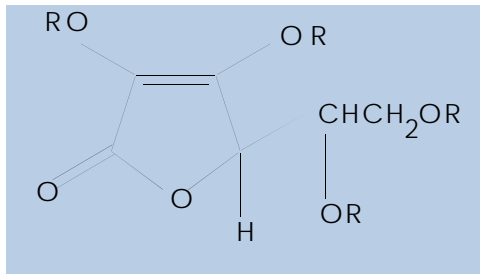


BARNET



BV-OSC



- A Quasi Drug Whitening Benchmark
- Superior Skin Penetration Compared to Other Whiteners
- Reduces Age Spots
- Great Stability in Formulas

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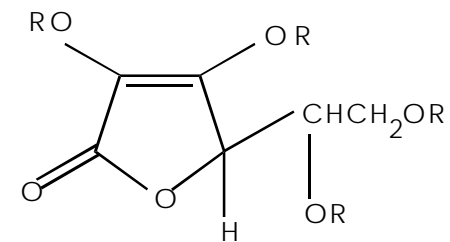
October 2015

CONCEPT

Whitening is the number one category in skin care in Asia. In Japan a whitening formula must contain a quasi-drug active (QD). The QD list is decided by the governmental administration in Japan. In Korea there is also an official list of Functional Actives.

To develop an active ingredient for whitening it is important to have it on those lists. The active also has to be very easy to use, stable, and with good delivery potential to penetrate into the skin. Ideally the whitening active could be used at or close to the skin's natural pH.

An ingredient with all the above would be a benchmark. This is BV-OSC, an oil-soluble ester of ascorbic acid.



BV-OSC at 3% (QD in Japan)

30 people – 3 weeks



Placebo

BV-OSC

<u>INGREDIENT</u>	<u>A</u>	<u>B</u>
PHASE A		
Ceteth-20	1.00	1.00
Sorbeth-30 Tetraoleate	0.50	0.50
Glyceryl Stearate	1.00	1.00
Cetanol	5.00	5.00
Squalane	10.00	10.00
Isocetyl Myristate	6.00	6.00
Triethylhexanoin	3.00	3.00
Jobba Oil	1.00	1.00
Dimethicone	0.20	0.20
Tocopherol	0.10	0.10
Preservative	0.10	0.10
BV-OSC	-----	3.00
PHASE B		
Water	61.90	58.90
Xanthan Gum (2% aq.)	5.00	5.00
Butylene Glycol	5.00	5.00
Preservative	0.20	0.20

BV-OSC was proven to have a strong whitening effect for UV induced pigmentation. The results showed that the formulation containing BV-OSC (3.00%) significantly reduced UV induced pigmentation compared to the placebo.

BV-OSC AT 10% ON AGE SPOTS

10 people – 16 weeks



Before



After

BV-OSC was tested in vivo to effectively remove age spots. The test was performed on 10 people for a sixteen week period at a concentration of 10%.

BV-OSC AT 10% AND 30% ON AGE SPOTS

NEW!

20 people, 12 weeks

The purpose of this clinical study was to evaluate the clinical efficacy of high concentration of BV-OSC in improving skin brightness of senile lentigo (aging spot) on female skin. * All volunteers recruited through the preliminary selection visited the research center for physical examination, medical history and visual evaluation of the test area by dermatologists.

Measurements:

1. L* value: Chromameter CR-400 (Minolta, Japan)

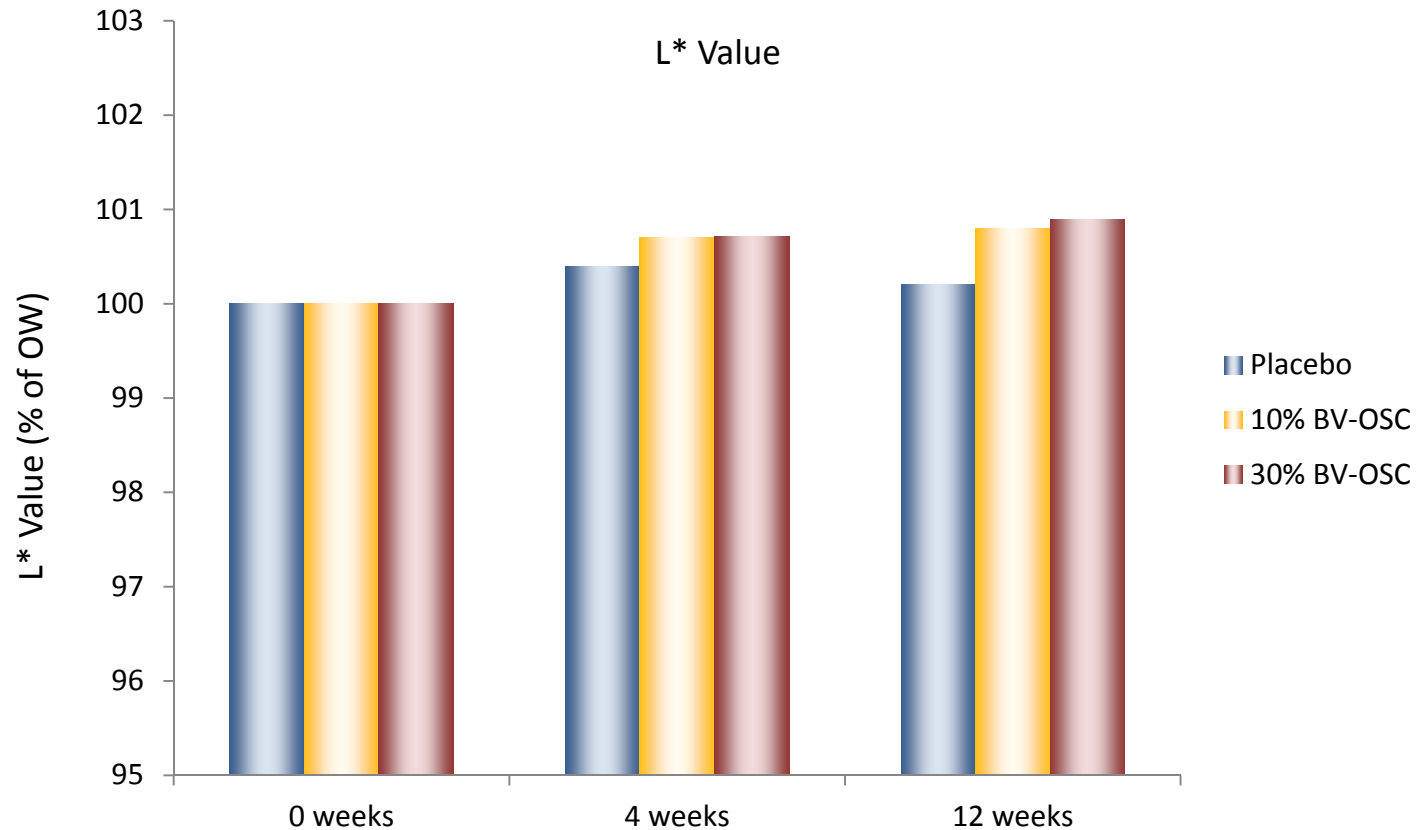


2. The selected tests are as of UV mode image and were evaluated by two experts using the grading scale below (0-7) at baseline, after 4 weeks, 8 weeks and 12 weeks.

- 0 None
- 1 None / mild hyper pigmented
- 2 Mild hyper pigmented
- 3 Mild / moderate hyper pigmented
- 4 Moderate hyper pigmented
- 5 Moderate / severe hyper pigmented
- 6 Severe hyper pigmented
- 7 Very severe hyper pigmented

Three formulas were used (Placebo, 10% BV-OSC and 30% BV-OSC). 20 female volunteers were selected for each formula.

BV-OSC AT 10% AND 30%

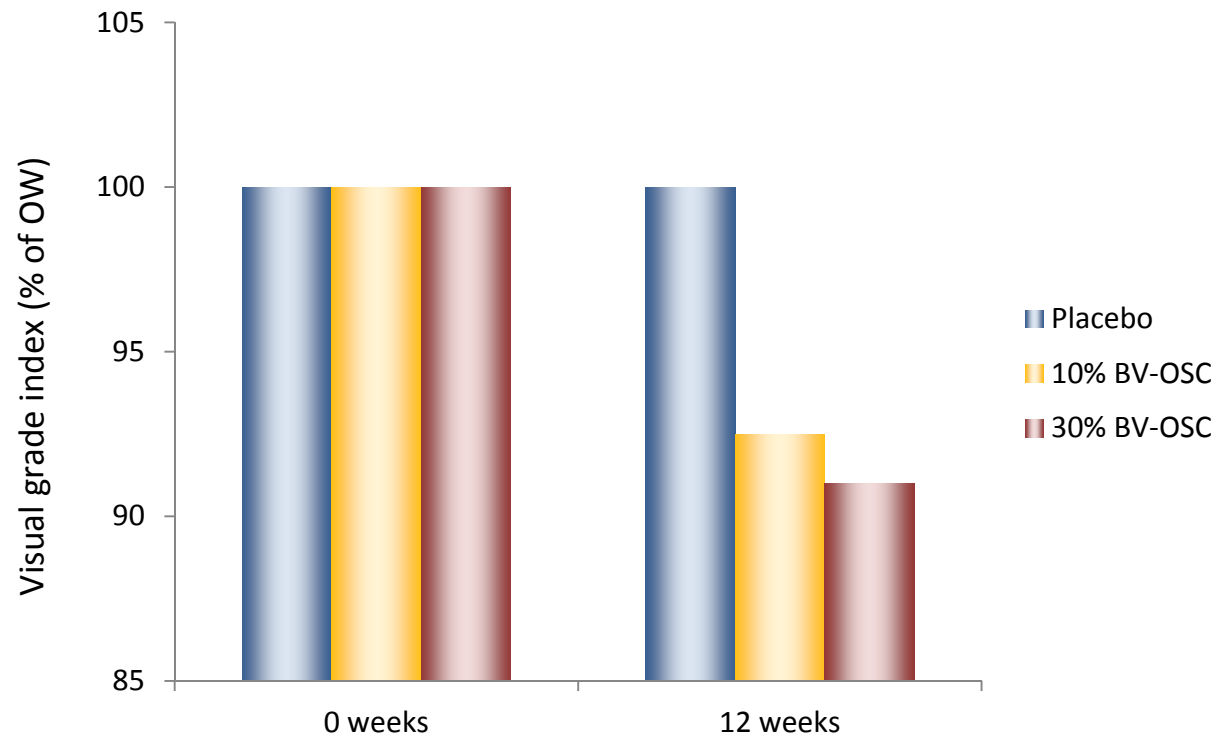


Results show that 10% BV-OSC was efficient in improving the lightening of the skin.

BV-OSC

Pigmentation of BV-OSC at 10% and 30% - 2015 test

Visual Assessment



BV-OSC significantly improved skin pigmentation in a dose dependent manner.

BV-OSC AT 30%



Baseline (0 week)

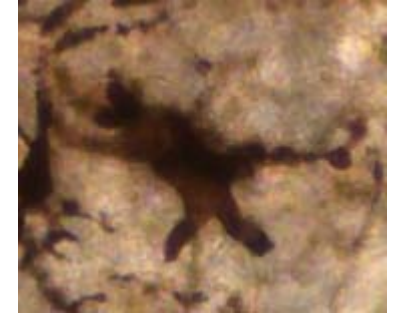
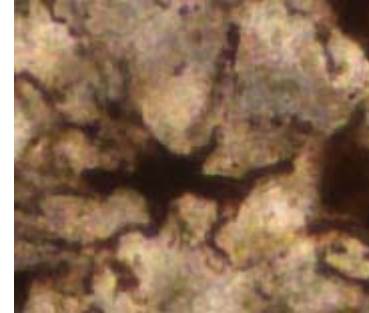
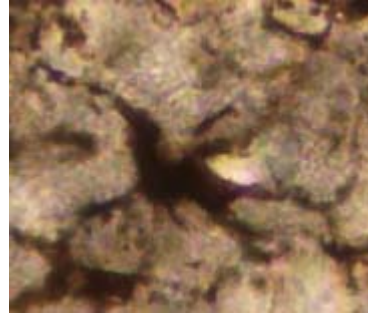
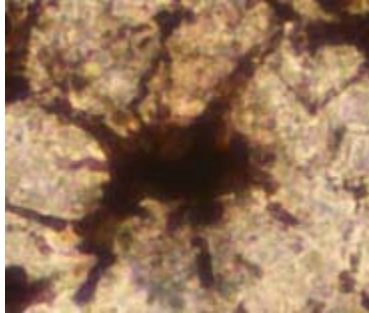


After 12 weeks

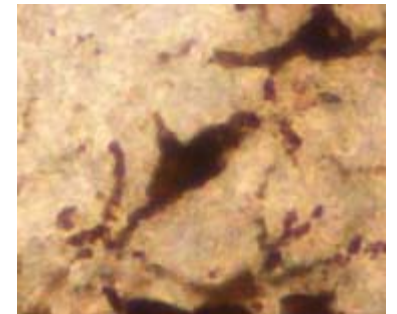
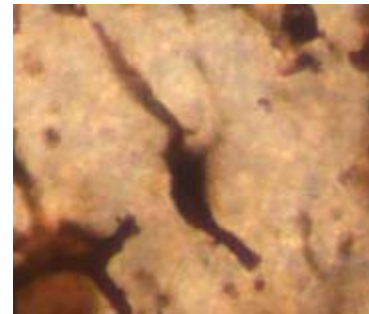
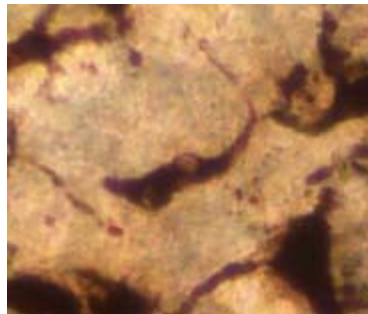
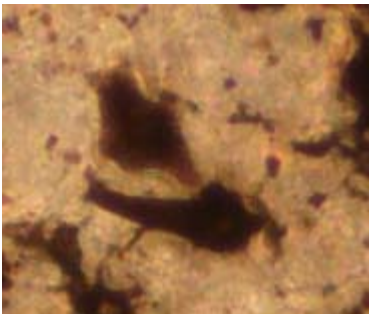
Pictures are an example of a significant reduction in the size of age spots.

BV-OSC REDUCES DENDRICITY AT 2%

Shape of melanocyte in 3D skin model



Control (0% BV-OSC)



2 % BV-OSC. Clearly less dendricity

Protocol:

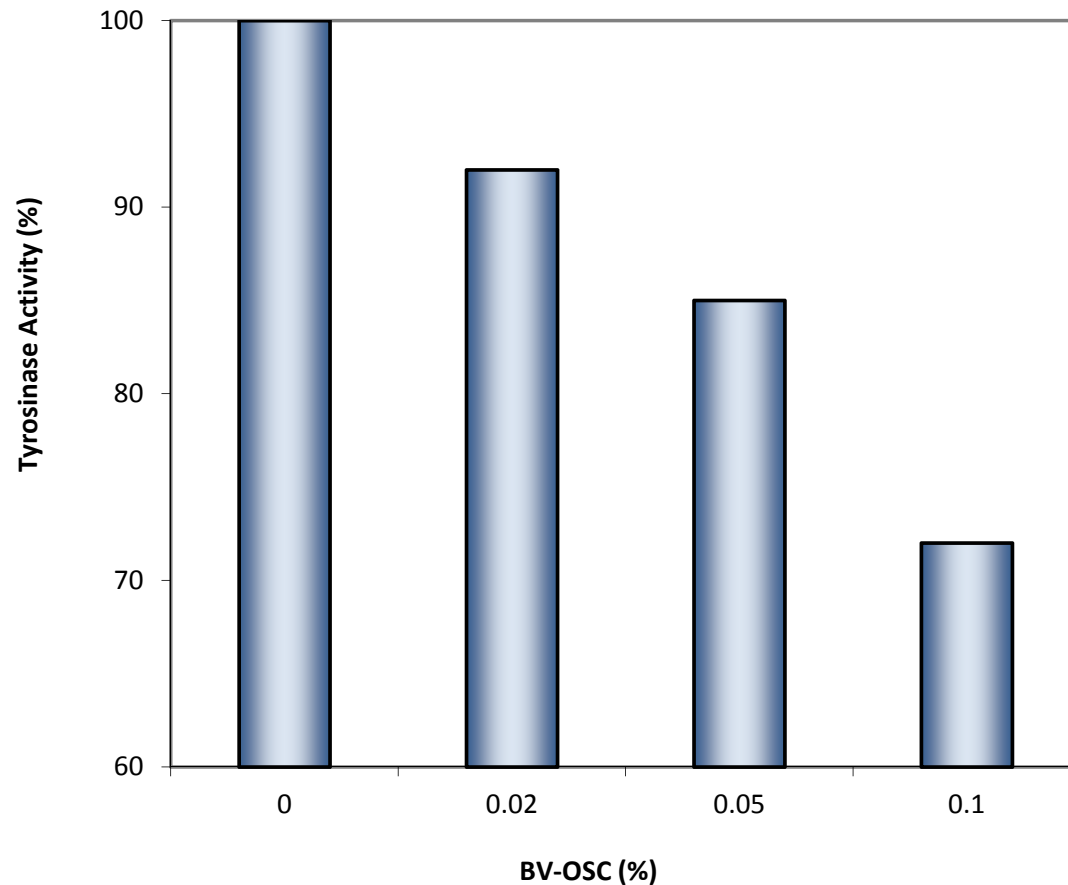
The 3D skin model containing melanocytes (MEL) was cultured EPI-100LLMM.

Test samples were applied from top of the skin model and melanocyte morphology was evaluated after 1 week. Melanocytes were stained with 0.1 % L-3,4-Dihydrophenylalanin (L-DOPA) and their shape was observed via microscope.

Test samples: BV-OSC 2%

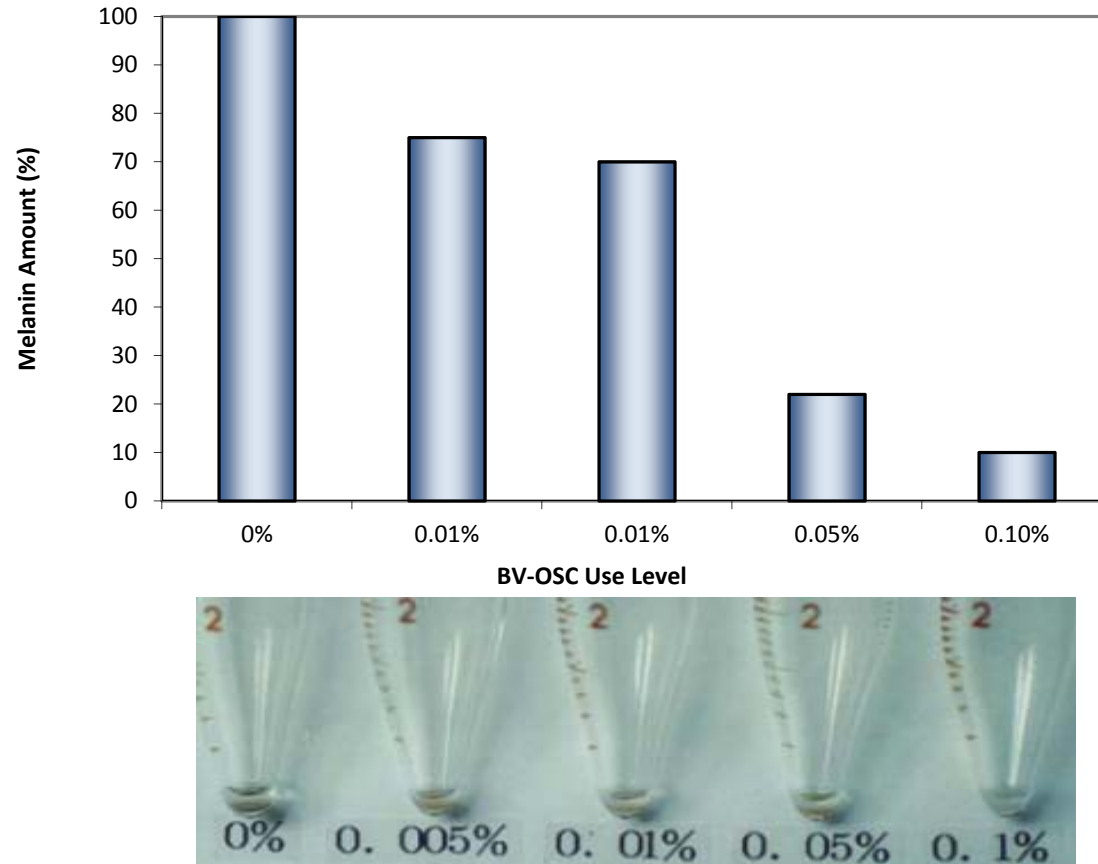
Vehicle: Ethylhexyl Palmitate (ester oil)

BV-OSC AND TYROSINASE INHIBITION



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

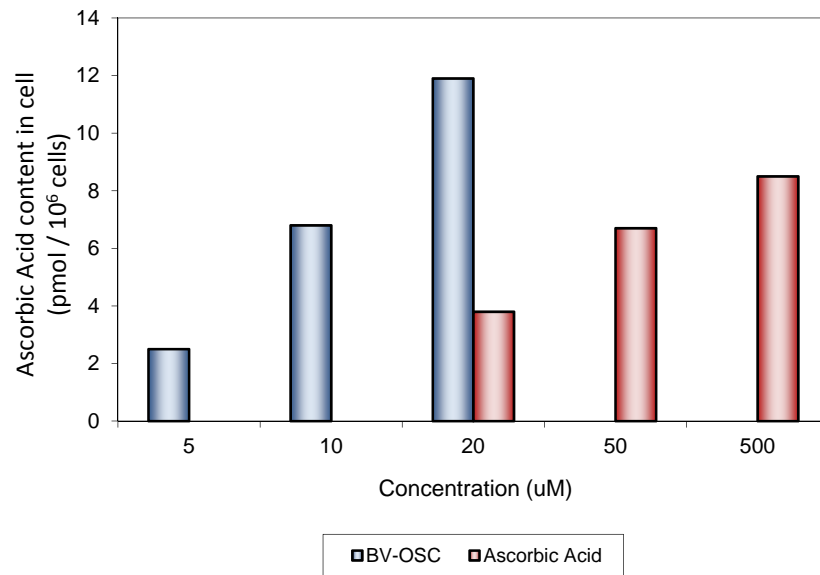
BV-OSC: INHIBITION OF MELANOGENESIS



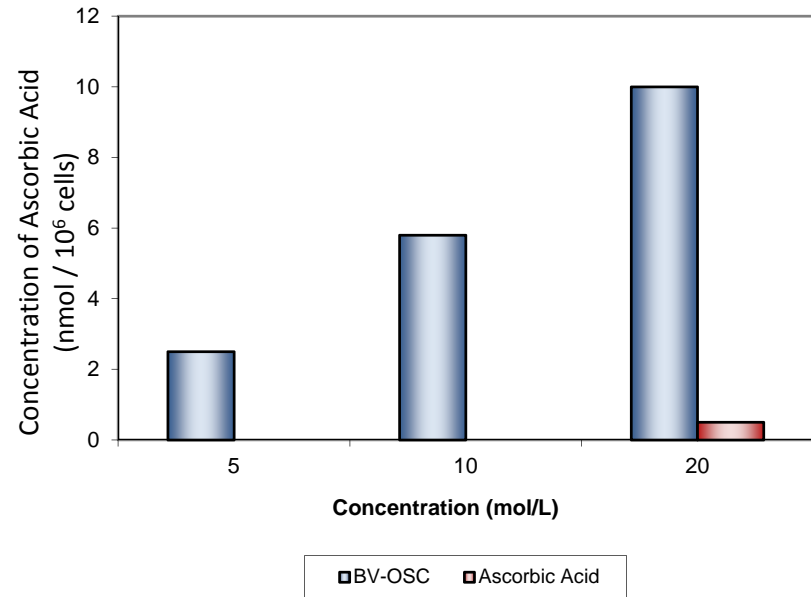
Various concentrations of BV-OSC were added to cultured human melanoma cells (HM-3-KO). After 4 days of cultivation, the amount of melanin produced was measured by observation of the color tone of each cell pellet. As shown, BV-OSC effectively inhibited melanogenesis in human melanoma cells in a dose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

BV-OSC: ABSORPTION EVALUATION

Keratinocyte Absorption



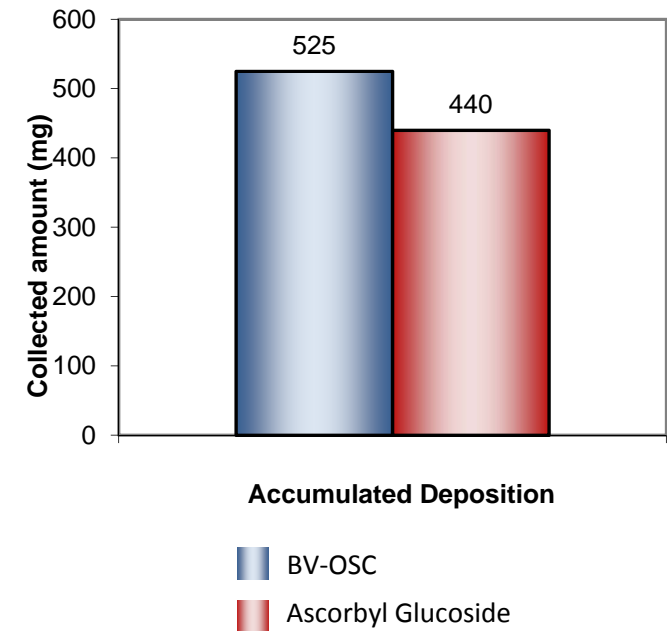
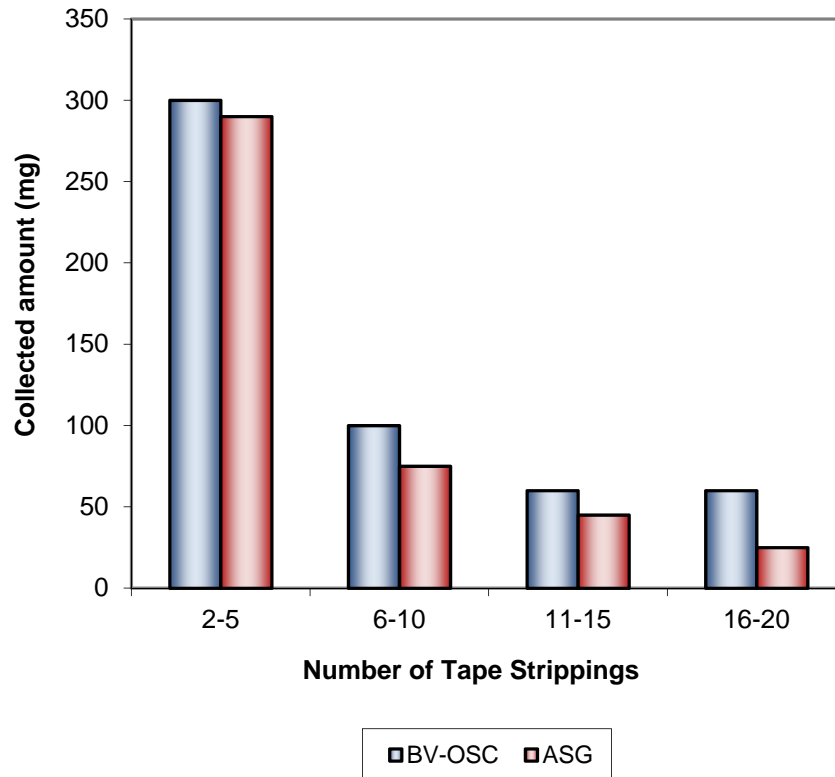
Fibroblast Absorption



The absorption of BV-OSC into human dermal fibroblasts and keratinocytes was measured as a concentration of ascorbic acid 2 hours after adding BV-OSC. As shown, the intake of ascorbic acid was much greater after the addition of BV-OSC as compared to L-ascorbic acid by itself. It was proven that BV-OSC breaks down into Ascorbic Acid inside the cells.

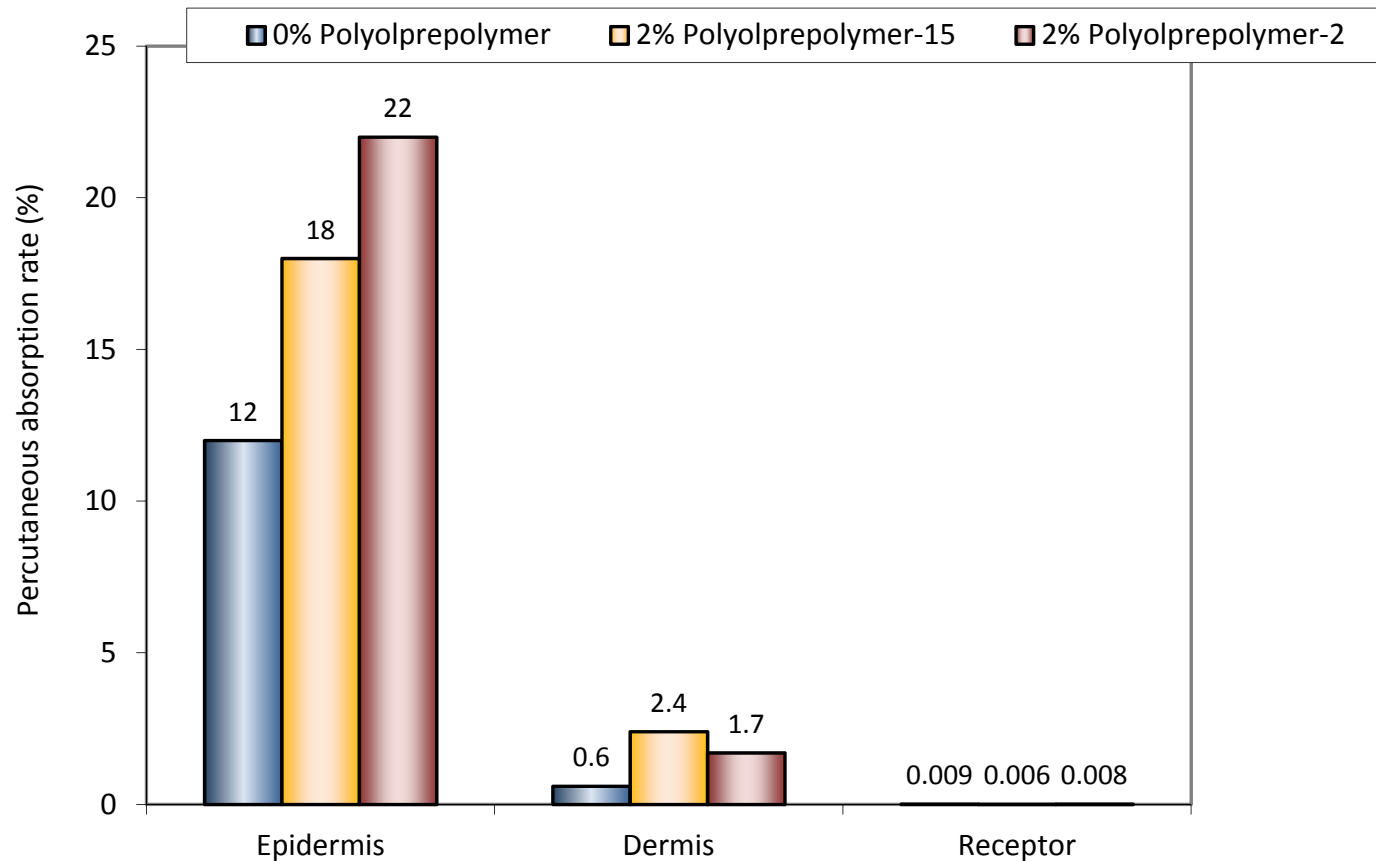
BV-OSC: SKIN PENETRATION EVALUATION (ASCORBYL GLUCOSIDE AND BV-OSC)

Skin Penetration Evaluation (Ascorbyl Glucoside and BV-OSC)



Skin penetration of BV-OSC and Ascorbyl Glucoside was evaluated on 8 volunteers. A cream containing 10% of each was applied on the forearm and let sit for 1 hour. Tape strippings were performed 20 times and the amount of BV-OSC and ASG collected was evaluated by HPLC. As seen in the graph above, BV-OSC had better penetration results.

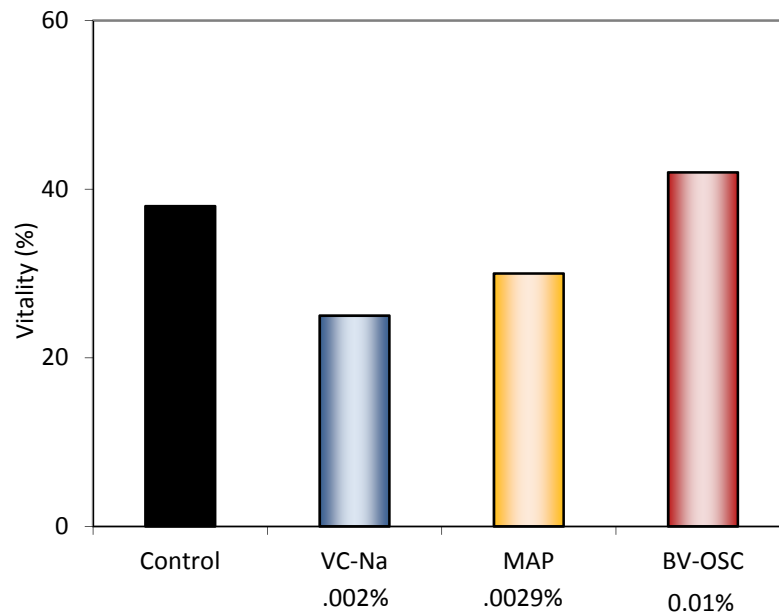
IMPROVING BV-OSC PENETRATION



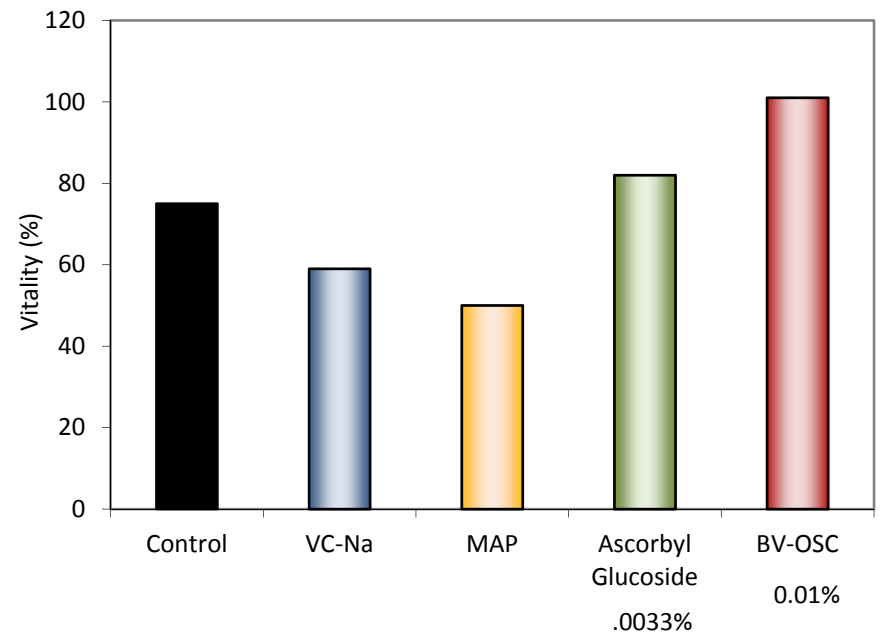
A study was conducted using human skin comparing the percutaneous absorption of topically applied BV-OSC (5 μM) from cream formulations containing 2% Polyolprepolymer-2 and 2% Polyolprepolymer-15 follow a 24 hour exposure. The results showed an increased presence of BV-OSC in the epidermis and dermis compared to the cream without Polyolprepolymers.

BV-OSC: ANTI-OXIDANT PROPERTIES

Protection of Cell Damage induced by H₂O₂



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

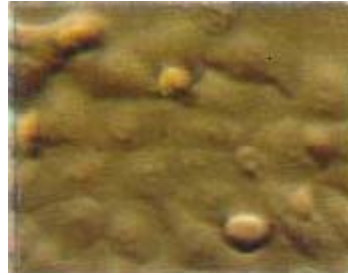


Human keratinocytes were treated with various vitamin C derivatives for 24 hours. After treatment of H₂O₂ and t-BHP cell survival was estimated. As seen above, BV-OSC showed the highest efficacy in cell viability.

BV-OSC: DNA PROTECTION – UV-A

Protection of Cell Damage Induced by UVA

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



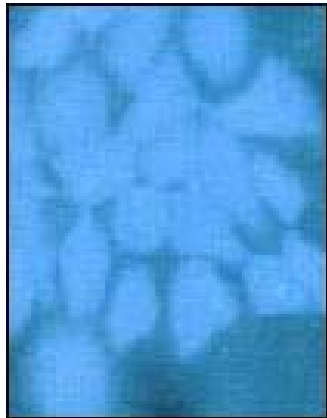
No UVA



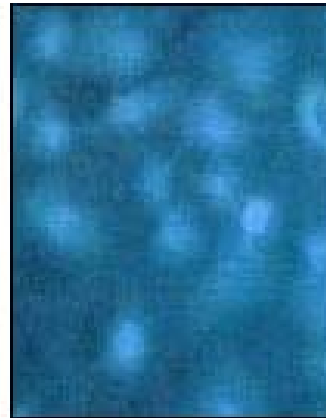
UVA



UVA + BV-OSC 80 mM



No treatment



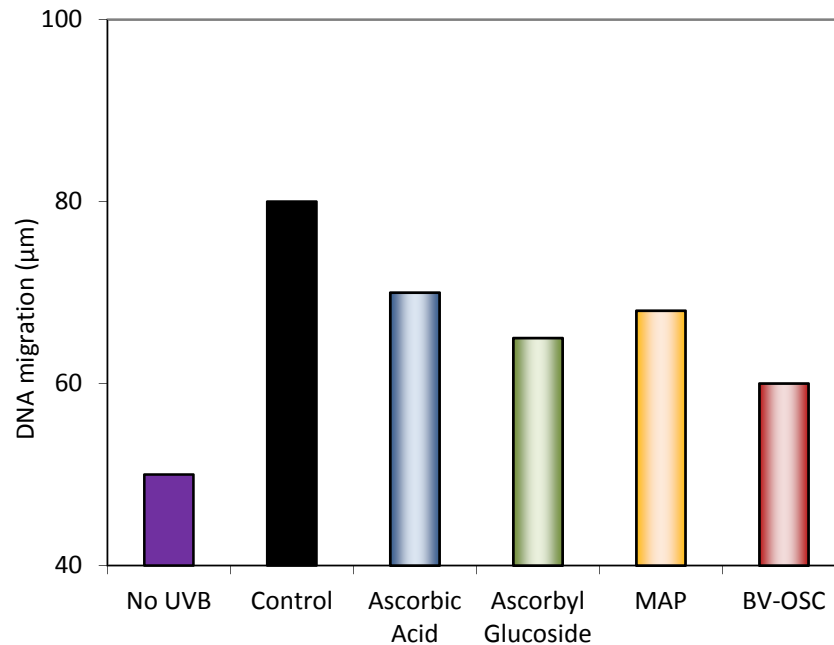
BV-OSC (80 μ M)

Inhibition of Keratinocytes DNA Damage Induced by UVA

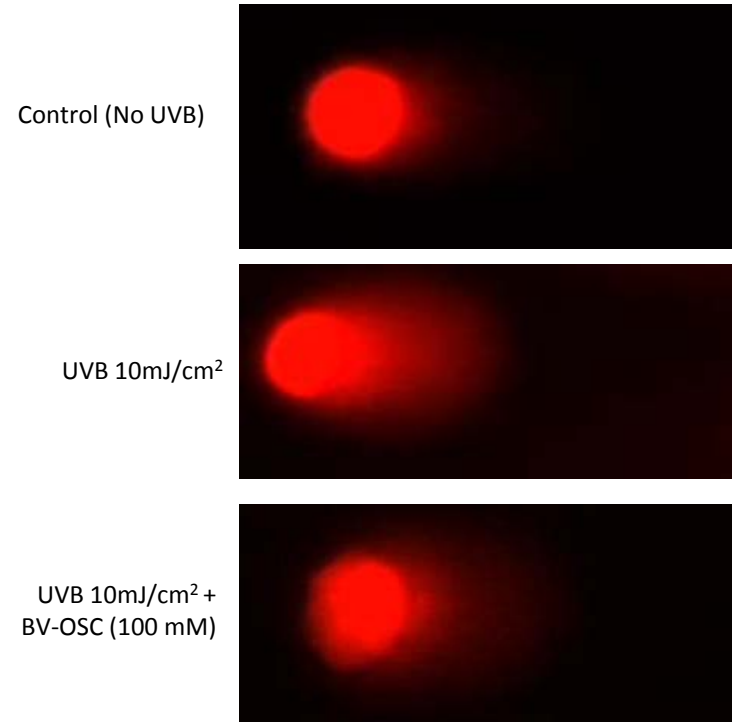
The light parts on the pictures (taken 1 hour after of UVA irradiation) indicate 8-hydroxyguanosine, an index of DNA damage. The application of BV-OSC inhibits the release of 8-hydroxyguanosine, thereby protecting the cell against UVA damage.

BV-OSC: DNA PROTECTION – UV-B

Suppression of DNA Damage Induced by UVB (Comet Assay)



BV-OSC Comet Assay



DNA damage was evaluated by the comet assay. HaCaT keratinocytes were treated with Vitamin C derivatives for 24 hours, were exposed to UVB at 100 mJ/cm². As seen in the results above, BV-OSC was the most effective form of Vitamin C for protection against DNA damage.

CONCLUSION

BV-OSC:

- Reduces melanin synthesis by 80%
- Reduces age spots
- Reduces UV-induced pigmentation
- Penetrates the skin 50 times better than Ascorbic Acid and 4 times better than MAP
- Tested in vivo to penetrate better than Ascorbyl Glucoside
- Superior stability in formulas

BV-OSC

INCI Name:	Tetrahexyldecyl Ascorbate
REACH Status:	Registered
Canada DSL:	Listed RICL (Revised In Commerce List)
China Registration:	All components are listed in the Inventory of Existing Chemical Substances in China (IECSC) and the Inventory of Existing Cosmetic Ingredients in China. (IECIC).
Suggested Use Level:	0.1% - 100.0%
Solubility:	Oil
Quasi-Drug:	Approved as a quasi-drug in Japan at 3% Approved as a quasi-drug in Korea at 2%

