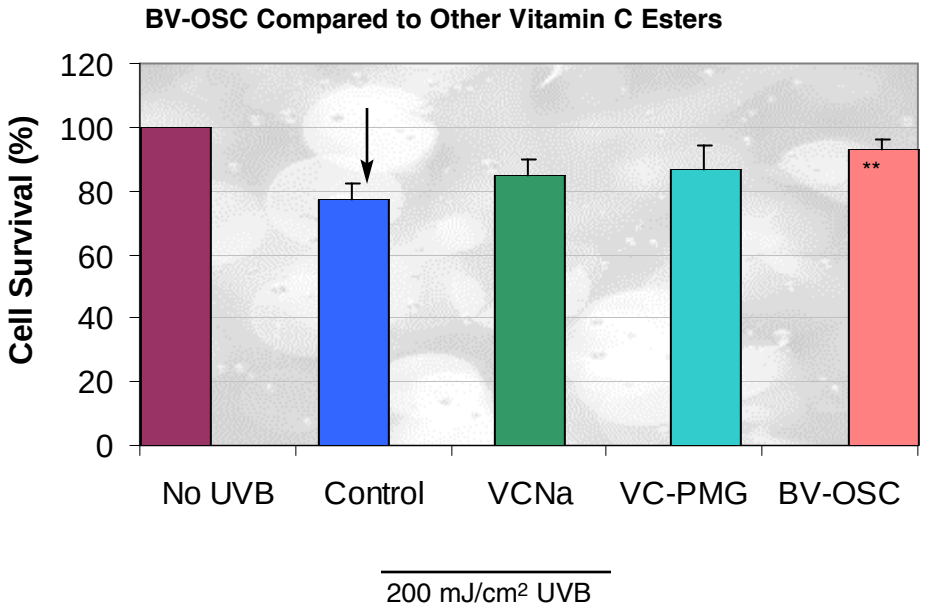
HaCaT keratinocytes were treated with various 100 µM of various Vitamin C derivatives for 24 hours. After treatment of 1.0 nM of t-BHP for 4 hours, cell survival was estimated.

C. Prevention of UV-B Damage with BV-OSC or Ascorbic Acid

BV-OSC has an excellent penetration in keratinocytes. As a result the cytoprotection against UV-B is increased. The cell viability is Increased up to 30% when BV-OSC is applied compared to pure Vitamin C.

Cytoprotective Effect Against Cell Mortality of UV-B Irradiated Skin Keratinocytes

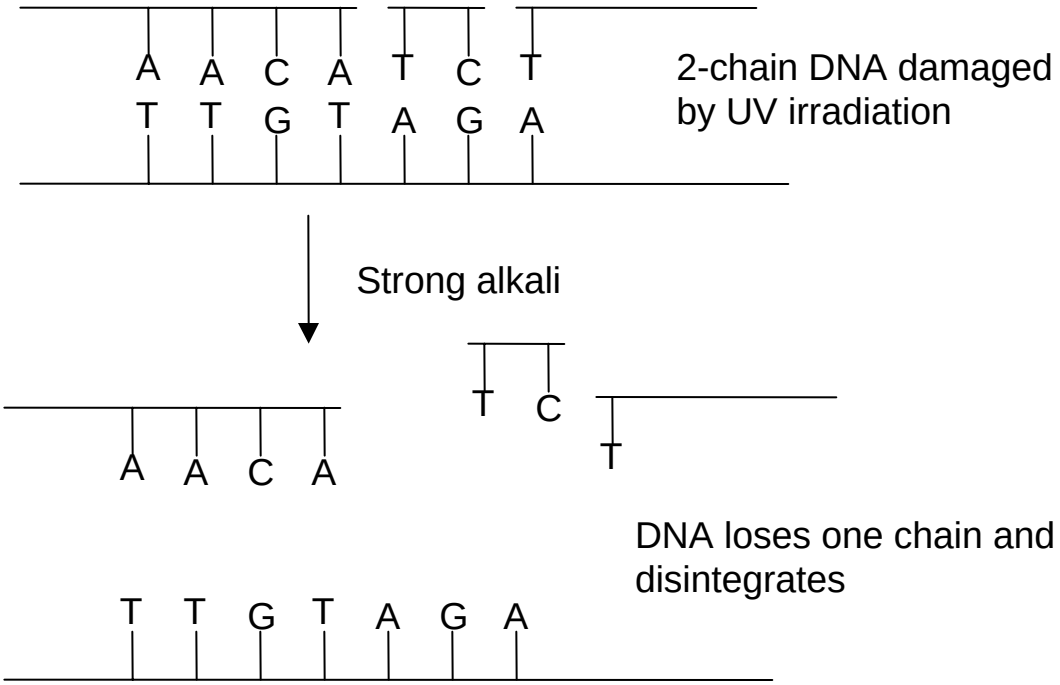




HaCaT keratinocytes were treated with various 100 µM of various Vitamin C derivatives for 24 h. After 24 h from UVB irradiation, cell survival was estimated. Significance: * $p<0.05$, ** $p<0.01$.

→ ***Comet Assay***

Idea

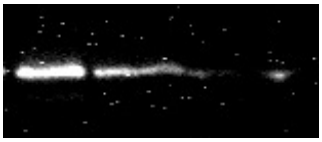


What is Comet Assay ?
The comet assay, also called the 'Single Cell Gel Assay', is the technique to detect DNA damage and repair at the level of single cells. The comet assay or single cell gel electrophoresis assay is based on the alkaline lysis of labile DNA at sites of damage. 'Comet Assay' is one of the most popular tests of DNA damage detection (e.g., single- and double-strand breaks, oxidative-induced base damage, and DNA-DNA/DNA-protein cross linking) by electrophoresis, developed in recent years.
Merits Of Comet Assay :
•Very high sensitivity to detect DNA damage
•Rapid and easy to handle
•Little amount of cell samples needed
•Applied to most eukaryotic cells

Western Blot (p53 expression suppressed by BV-OSC)

Result:
BV-OSC @ 0.005% : p53 expression decreased to 50%
BV-OSC @ 0.01% : p53 expression decreased to 10%

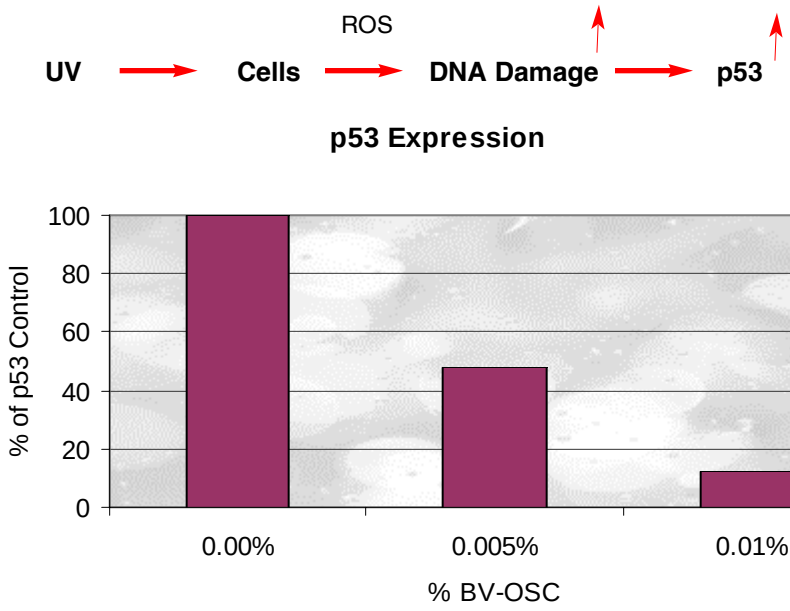
Concentration of BV-OSC:



0 0.005 0.01 (%)

→ *Quantitative Evaluation of Protection*

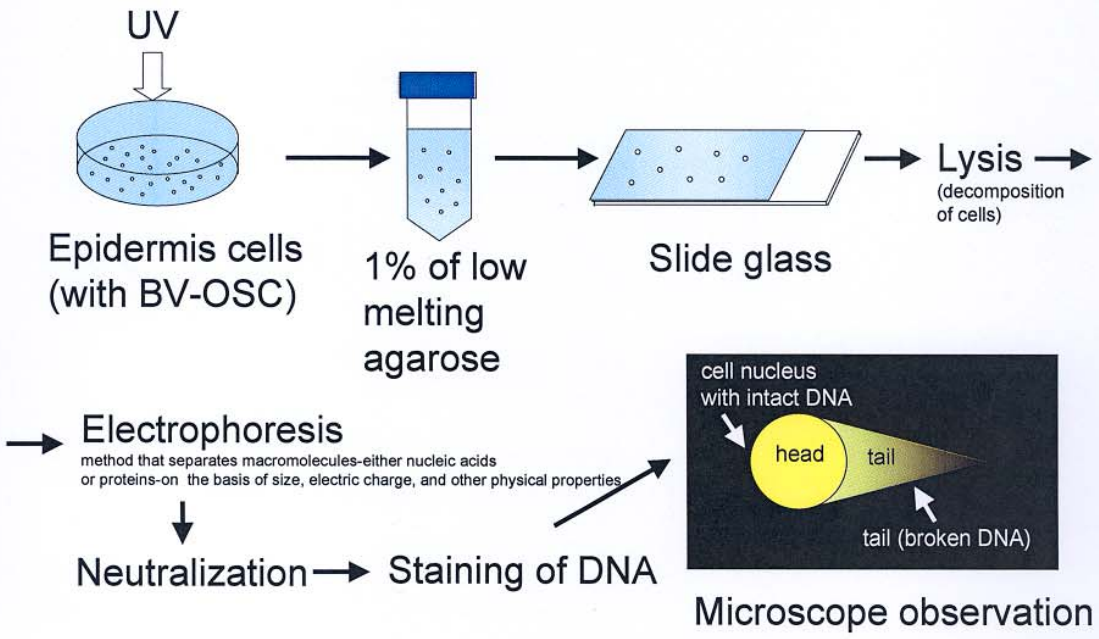
UVB Induces p53 Synthesis



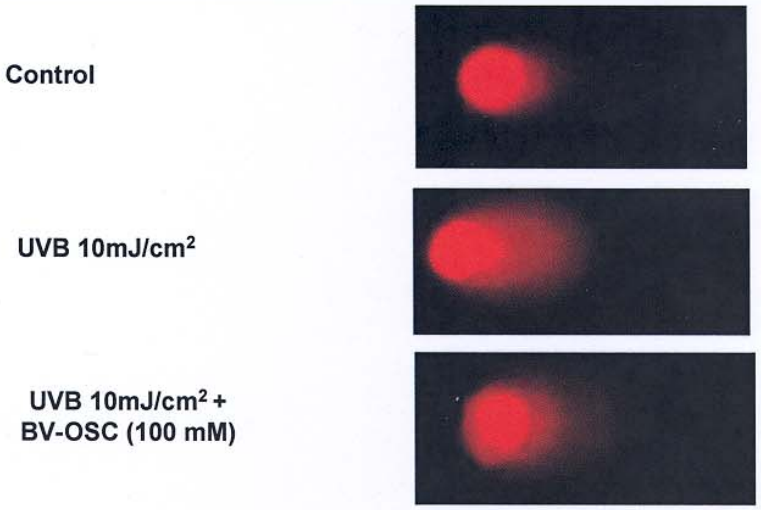
Human dermal fibroblasts were treated with various concentration of BV-OSC for 24 h. 100 mJ/cm2 UVB was irradiated following additional 24h cultivation. p53 (proteins that cause apoptosis, or cell death) is secreted in the cell. The cells were then lysed and the medium was tested for for p53 expression by Western blotting.

BV-OSC limits p53 synthesis. It reduces UV-B damage.

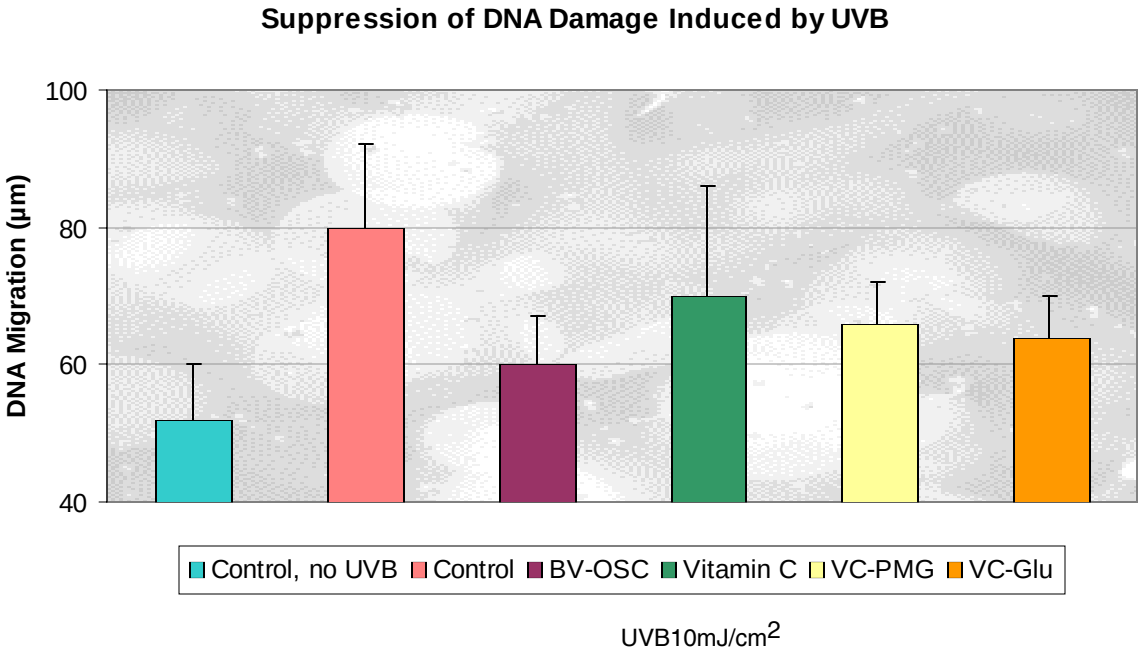
Test method



Suppression of DNA damage induced by UVB



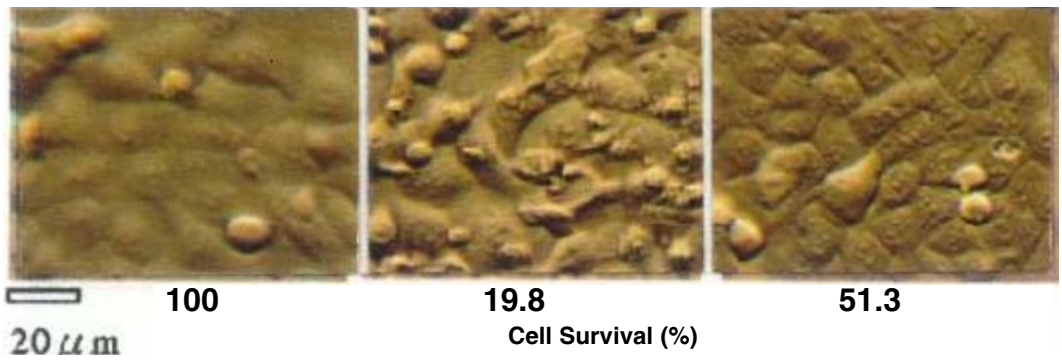
DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with VC derivatives for 24 h, were exposed to UVB at 100 mJ/cm². Cells are stained with etidium bromide.



DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with VC derivatives for 24 h, were exposed to UVB at 10 mJ/cm2, n=50.

D. BV-OSC Prevents UV-A Damage

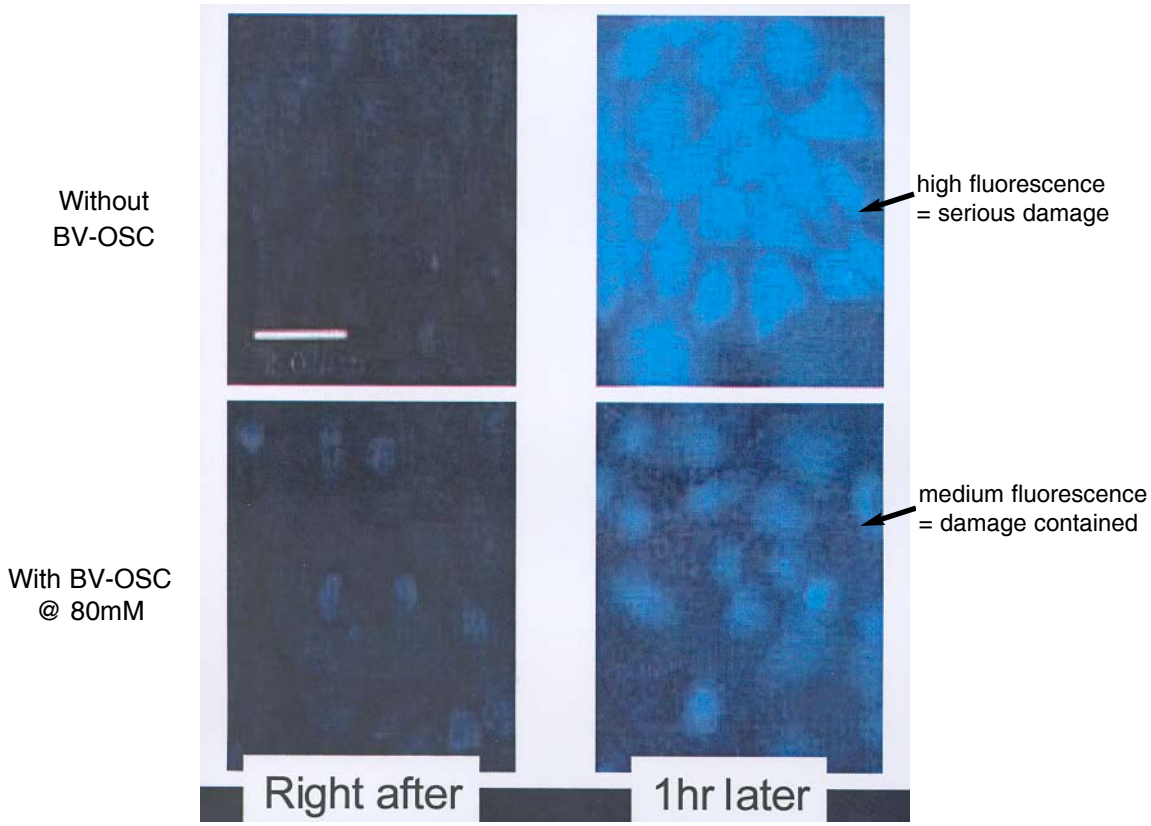
Cyto-Protective Effect of BV-OSC Against UVA Irradiation



→ *Quantitative evaluation*

UVA damage can be measured by the quantity of 8-OHdG released.

Inhibitory Effect on 8-OHdG Production
Induced by UV-A
8-OHdG (8-hydroxy-2'-deoxyguanosine)



HaCaT cells were treated with 80 mM BV-OSC. After UVA irradiation, 8-OHdG was detected immunohistochemically using anti-8-OHdG antibody.

The application of BV-OSC inhibits the release of 8-OHdG, thereby protecting the cell against UV-A damage.

3. BV-OSC has anti-aging properties.

A. BV-OSC and Collagen Synthesis

First we observed that by adding 0.1% of BV-OSC in a fibroblast culture, the proliferation of the cells is increased by 50% (Figure 1).

Furthermore, the fibroblasts are significantly increasing collagen synthesis.
It doubles with the use of 50µM of BV-OSC. The same dosage of Ascorbic Acid increases collagen synthesis by only 25% (Figure 2).

Figure 1: BV-OSC and Cell Proliferation

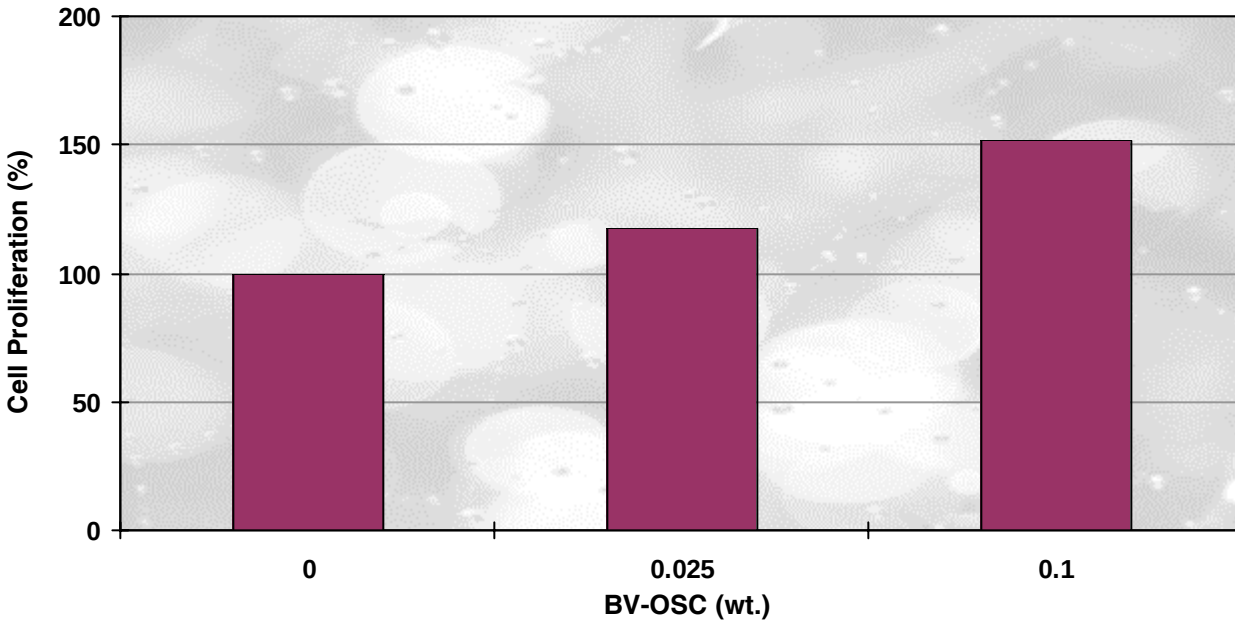
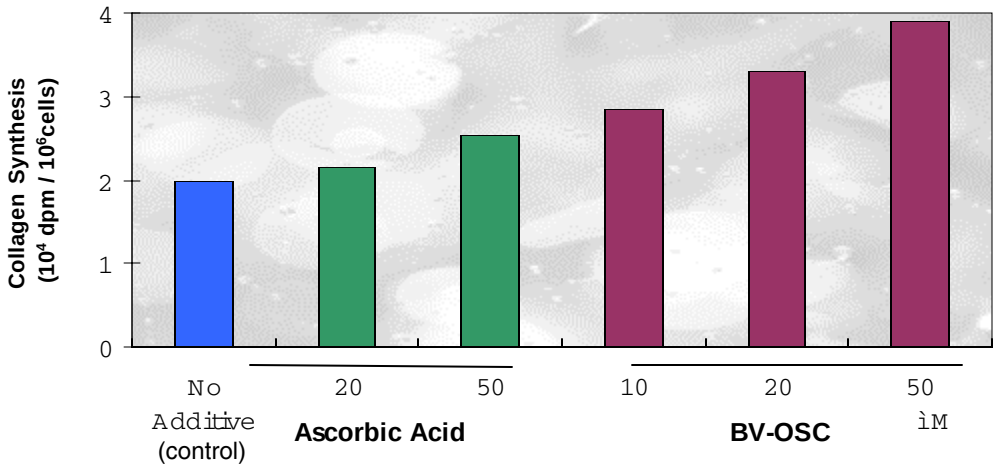
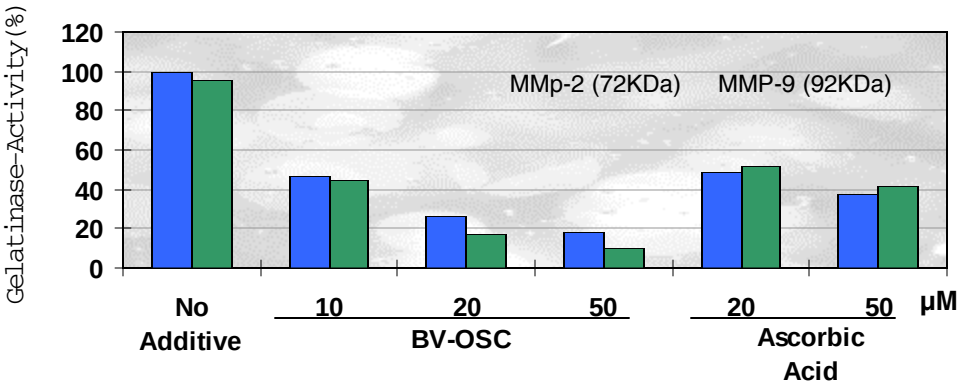


Figure 2: Comparison of Ability for Collagen Synthesis



B. BV-OSC has MMP Inhibition Effect.

Figure 3: The Ability of Inhibition of Gelatinase Activity



Measurement of MMPs:

Serum-free condition media of NHDF cells cultured for 48 hours in the presence or absence of 50 μM BV-OSC were concentrated by ultra-filtration, and were electrophoresed under non-reduced conditions on a SDS-Polyacrylamide gel containing 0.2% gelatin, followed by staining with Coomasie Brilliant Blue R250 and subsequent measurement by laser densitometry.

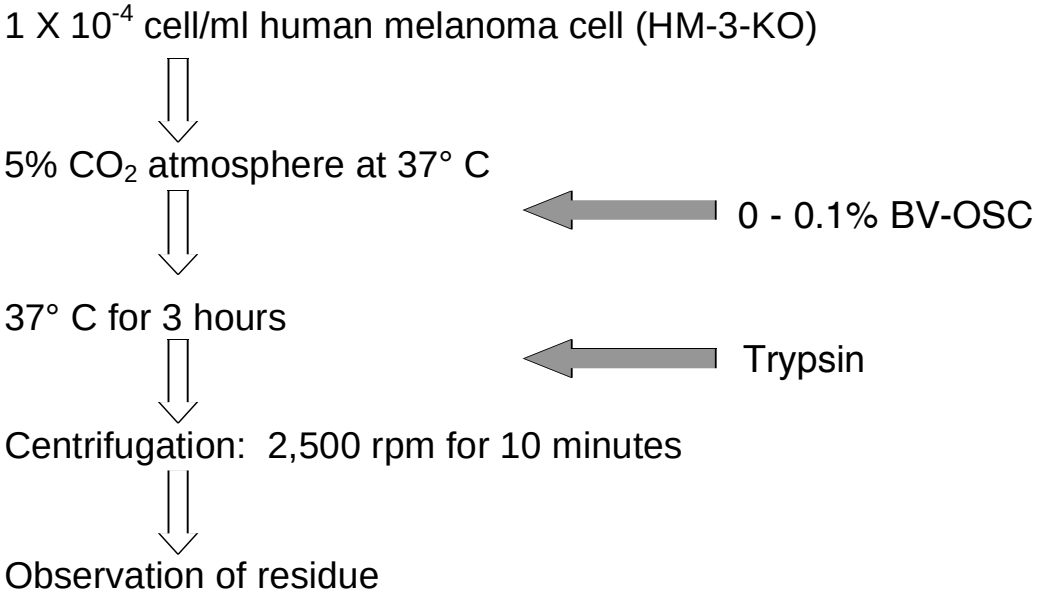
4. BV-OSC has whitening properties.

A. Inhibition of Melanogenesis *in vitro* test with BV-OSC

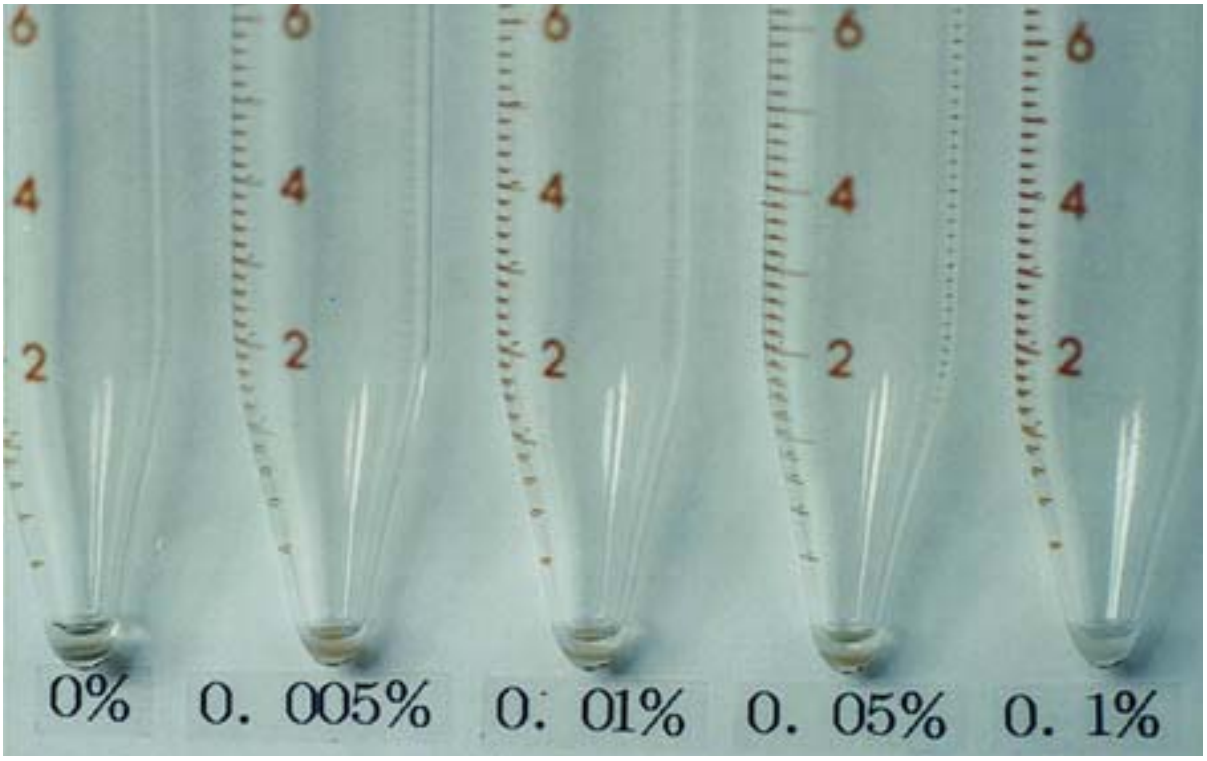
One of the many benefits of Vitamin C in cosmetic formulations is its ability to provide a more even skin tone. Occidental countries describe the activity as a "clarifying and brightening" effect, while in Asia the term "whitening" is used.

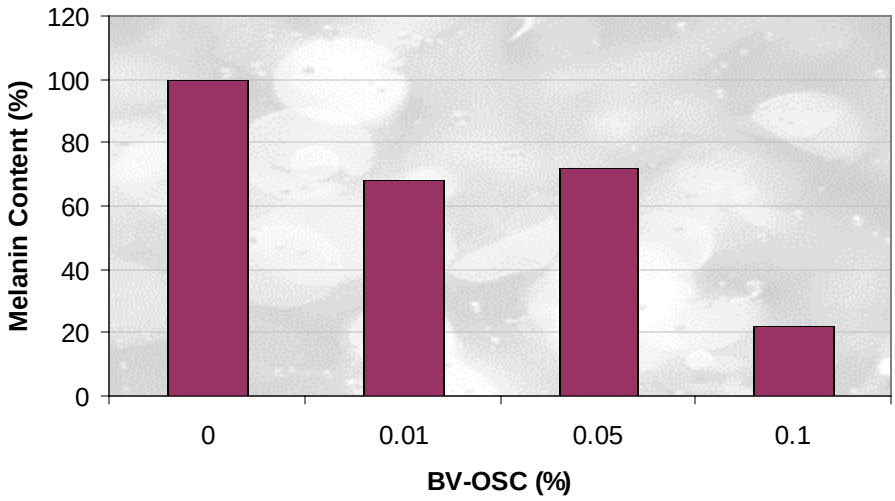
The following in vitro test shows that 0.1% - 0.2% of BV-OSC reduces melanogenesis by more than 80%.

Protocol for Evaluation of Inhibitory Effect of Melanogenesis



Inhibitory Effect on Melanogenesis In Cultured Human Melanoma Cell





Human melanoma cells were treated with the medium containing BV-OSC for 4 days. After harvesting the cells, melanin contents were estimated using slot-blot method. Values were expressed as % of control.

5. BV-OSC Doctor's Application

An aqueous gel with 10% BV-OSC was applied to 10 patients with acne (16-45 years old) for 2-10 months. Efficacy was evaluated according to the following scale:

> 75% improvement: Excellent

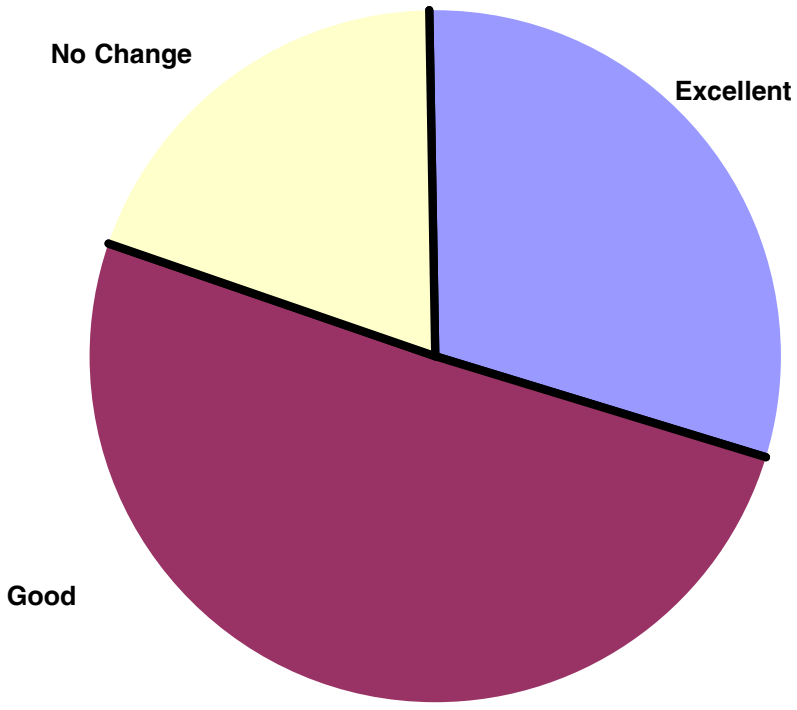
> 50% improvement: Good

< 50% improvement: No Change

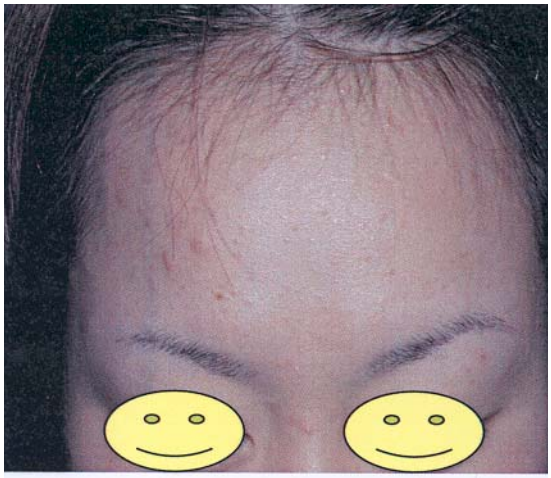
10% GEL FORMULATION

Water	q.s. 100%
Concentrate Glycerin	17.0%
Carbomer	0.5%
Sodium Polyacrylate	0.25%
Butylene Glycol	2.5%
BV-OSC	10.0%
Methyl paraben	0.05%
Phenoxyethanol	0.6%

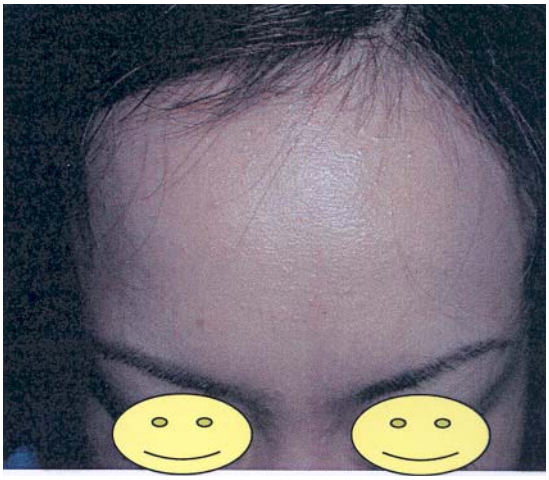
TEST RESULT - ACNE TREATMENT BY 10% BV-OSC GEL



BEFORE



AFTER 12 WEEKS



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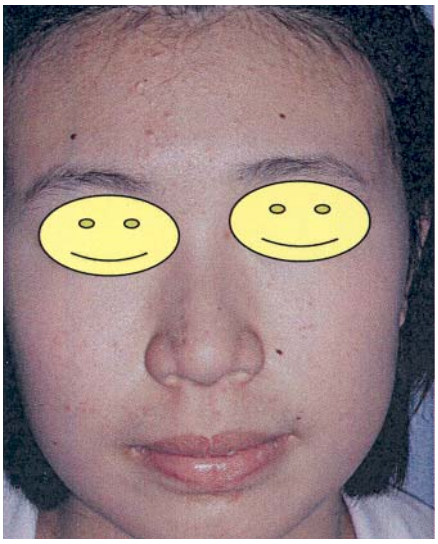
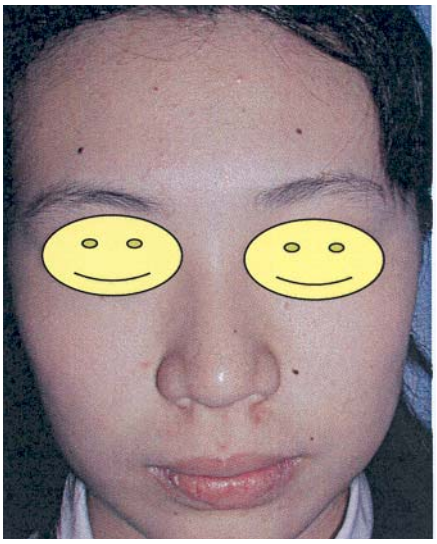


AFTER 9 WEEKS



BEFORE **AFTER 16 WEEKS**