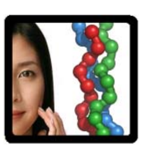
BARNET

Stabilized oil-soluble vitamin C derivative

BV-OSC

Vitamin C

Whitening



Collagen Synthesis

Anti-oxidant

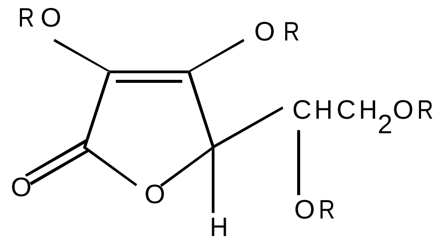
DNA Protection

UV Protection

Stable

Oil-Soluble Vitamin C Derivative

BV-OSC



INCI Name : Tetrahexyldecyl Ascorbate

Vitamin C has many functions as a cosmetic ingredient, including skin lightening, promoting collagen synthesis and inhibiting lipid peroxidation. BV-OSC (tetrahexyldecyl ascorbate) is stable at high temperatures and has good solubility in oils. BV-OSC exhibits excellent percutaneous absorption and effectively converts into free vitamin C in the skin to perform various physiological functions. BV-OSC is approved as a quasi-drug active in Japan (at 3%). It is also registered in Korea as a functional ingredient for skin lightening at 2% concentration.

Properties of BV-OSC

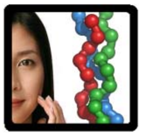
Superior percutaneous absorption
Inhibits activity of intracellular tyrosinase and melanogenesis (whitening)
Reduces UV-induced cell / DNA damage (UV protection / anti-stress)
Prevents lipid peroxidation and skin aging (anti-oxidant)
Good solubility in common cosmetic oils
SOD-like activity (anti-oxidant)
Collagen synthesis and collagen protection (anti-age)
Heat- and oxidation-stable

Physical properties of NIKKOL BV-OSC

Appearance	:	Colorless to pale yellow liquid
Specific gravity (d^{2020})	:	0.930 - 0.943
Refractive Index (n^{25D})	:	1.459 - 1.465


Vitamin C


Whitening


Collagen Synthesis

Anti-oxidant


DNA Protection

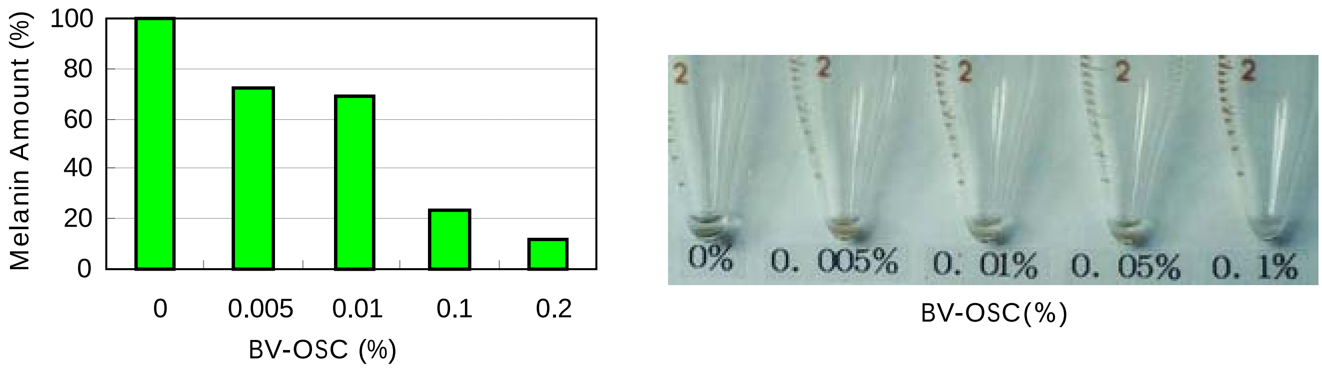

UV Protection


Stable

Skin Lightening / Anti-Pigmentation

Inhibition of Melanogenesis

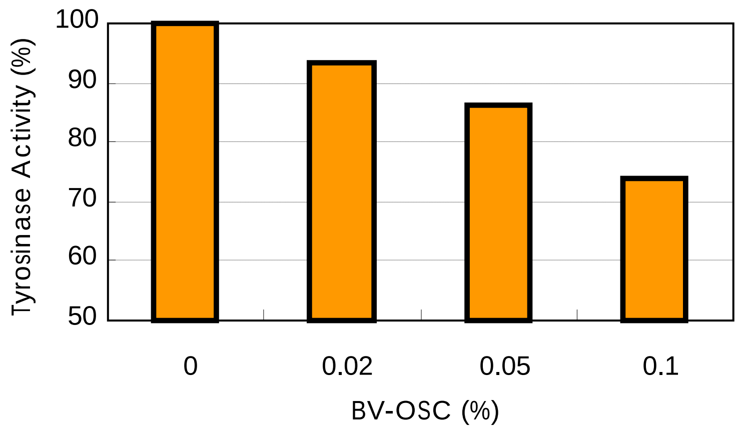
Various concentrations of BV-OSC were added to cultured human melanoma cells (HM-3-KO). After 4 days of cultivation, the amount of melanin produced was measured by observation of the color tone of each cell pellet. As shown below, BV-OSC effectively inhibited melanogenesis in human melanoma cells. Results were dose-dependent.



Melanogenesis Inhibition of BV-OSC (in Human Cells)

Inhibition of Intracellular Tyrosinase Activity

BV-OSC was added into mouse melanoma cells (B16-4A5) at various concentrations. After a 72-hour cultivation, the cells were dissolved and extracted. L-Dopa was then added to the extract. After 60 minutes at 37℃, the amount of dopachrome formed by the activity of tyrosinase was evaluated by measuring its absorbance at 540 nm. Figure below shows that at a concentration of 0.02% and above BV-OSC inhibited the activity of intracellular tyrosinase.



Inhibitory Effect of BV-OSC on Intracellular Tyrosinase Activity

Clinical In-Vivo Study on BV-OSC (3%)

Number of volunteers: 30

Testing site: Inner side of volunteer's upper arm

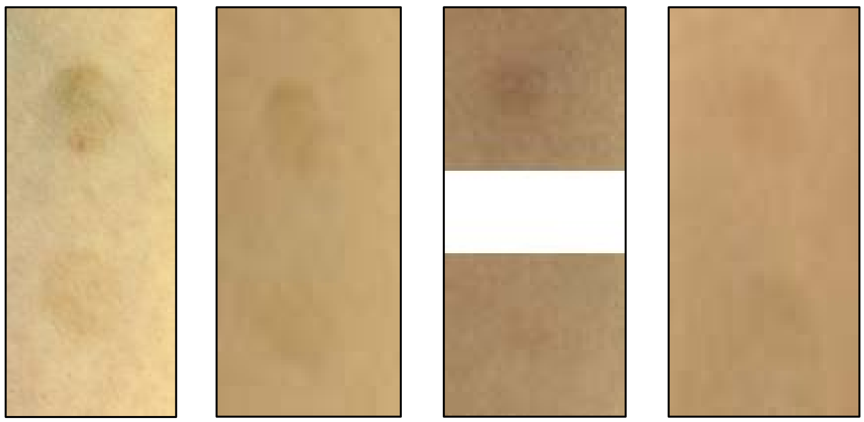
Testing period: 3 weeks

Procedure: For the first step of the test, minimal erythema dose (MED) of each volunteer is measured using solar simulator. Briefly, 6 dosed of UV ray are irradiated to the inner side of right upper arm. After 24 hours from irradiation, MED is judged. For the second step, 1.5 MED of UV ray is irradiated on the inner side of left upper arm of each volunteer in order to make pigmentation. Sample application is started just after irradiation. Sample is applied twice a day during test period. Sample application sites are randomly changed in every volunteer in order to do justice.

Results

Placebo

3% BV-OSC



Formulations used in the evaluation

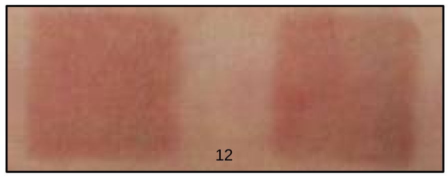
	BLANK	TEST Sample (BV-OSC 3%)
(A)		
NIKKOL BC-20TX (Ceteth-20)	1.0	1.0
NIKKOL GO-440 (Sorbeth-40 Tetraoleate)	0.5	0.5
NIKKOL MGS-B (Glyceryl Stearate)	1.0	1.0
Cetanol	5.0	5.0
NIKKOL Squalane	10.0	10.0
NIKKOL ICM-R (Isocetyl Myristate)	6.0	6.0
NIKKOL Trifat S-308 (Triethylhexanoin)	3.0	3.0
NIKKOL Jojoba Oil	1.0	1.0
Dimethicone	0.2	0.2
Tocopherol	0.1	0.1
Propylparaben	0.1	0.1
(B)		
BV-OSC	-	3.0
Distilled Water	-	-
(C)		
Xanthan Gum (2% soln.)	5.0	5.0
1,3-BG	5.0	5.0
Metylparaben	0.2	0.2
Distilled Water	61.9	58.9

Skin Lightening / Anti-Pigmentation

Other Clinical In-Vivo Studies with BV-OSC

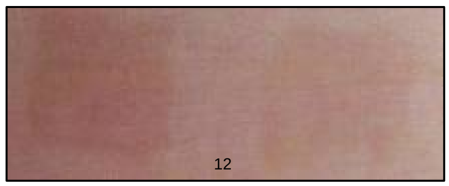
Reduction of UV-induced pigmentation

Test period: 56 days.
Concentration: 2%.



Before

After 56 days



Control BV-OSC (2%)

Before

After 56 days

Control BV-OSC (2%)

Pigmentation reduction effect

Test period: 16 weeks.
Concentration: 10%.
Result: complete removal of pigmentation (age spot).

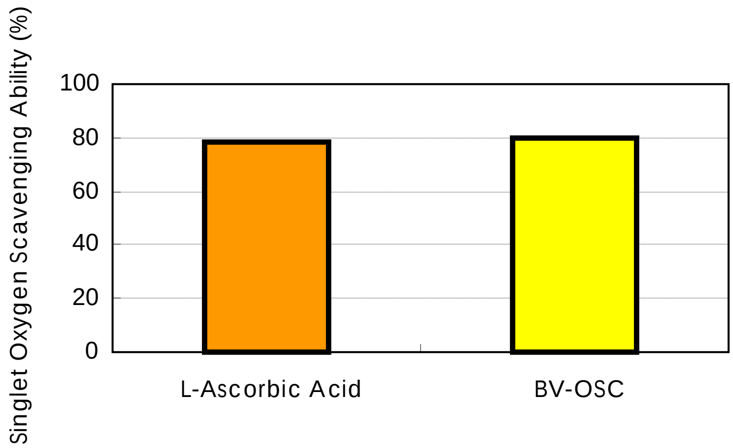
Before

After

Anti-oxidant

Singlet Oxygen Scavenging Ability

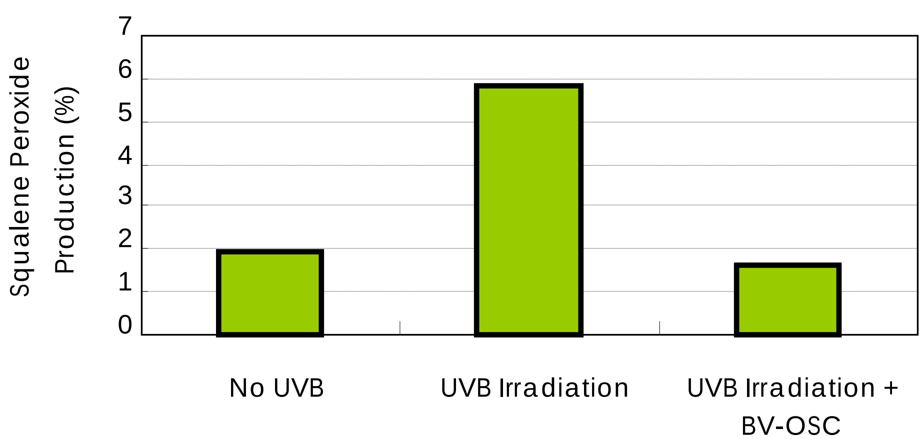
BV-OSC can scavenge singlet oxygen and prevent it from oxidizing L-Dopa to dopachrome. In order to evaluate this ability, UVB was used to irradiate mixture of Hematoporphyrin and L-Dopa with BV-OSC/ L-ascorbic acid to produce singlet oxygen and subsequently dopachrome. The amount of produced dopachrome was then determined through its absorbance. Using this measured value, the singlet oxygen scavenging ability of each BV-OSC/ L-ascorbic acid was calculated. As shown in figure below, BV-OSC and L-ascorbic acid scavenged 80% and 78% of singlet oxygen, respectively.



Singlet Oxygen Scavenging Ability of BV-OSC
(UVB Irradiation: 4 hours, Concentration: $2.5x10^{-4}$mol/L, Amount of dopachrome was determined through its absorbance at 475nm.)

Inhibition of Sebum Oxidation

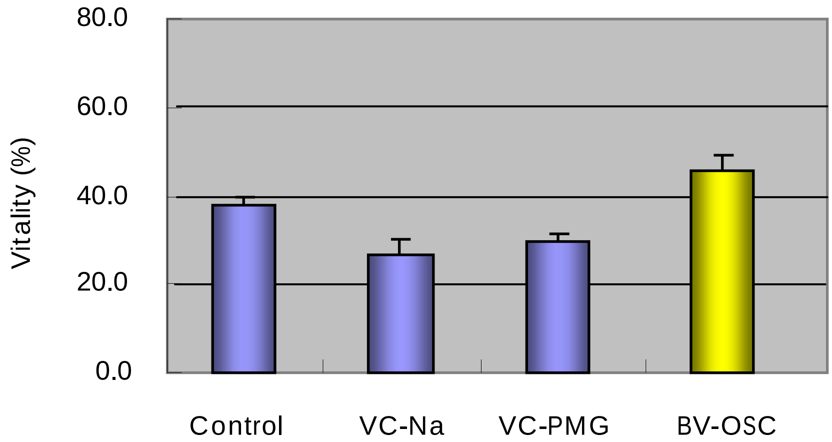
BV-OSC (10% in mineral oil) was applied to the inner forearms of 6 volunteers. UVB (2.05 J/cm^2) was used to irradiate the test site 4 hours after application. Then the production rate of squalene peroxide was measured as an indicator of sebum oxidation. As shown below, BV-OSC inhibited the production of squalene peroxide at the same level as the control without UVB irradiation.



Inhibition of Sebum Oxidation

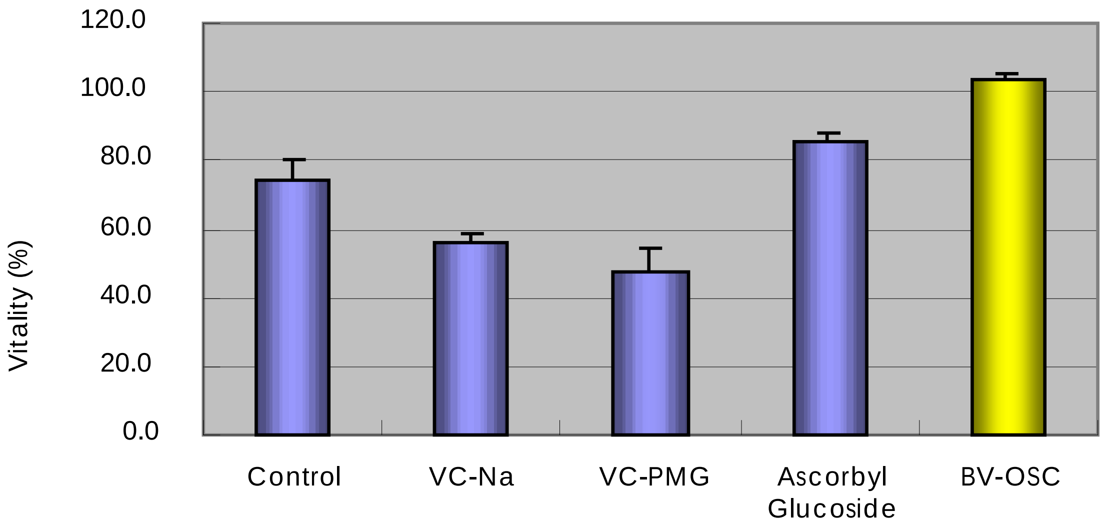
Anti-oxidant

Protection of Cell damage induced by H_2O_2



HaCaT keratinocytes were treated with various 100 mM of various vitamin C derivatives for 24 h. After treatment of 20 mM H_2O_2 for 2 h, cell survival was estimated. Significance: * $p<0.05$.

Protection of Cell damage induced by t-BHP (tert-butylhydroperoxide)



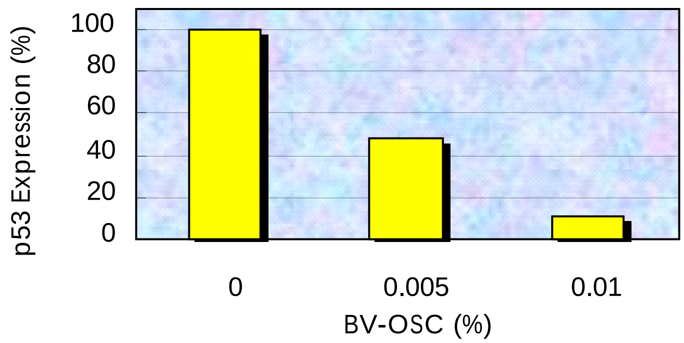
HaCaT keratinocytes were treated with various 100 mM of various Vitamin C derivatives for 24 h. After treatment of 1.0 mM of t-BHP for 4 h, cell survival was estimated. Significance: ** $p<0.01$.

SOD-Like Activity

The amount of Diformazan formed by the reduction of Nitro Blue Terazolium by superoxide anions (O2-) was determined by measuring the absorbance (NBT reduction method). This was then used as the indicative value for SOD-like activity of BV-OSC. Before the measurement, 0.1mL of BV-OSC was added to 2mL of the sample. As a result, BV-OSC inhibited 40% of Diformazan formation. This confirms the SOD-like activity of BV-OSC.

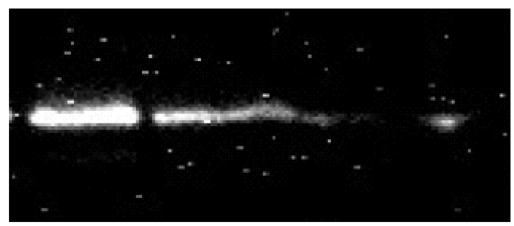
BV-OSC protects skin from UVB

Protection of DNA from UVB irradiation



When cells are exposed to UVB rays, a cancer suppressor, p53 protein, is activated in response to DNA damage. The amount of expressed p53 was measured as an indicator of the amount of damage to DNA. Human fibroblasts (NB1RGB); UVB (0.1J/cm^2).
BV-OSC significantly inhibited the expression of p53.

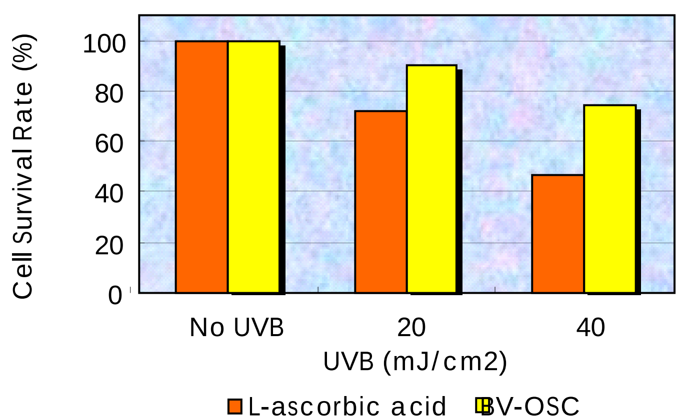
p53 Expression (Western Blot)



0 0.005 0.01 (%)

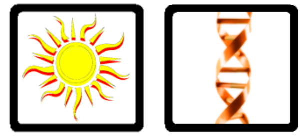
BV-OSC (%)

Reduction of cell damage caused by UV Irradiation



BV-OSC and L-ascorbic acid were added at the concentration of 10μmol/L to epidermal cells. The cells were then irradiated with UVB. After a 24-hour cultivation, cell survival rate was measured by WST-1 assay. Protective effect of BV-OSC was higher than that of L-ascorbic acid due to the fact that the conversion rate of BV-OSC into the cells is higher than that of pure ascorbic acid.

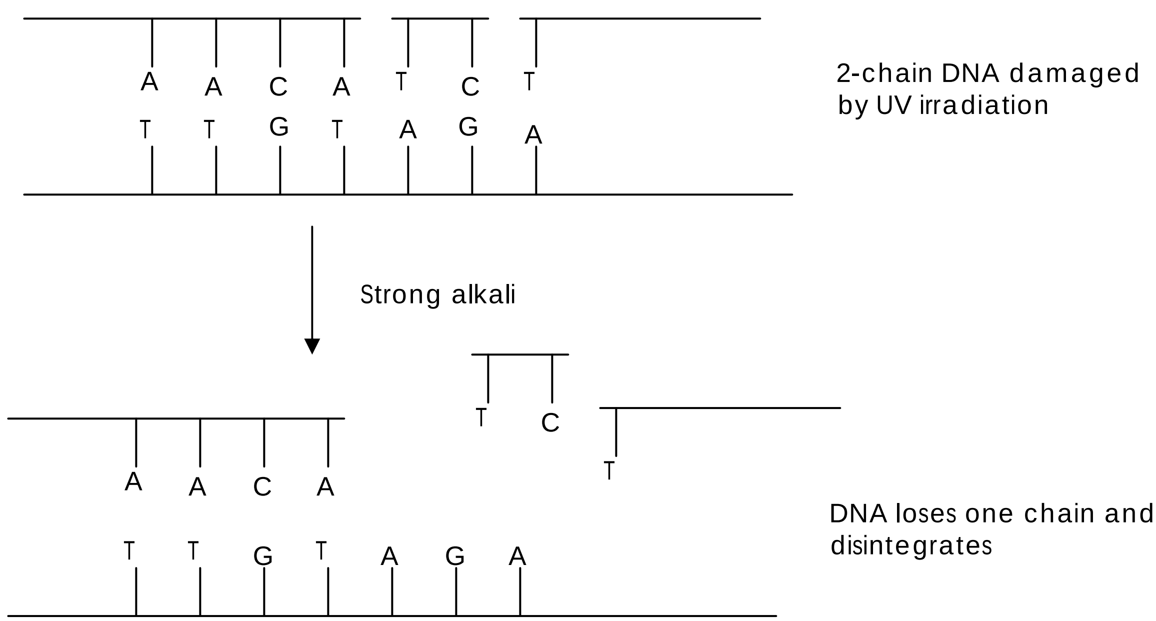
BV-OSC protects skin from UVB



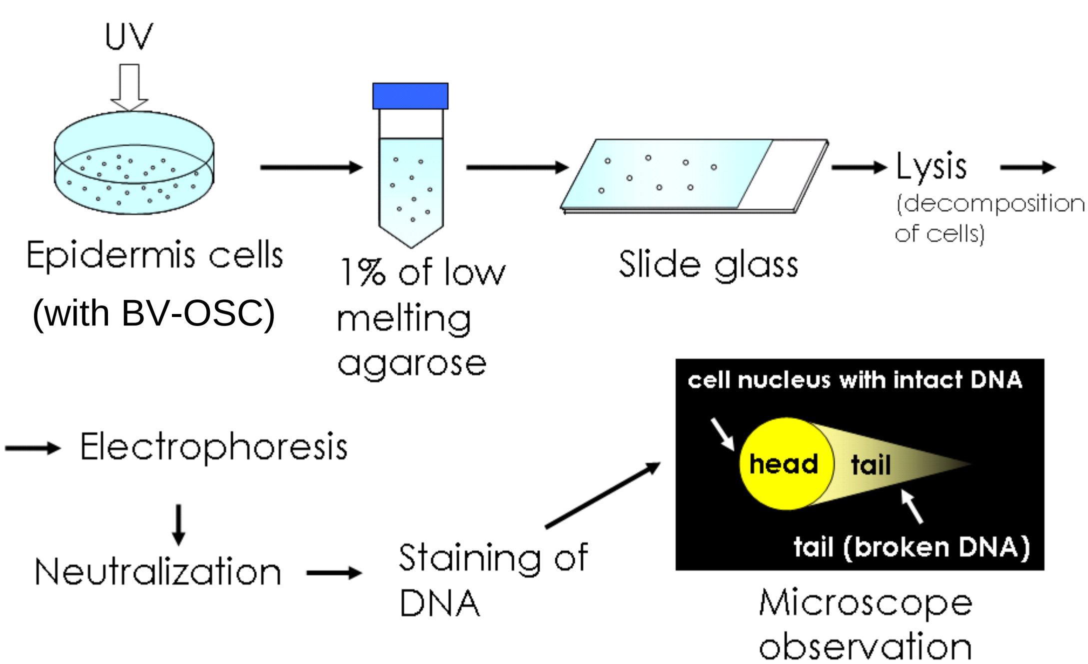
Comet Assay Test

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.
Comet Assay has very high sensitivity to detect DNA damage.

Idea

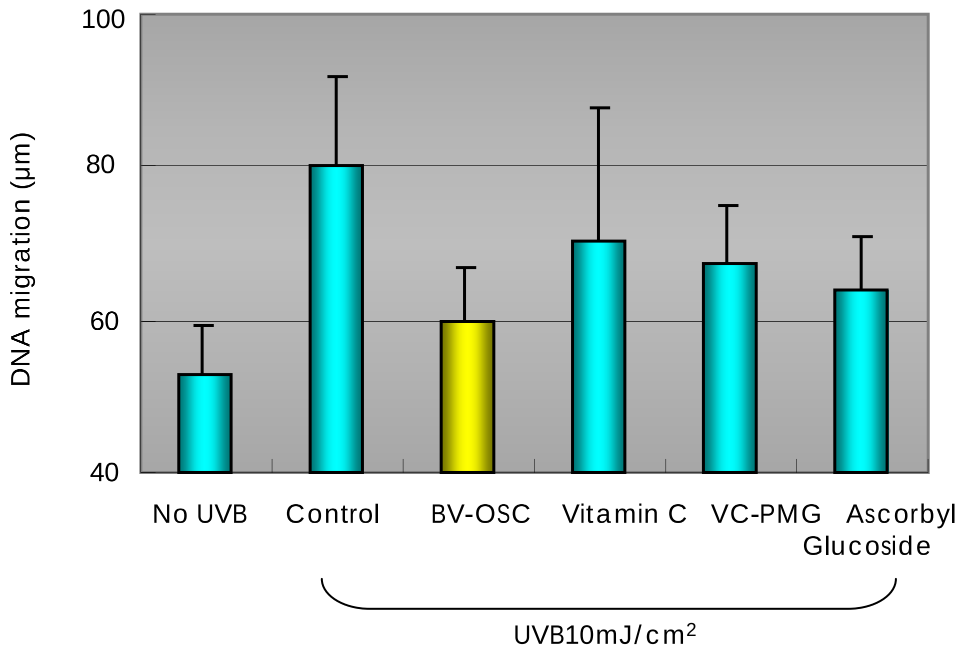


Test method



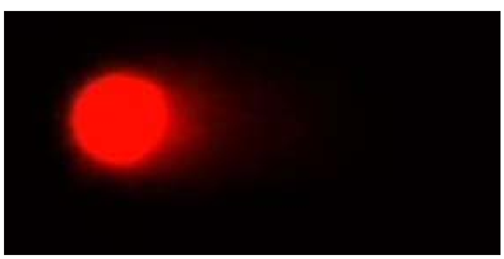
BV-OSC protects skin from UVB

Suppression of DNA damage induced by UVB (Comet Assay test)



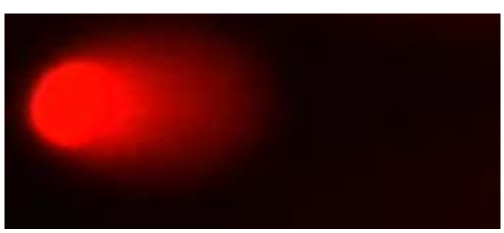
DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with VC derivatives for 24 h, were exposed to UVB at 100 mJ/cm^2.

Control (No UVB)

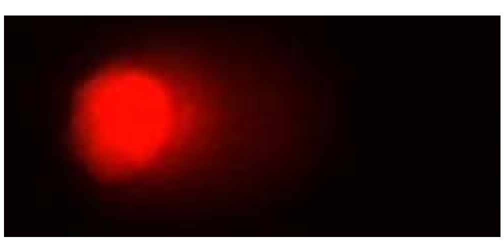


DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with BV-OSC for 24 h, were exposed to UVB at 10 mJ/cm^2. Cells are stained with etidium bromide.

UVB 10mJ/cm^2



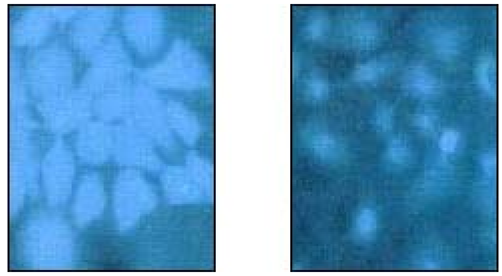
UVB 10mJ/cm^2 + BV-OSC (100 mM)



BV-OSC protects skin from UVA

Inhibition of keratinocytes' DNA damage induced by UVA

The light parts on the pictures (taken 1 hour after of UVA irradiation) indicate 8-hydroxyguanosine, an index of DNA damage. The application of BV-OSC inhibits the release of 8-hydroxyguanosine, thereby protecting the cell against UVA damage.



No treatment BV-OSC (80 μM)

Protection of cell damage induced by UVA

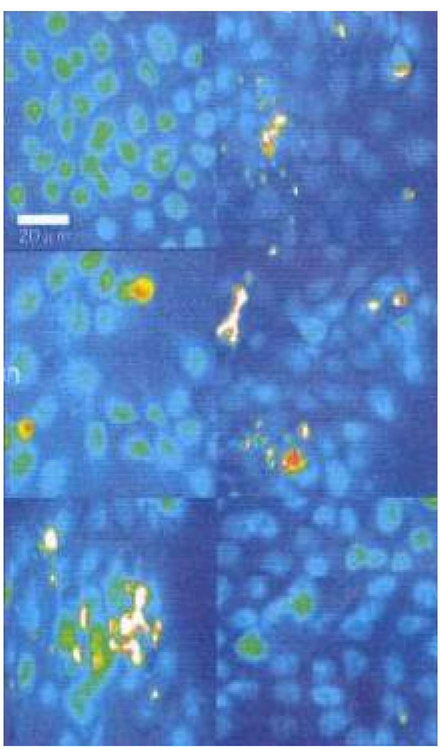
Microscopic pictures of keratinocytes 24 hours after irradiation.
BV-OSC treatment reduces cell death by 31.5%.

No UVA UVA UVA + BV-OSC

Inhibition of DNA Fragmentation induced by UVA

Control

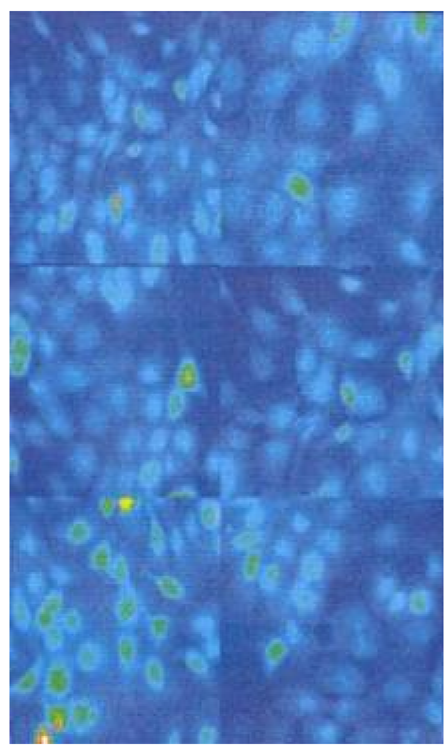
0 hr
0.5 hr
1 hr



2 hr
4 hr
8 hr

Treated with BV-OSC

0 hr
0.5 hr
1 hr



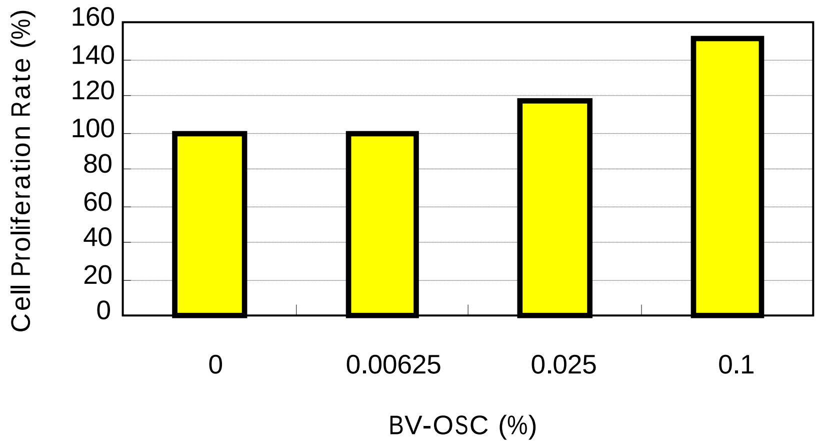
2 hr
4 hr
8 hr

HaCaT cells were treated with 80 μM BV-OSC. After UVA irradiation, DNA fragmentation was detected by nick end labeling method (TUNEL). BV-OSC significantly suppressed DNA fragmentation (shown with fluorescent staining).

Anti-aging Properties

Cell Revitalizing Activity / Cell Proliferation

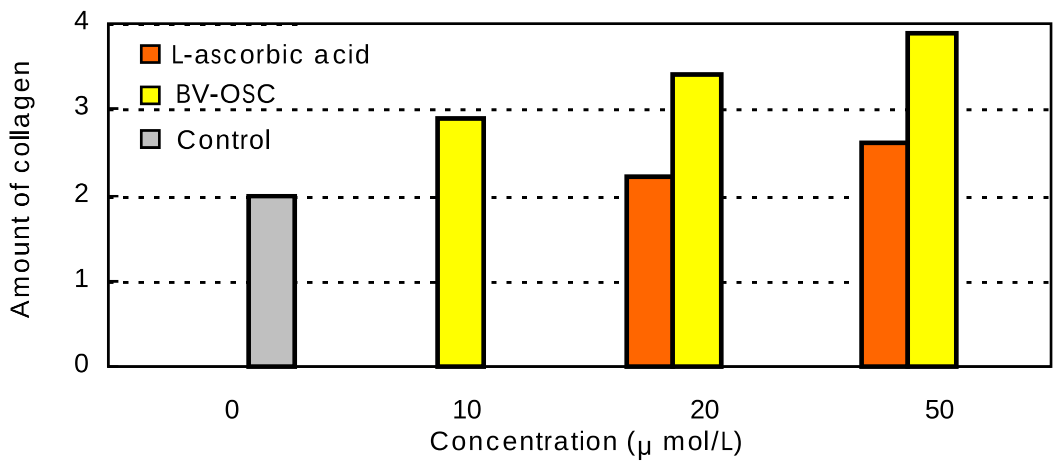
BV-OSC was added at various concentrations to human fibroblasts (NB1RGB). After 3 days of cultivation, the cell growth rate was measured by MTT reduction assay. As shown below, BV-OSC proliferated human fibroblasts. And the result was dose-dependent.



Cell Revitalizing Activity of BV-OSC

Promotion of Collagen Synthesis

Proline involved in collagen synthesis was labeled by ^{3}H and added to human dermal fibroblasts (NHDF) with various concentrations of BV-OSC/L-ascorbic acid and cultivated for 24 hours. Then collagen fractions were obtained. The amount of ^{3}H taken into the collagen fraction was measured by using a liquid scintillation counter and slot blotter. As shown below, BV-OSC significantly promoted collagen synthesis.

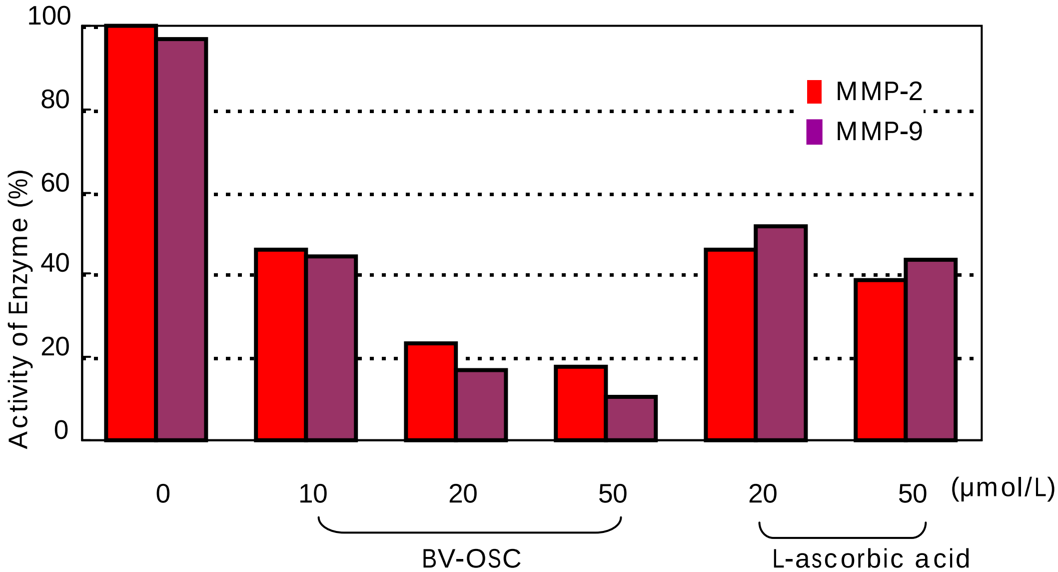


Promotion of Collagen Synthesis

Anti-aging Properties

Inhibition of Activity of Collagen Degrading Enzyme

BV-OSC was added at concentrations of 10-50μmol/L to human dermal fibroblasts (NHDF). After a 48-hour cultivation, secreted material was obtained. The activity of two types of collagen degrading enzymes, MMP-2 (72kDa) and MMP-9 (92kDa), in the secreted material were evaluated by gelatin zymography. BV-OSC drastically inhibited the activity of both MMP-2 and MMP-9. The inhibitory effect was considerably higher than that of L-ascorbic acid.

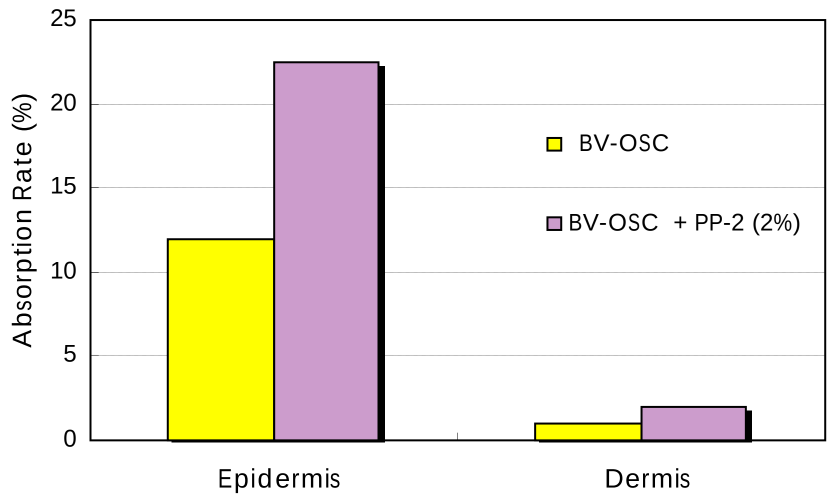


Inhibition of Collagen Degrading Enzyme Activity

Superior Skin Penetration

Percutaneous Absorption of BV-OSC

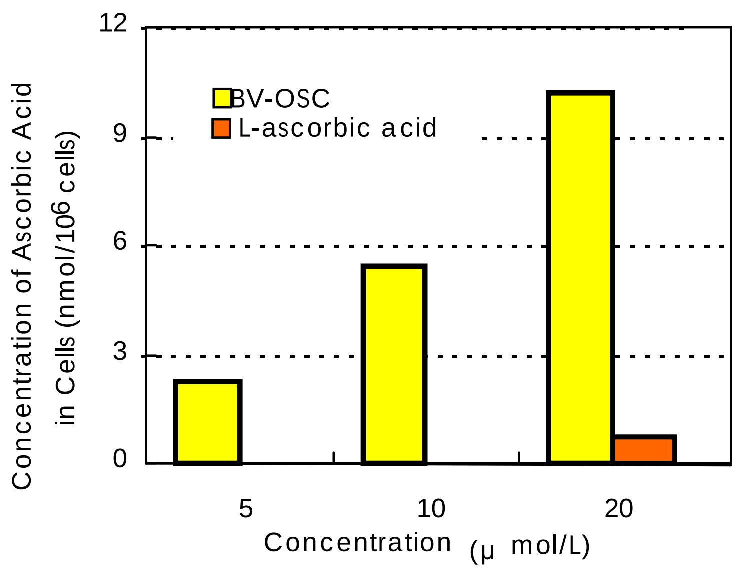
Percutaneous absorption of BV-OSC was measured with a tissue section isolated from human skin. BV-OSC showed superior penetration ability into the epidermis.
BV-OSC is an oil-soluble liquid and has a high affinity for the skin. This seems to explain the excellent percutaneous absorption. This ability can be enhanced when BV-OSC is used together with Polyolprepolymer-2 (PP-2, Bertek Pharmaceuticals).



Percutaneous Absorption of BV-OSC

Efficient Absorption into Human Dermal Fibroblasts

The absorption of BV-OSC into human dermal fibroblasts (NHDF) was measured as a concentration of ascorbic acid 2 hours after adding BV-OSC. As shown below, the intake of ascorbic acid into the skin after the addition of BV-OSC was considerably higher than that after the addition of L-ascorbic acid by itself. It was proven that BV-OSC was rapidly broken down into ascorbic acid at a high conversion rate.



Absorption of BV-OSC into Human Fibroblasts

Superior Skin Penetration

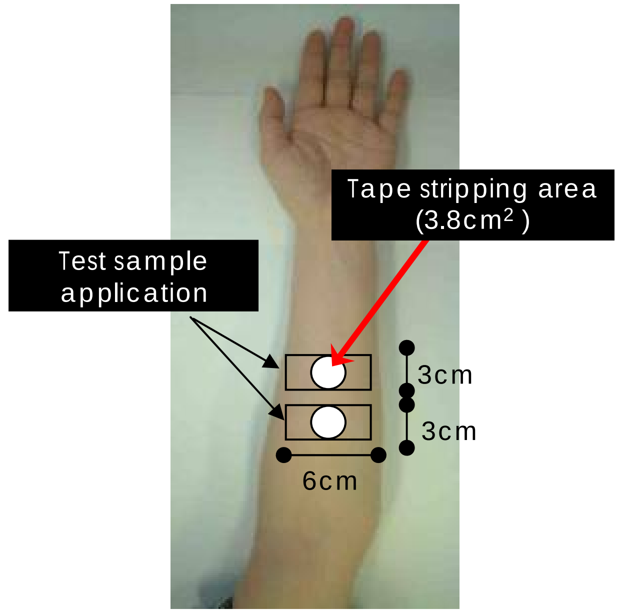
Evaluation of human skin penetration of BV-OSC and Ascorbyl Glucoside

Subjects: 8.
Test site: forearm.

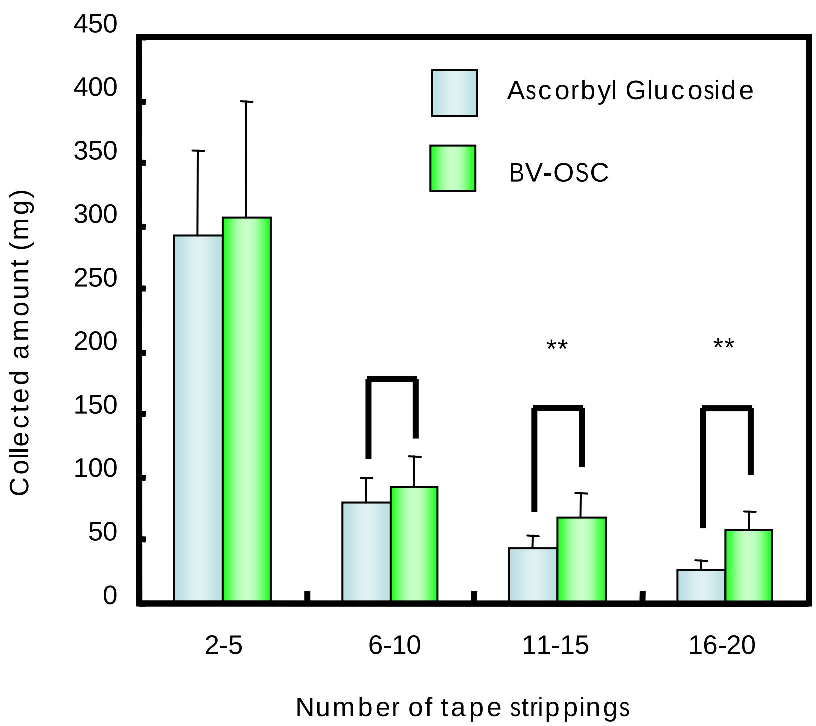
Cream with 10% BV-OSC
Cream with 10% Ascorbyl Glucoside

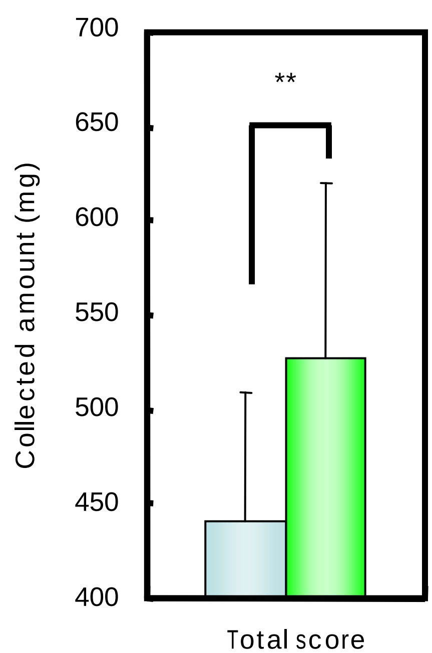
Application time: 1 hour

Tape stripping from test sites is performed 20 times



Amount of BV-OSC and Ascorbyl Glucoside collected by tape stripping is evaluated by HPLC





** : $p < 0.05$

BV-OSC showed higher skin penetration than Ascorbyl Glucoside.

Solubility of BV-OSC

The solubility of BV-OSC in various cosmetic raw materials was measured at 25, 50 and 75℃. BV-OSC is soluble in most oils, whether polar oil or non-polar, except polyhydric alcohols.

Concentration of BV-OSC (wt%)	5			10			50		
Temperature (℃) Cosmetic Ingredient	25	50	70	25	50	70	25	50	70
Glycerin	I	I	I	I	I	I	I	I	I
Propylene Glycol	I	I	I	I	I	I	I	I	I
1,3-Butylene Glycol	I	I	I	I	I	I	I	I	I
Ethanol	S	S	S	S	S	S	I	S	S
Propyleneglycol Monocaprylate	S	S	S	S	S	S	S	S	S
Castor Oil	S	S	S	S	S	S	S	S	S
Triethylhexanoin	S	S	S	S	S	S	S	S	S
Olive Oil	S	S	S	S	S	S	S	S	S
Cetyl Ethylhexanoin	S	S	S	S	S	S	S	S	S
Mineral Oil	S	S	S	S	S	S	S	S	S

S : Soluble I : Insoluble

Instructions for Use

BV-OSC has superior stability compared with other vitamin C derivatives. However, it may discolor under some specific conditions. Design preparations in the pH range not higher than 6.0. The use of chelating agents and anti-oxidants (tocopherol) in the formulation is recommended because they are effective to prevent discoloration. Since contact with water may induce oxidation of BV-OSC, add surfactants with long chain polyoxyethylenes to stabilize the system, strengthening the interfacial membrane.
Avoid heating for long periods of time.

Stability of BV-OSC in cream

Stability of BV-OSC assay and pH of cream (10% aq. solution) at 45 degrees Celsius x 3 months.

Emulsifier: glyceryl stearate.
BV-OSC: 3.3%. Oil phase: 10%.
PH adjustment: citric acid / sodium citrate.