



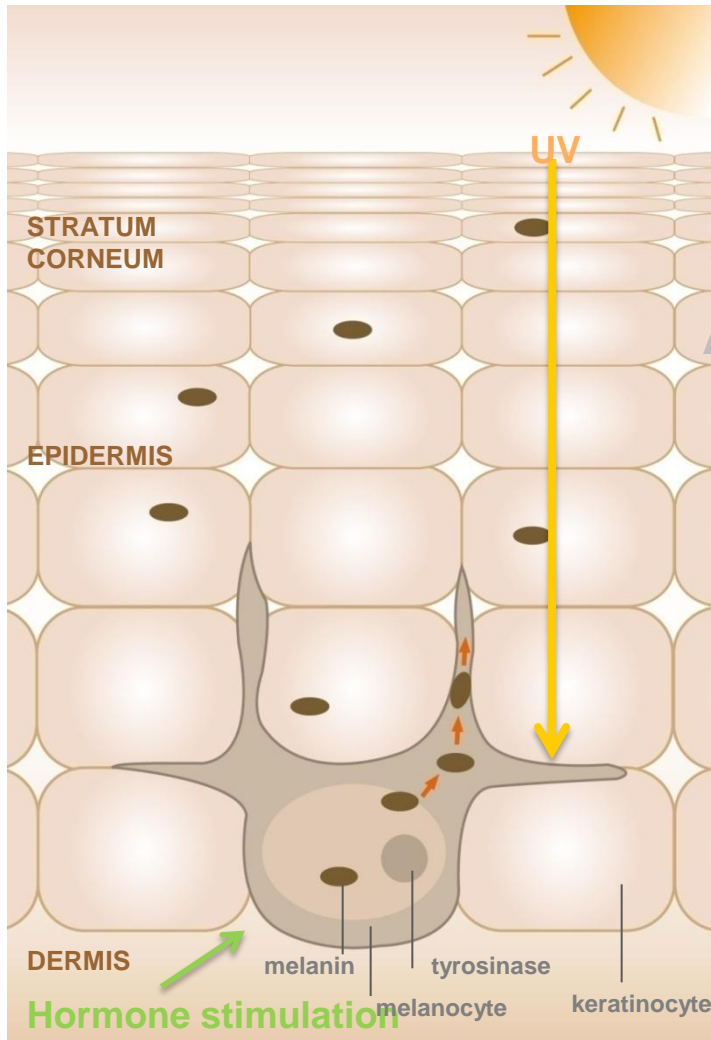
Corum 9515

3-O-Ethyl Ascorbic Acid

**AS POTENT AS VITAMIN C
WITHOUT THE INSTABILITY**

CORUM
For brighter, younger-looking skin

Melanogenesis



3. Migration to the skin surface

2. Distribution to keratinocyte

1. Melanin production

- Tyrosinase inhibition
- Inhibition of melanin stimulating factor
e.g. alpha-MSH

Whitening agents examples

- **Hydroquinone**
 - Effective BUT cytotoxic and mutagenic
 - Cannot be used in cosmetics
- **α -Arbutin**
 - A hydroquinone derivative
 - Potential ban in Europe
- **Kojic acid**
 - Possible mutagenic
 - Japan: 1% limitation
- **Ascorbic acid**
 - Effective BUT easily oxidized in aqueous solution

Ref: Petit, L., Pierard, G., *Skin-lightening products revisited*, Int. J. Cosm. Sc, (2003), 25, 169-181

Whitening agents – Ascorbic Acid Derivatives

- **Magnesium L-ascorbyl-2-phosphate (MAP)**
 - Stable
 - Easily dissociates to anions
 - Difficult to penetrate the epidermis
- **L-Ascorbic Acid 2-Glucoside (AA2G)**
 - Higher molecular weight
 - Lesser activity on tyrosinase inhibition
 - Lesser activity on monomer aggregation
- **Ethyl ascorbic acid**
 - Stable
 - Penetrates epidermis

Ref: Petit, L., Pierard, G., *Skin-lightening products revisited*, Int. J. Cosm. Sc, (2003), 25, 169-181

CORUM 9515

INCI: 3- O – Ethyl ascorbic acid

Also known as:

3- O – ethyl ascorbic ether

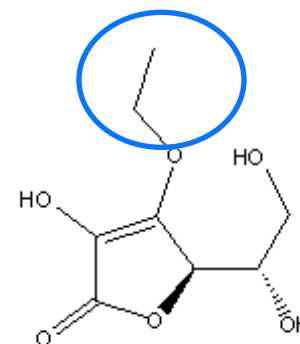
3- O – ethyl ascorbic acid

CAS: 86404-04-8

MW: 204.18

Physical Properties

- Purity > 99.0%
- White crystalline powder
- Water soluble
- Metabolized by the human body in the same manner as L-ascorbic acid (Vitamin C)
- Excellent stability



The Functions of Corum 9515



Skin lightening & Balance skin tone

Reduce dark spot & age spot

Collagen synthesis

Reversing auto-oxidation

Radical scavenging

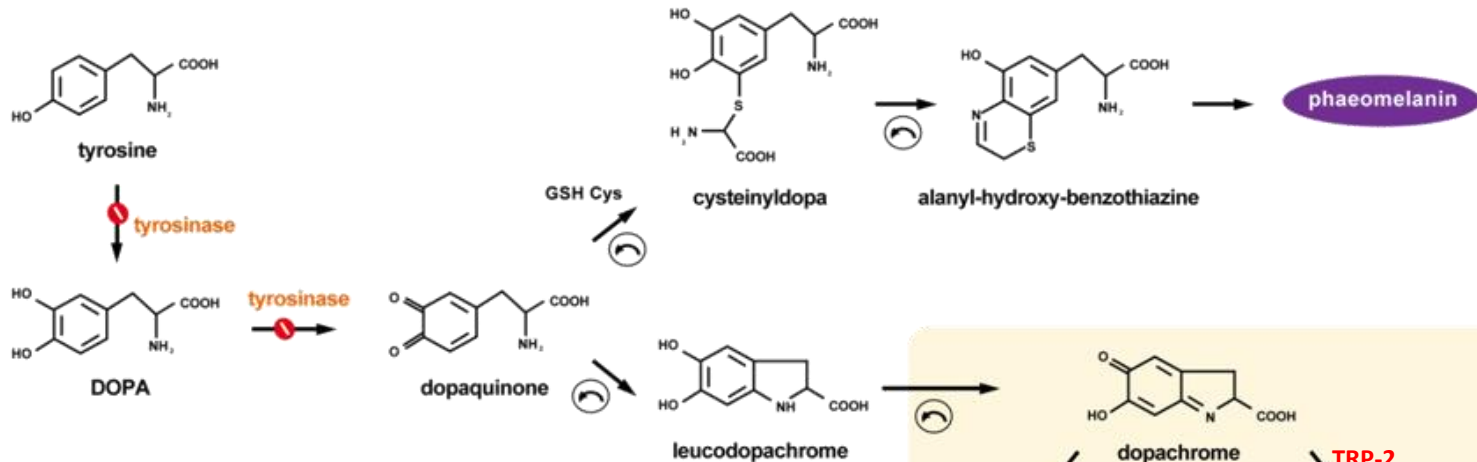
DNA protection

CORUM 9515 Efficacy Studies

- **In- vitro tyrosinase inhibition**
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- In-vivo whitening efficacy
- Anti-inflammation test
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA Protection



CORUM 9515 Whitening Mechanism



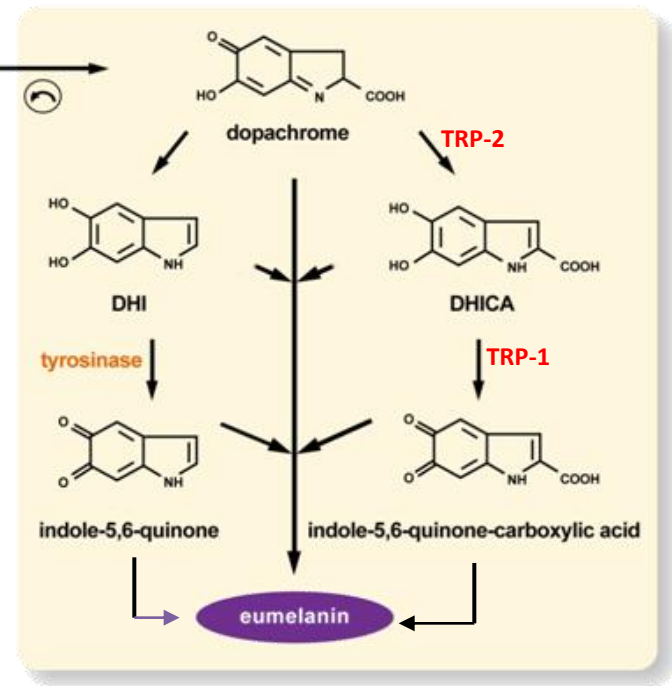
Three melanin-generating enzymes :

1. Tyrosinase
2. TRP-2 (DOPochrome tautomerase)
3. TRP-1 (DHICAoxidase)

-Inhibit the reaction center Cu^{2+} ion of tyrosinase

-inhibit the activity of TRP-2

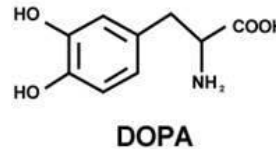
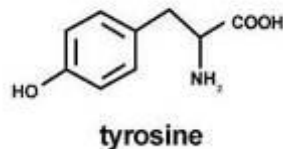
- Reduce oxidized melanin pigments



Ref: Briganti, S et al. (2003) *Chemical and Instrumental Approaches to Treat Hyperpigmentation*
 Pigment Cell Res 16:101–110.

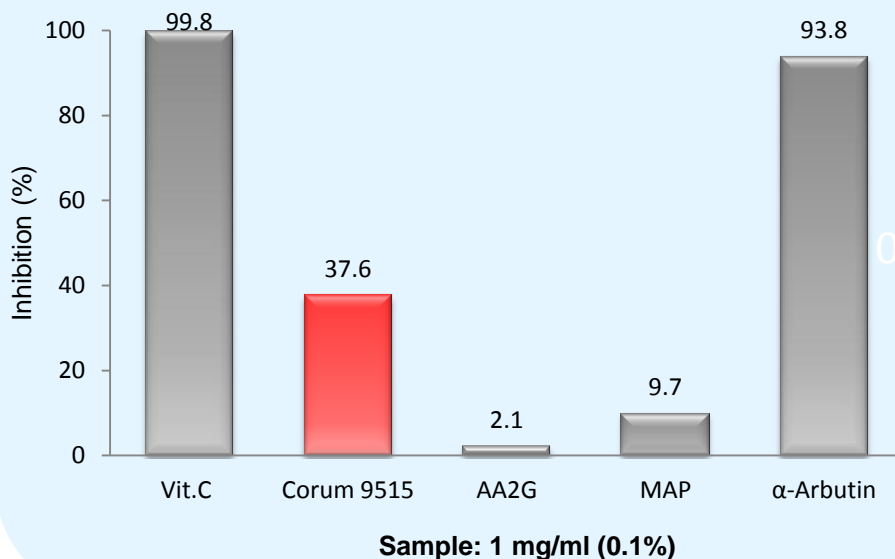
Efficacy Test: *In- vitro* tyrosinase (I) inhibition

Reaction:

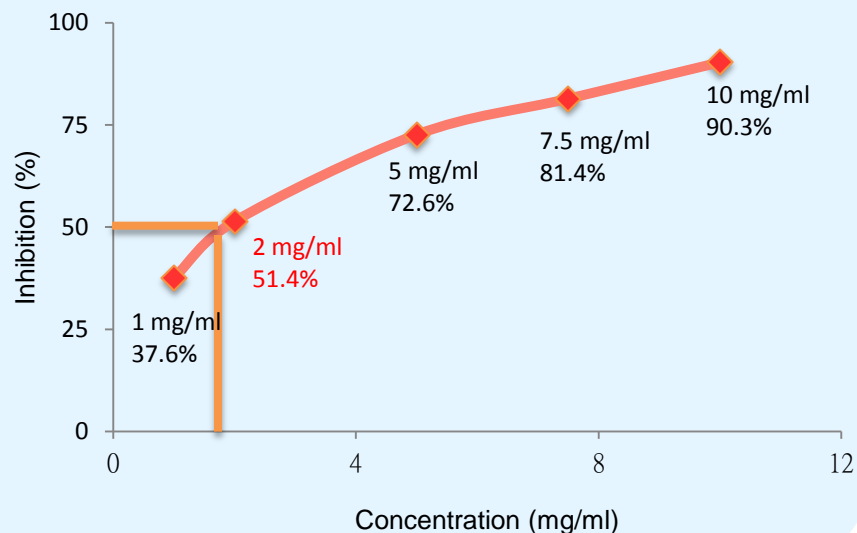


Enzyme level

Tyrosine Assay



Tyrosine Inhibition Activity IC50

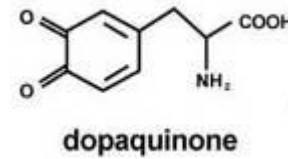
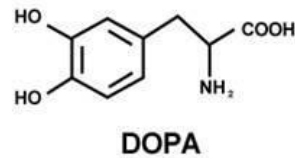


Result : 0.1 % Corum 9515 can inhibit tyrosinase activity up to 37.6 %,

2 mg/mL concentration of Corum 9515 can reach 50% inhibition

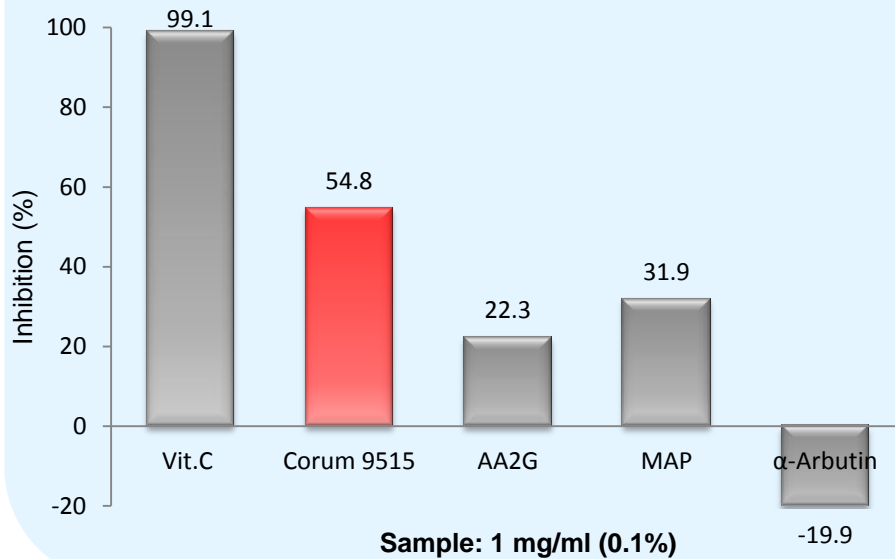
Efficacy Test: *In- vitro* tyrosinase (II) inhibition

Reaction:

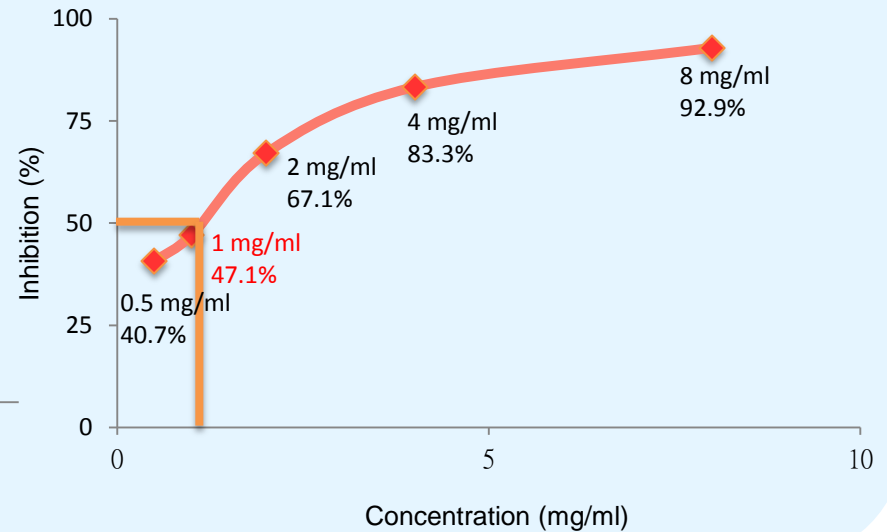


Enzyme level

DOPA Assay



Tyrosine Inhibition Activity IC50



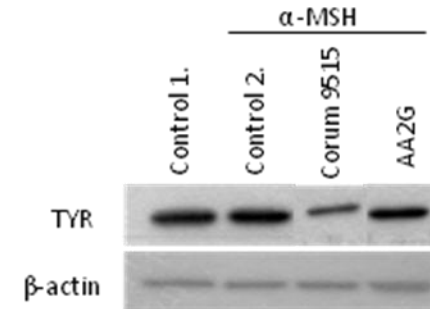
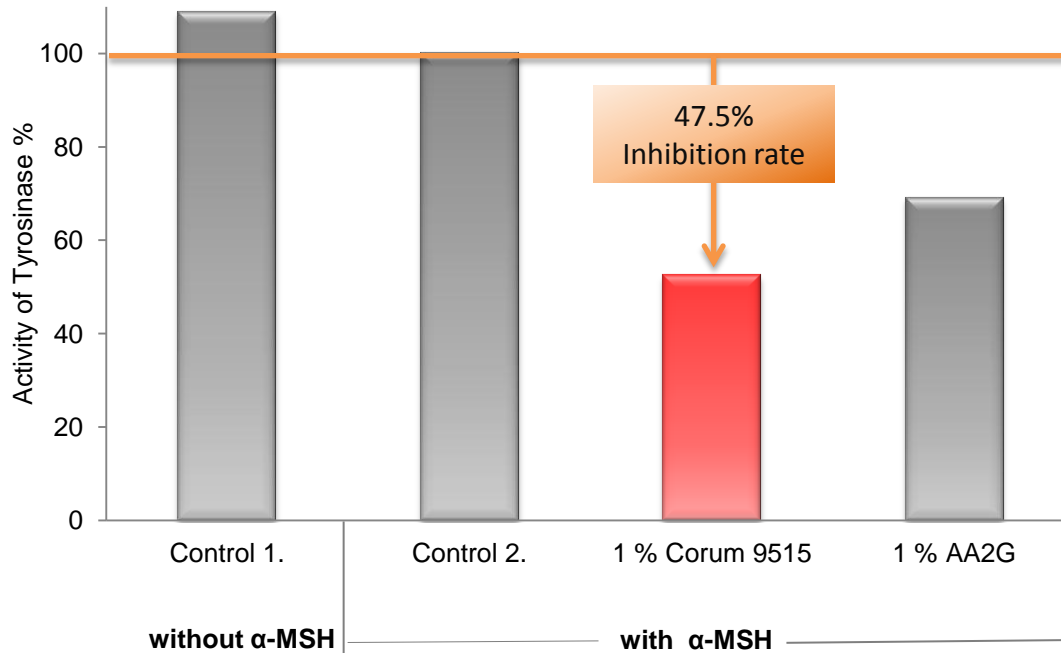
Result: 0.1 % Corum 9515 can inhibit tyrosinase activity up to 54.8%,

1 mg/mL concentration of Corum 9515 can reach 50% inhibition

Efficacy Test: *In- vitro* Tyrosinase, Trp-2 inhibition

Protein level

Tyrosinase inhibition



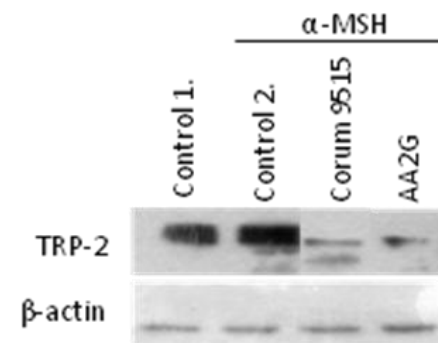
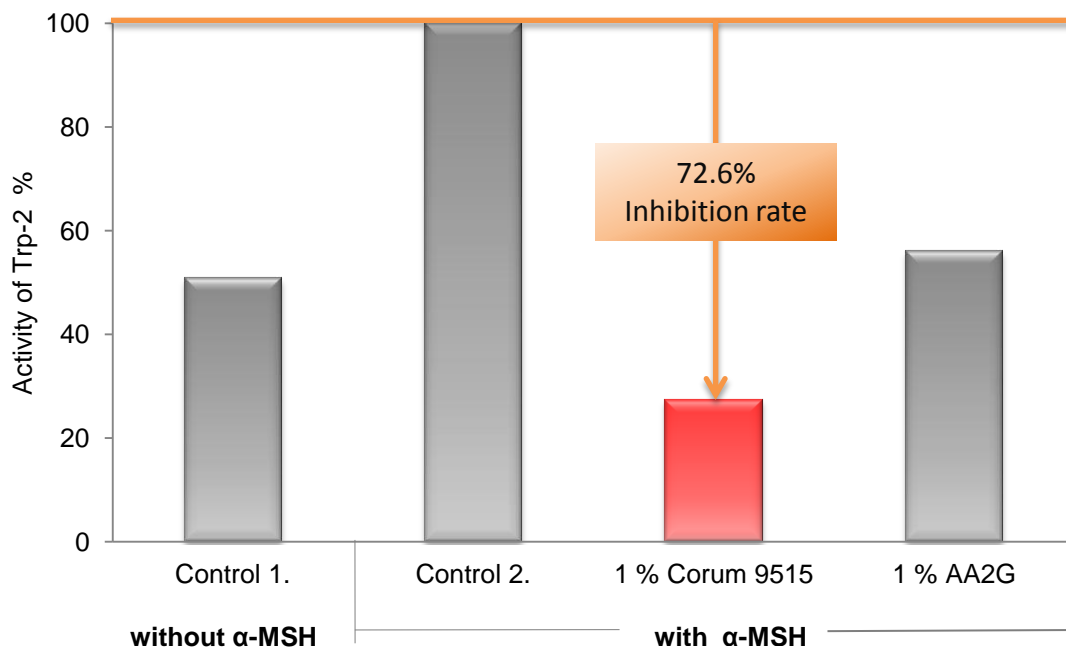
Tests:
1. Western blot – Tyrosinase, Trp-2
2. In-vitro melanogenesis inhibition (with alpha-MSH)

Result : 1 % Corum 9515 can inhibit tyrosinase activity up to 47.5 % in protein level.

Efficacy Test: *In- vitro* Tyrosinase, Trp-2 inhibition

Protein level

Trp-2 inhibition



Tests:
1. Western blot – Tyrosinase, Trp-2
2. In-vitro melanogenesis inhibition (with alpha-MSH)

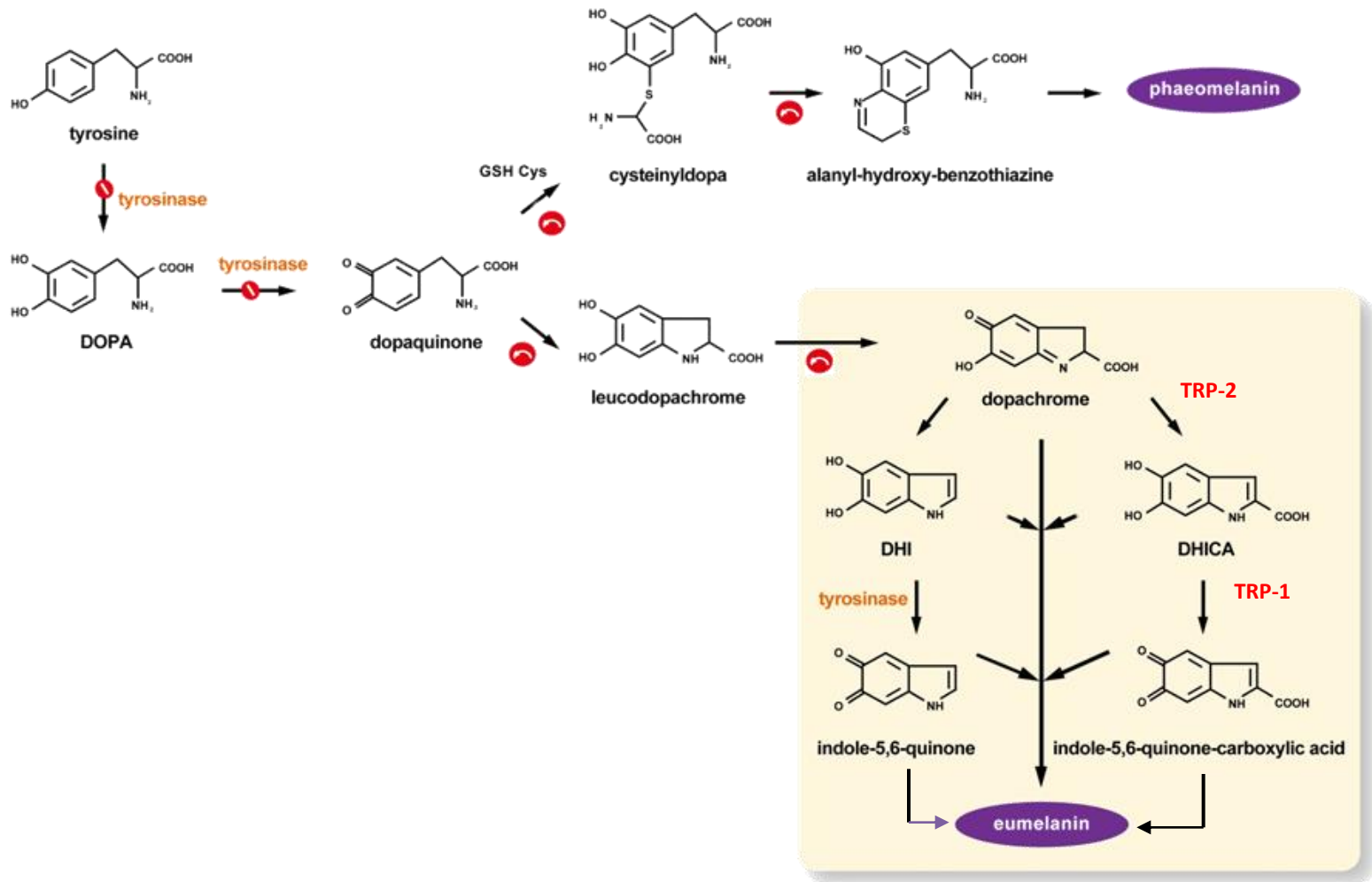
Result : 1 % Corum 9515 can inhibit trp-2 activity up to 72.6 % in protein level.

CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
- **Reducing activity**
- Ex-vivo melanin assay
- In-vitro whitening activity
- In-vivo whitening efficacy
- Anti-inflammation test
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA Protection



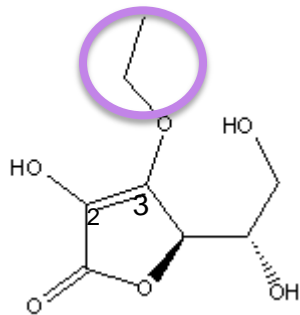
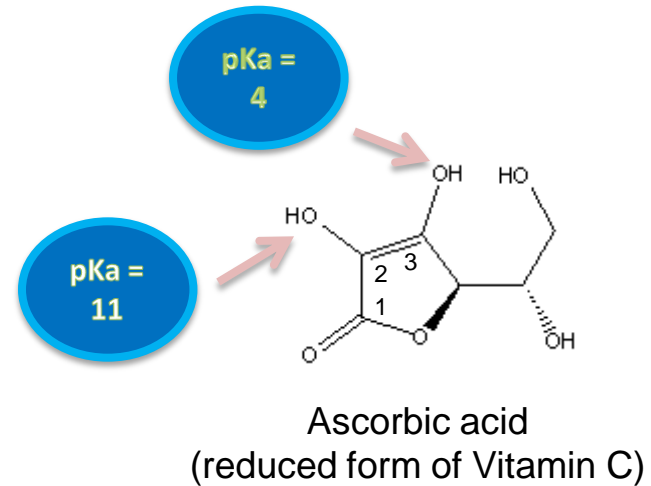
Efficacy Test: Ferric Reducing Ability



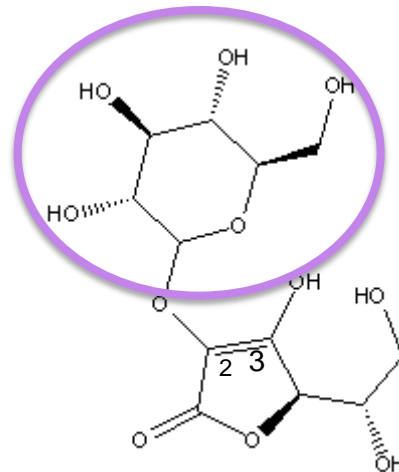
Ref: Briganti, S et al. (2003) *Chemical and Instrumental Approaches to Treat Hyperpigmentation*
 Pigment Cell Res 16:101–110.

Efficacy Test: Ferric Reducing Ability

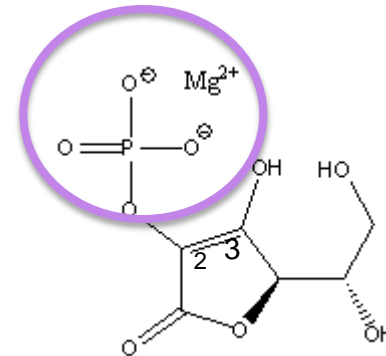
- pKa value greater – less acidic
- better reducing ability
- 2' carbon without occupying by the ethyl group has better reducing ability



Ethyl Ascorbic Acid

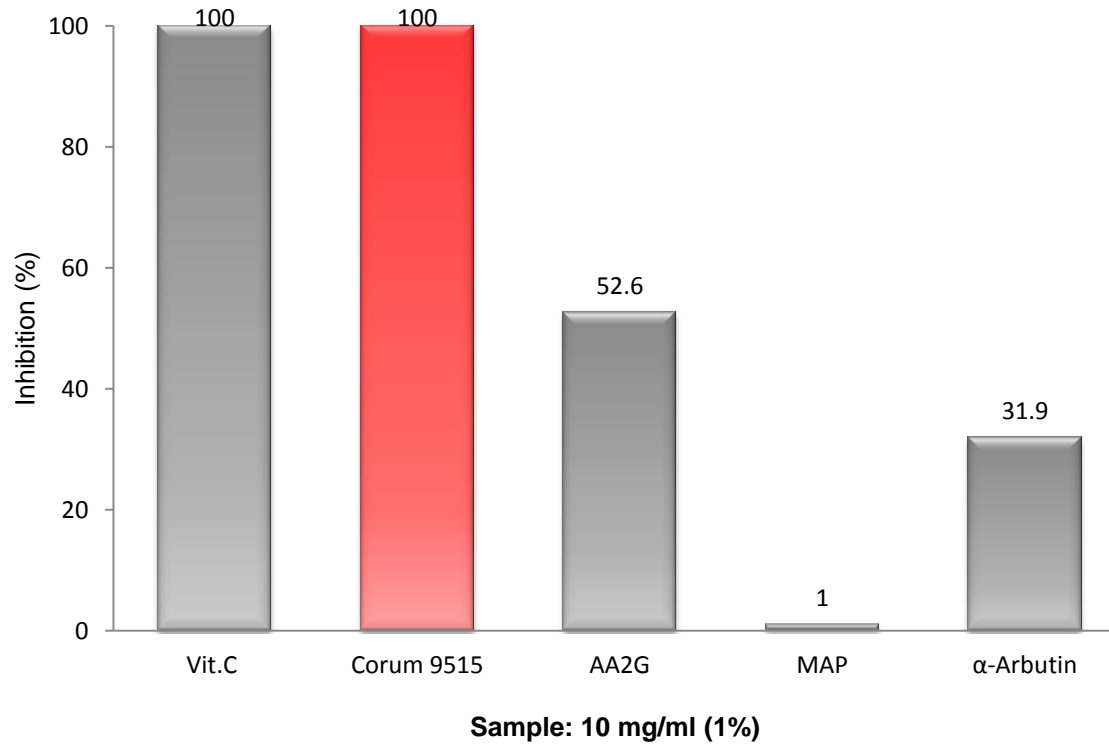
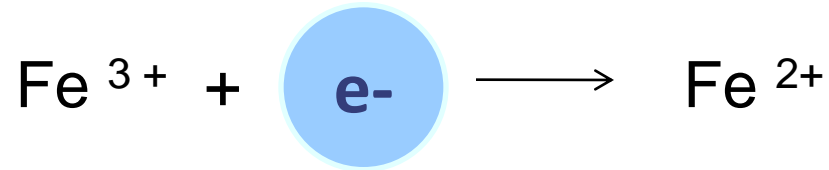


AA2G



MAP

Efficacy Test: Ferric Reducing Ability



CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
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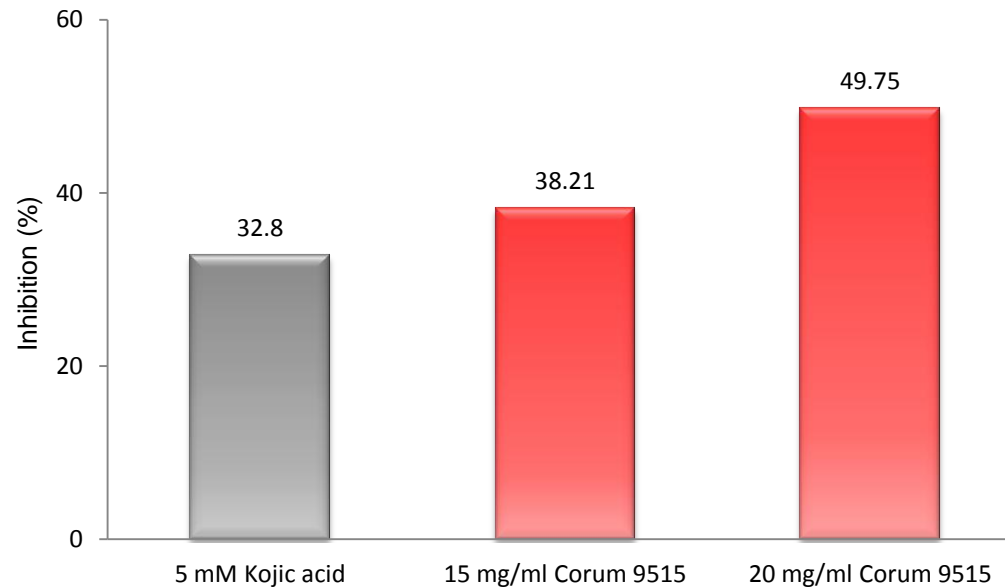
Efficacy Test:

In- vitro whitening activity study - melanin assessment

Tested by IDEA

Method: 12 hours contact with the product and stimulation during 48 hours with theophylline at 0.5 mM.

Result: 49.75% whitening effect, 20mg/ml Corum 9515



Ref: "Study of the effect of a test item on depigmentation of cells in culture: melanin assessment", IDEA, Aug, 2008.

CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
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- In-vitro whitening activity
- **Ex-vivo melanin assay**
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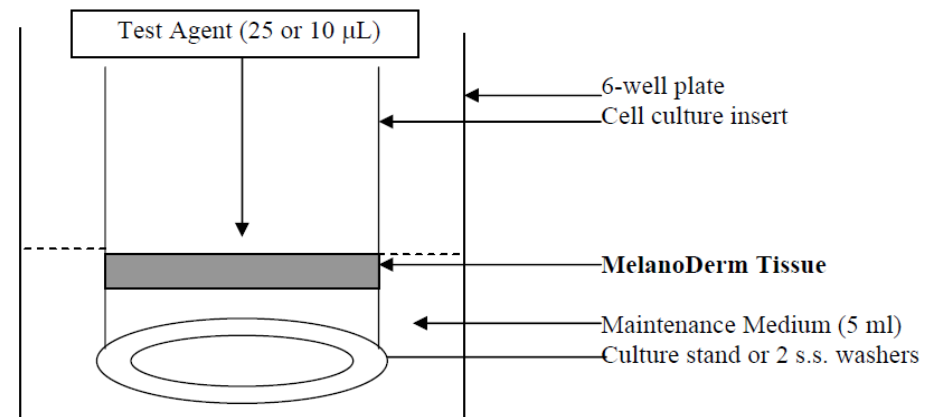
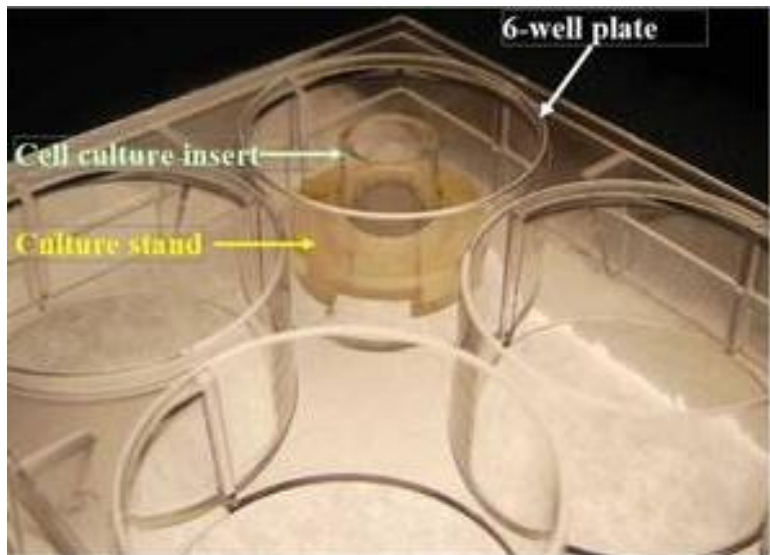


Efficacy Test: Ex-vivo Melanin Assay (MelanoDerm™)

Tested by BioInnovation Laboratories, Inc (USA)

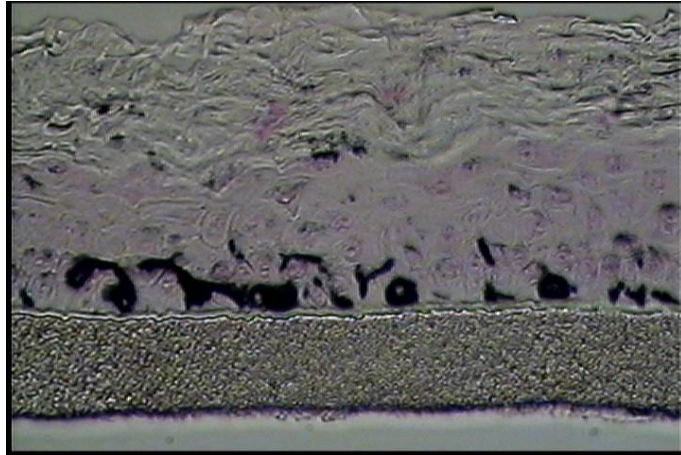
Purpose

- using an *in vitro* tissue model of the human epidermis prepared from cultured human keratinocytes and melanocytes.
- both water-soluble and water-insoluble materials
- skin darkening agents or skin lightening agents



Efficacy Test: Ex-vivo Melanin Assay

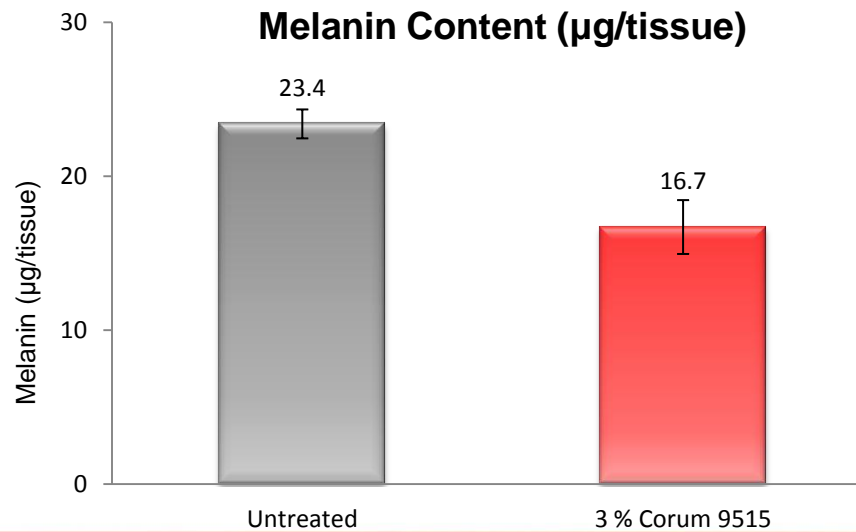
Test day- 9 days



Untreated Tissue



3 % Corum 9515



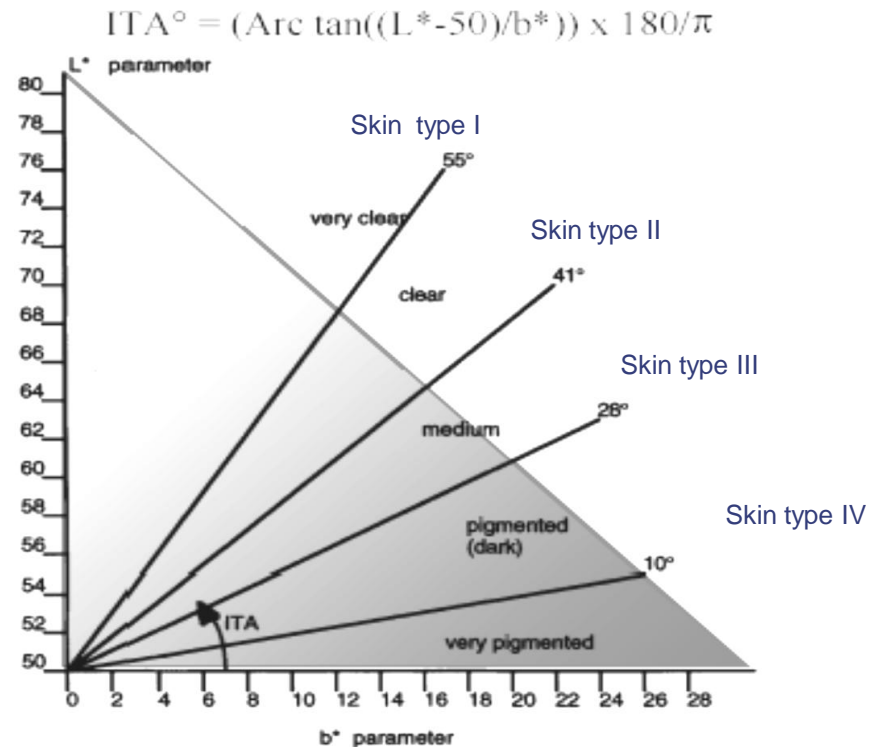
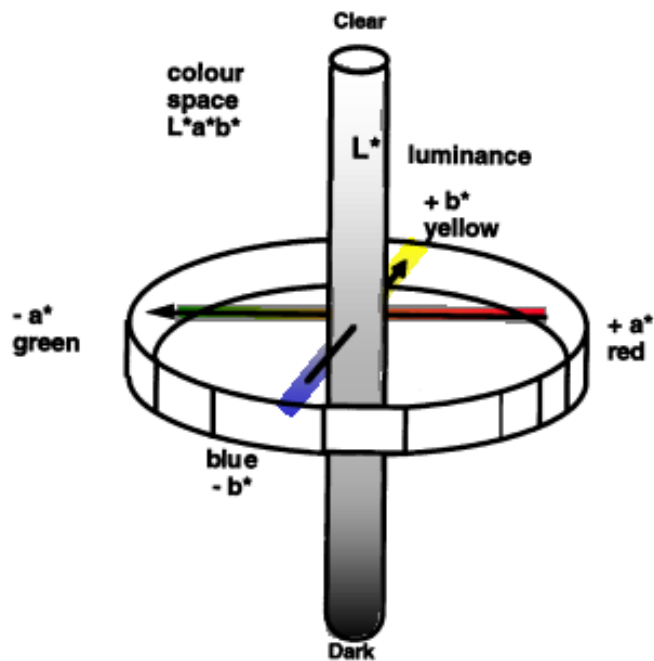
CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- **In-vivo whitening efficacy**
- Anti-inflammation test
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA Protection



Efficacy Test: *In-vivo* whitening efficacy

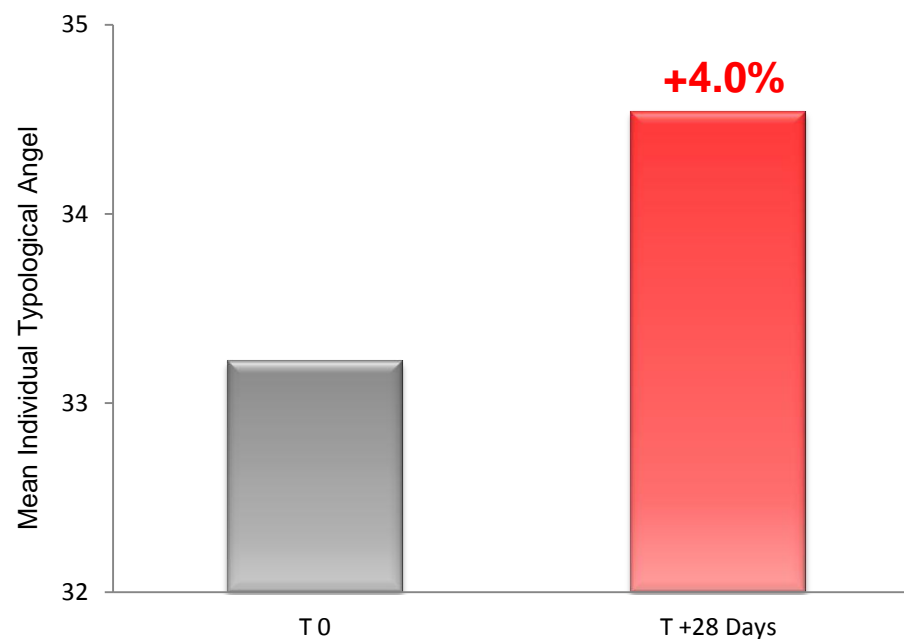
- Tested by : SPINCONTROL
- Test Method : Chromametric Analysis



Ref: Chardon, A., Cretois, I., Hourseau, C., *Skin colour typology and sun tanning pathways* Int. J. Cosm. Sci, (1991), 13, 191-208

Efficacy Test: *In-vivo* whitening efficacy

- Tested by SPINCONTROL
- Subjects: 20 healthy Asian female 25-40 years old with skin type III
- Method: Chromametry (CR-300), 2% C-9515 cream



Result: Significant increase

Ref: "In vivo evaluation of the efficacy of one whitening lotion in healthy Asian subjects by chromatography", Spincontrol Asia, November, 2007.

Efficacy Test: *In-vivo* whitening efficacy



Ref: *"In vivo evaluation of the efficacy of one whitening lotion in healthy Asian subjects by chromatography"*, Spincontrol Asia, November, 2007.

Efficacy Test: *In-vivo* whitening efficacy



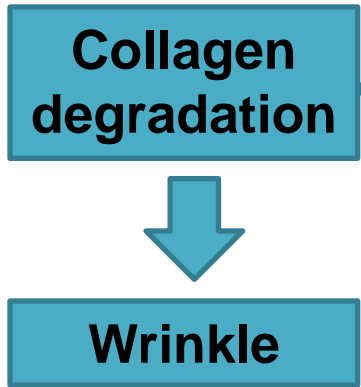
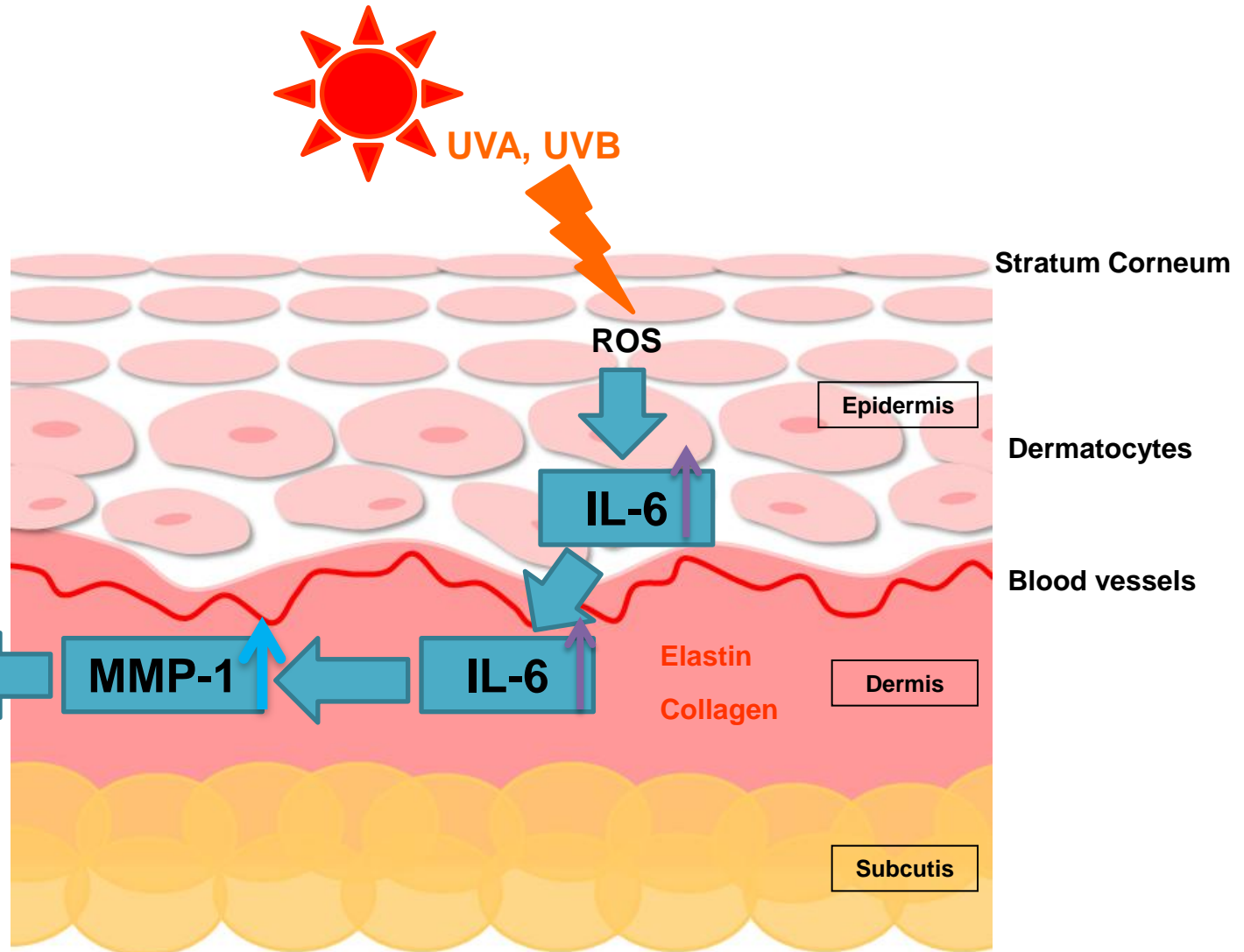
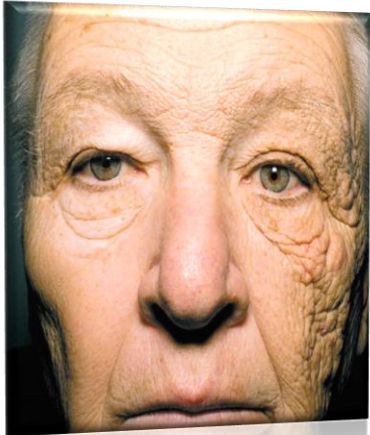
Ref: *"In vivo evaluation of the efficacy of one whitening lotion in healthy Asian subjects by chromatography"*, Spincontrol Asia, November, 2007.

CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
- Reducing activity
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- Ex-vivo melanin assay
- In-vivo whitening efficacy
- **Anti-inflammation test**
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA Protection



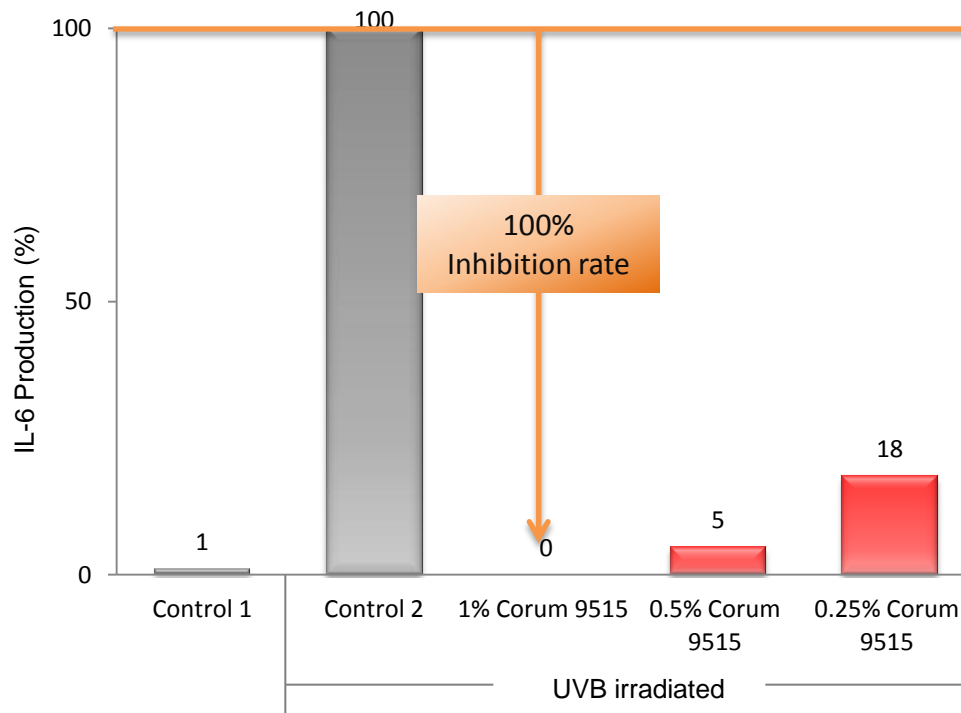
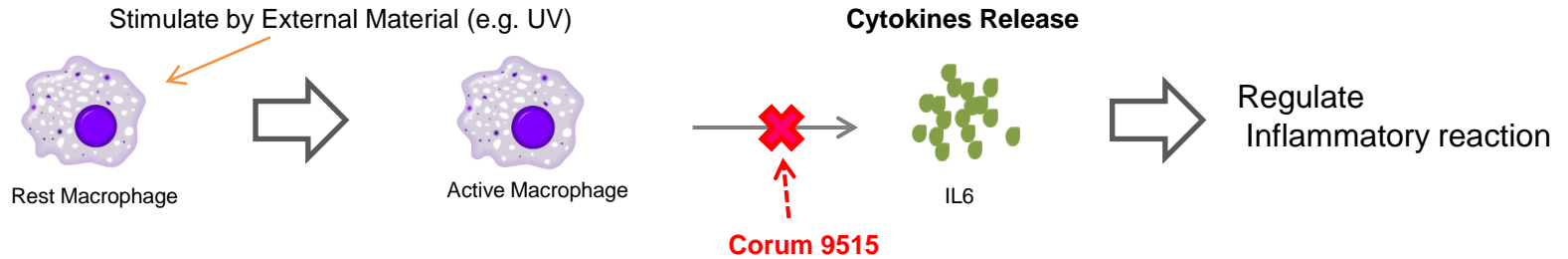
Mechanism of Photoaging



Efficacy Test

Anti-inflammation test – “IL-6”

Principle



Ref: Corum Internal Report.Sep 2012.

CORUM 9515 Efficacy Studies

- In- vitro tyrosinase inhibition
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- Anti-inflammation test
- **Stimulation of collagen synthesis**
- Radical scavenging effect
- DNA Protection

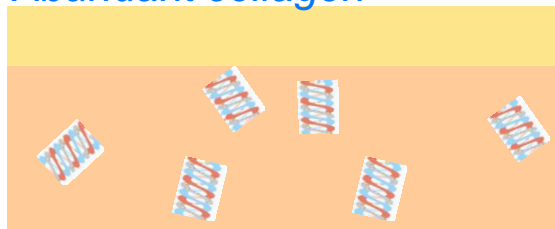


Collagen and its function

What is collagen

- The main protein of connective tissue
- Make up 25% - 35% of the whole-body protein content
- Different types of collagen

Abundant collagen



Epidermis

Dermis

Less collagen



Functions

- Impart strengths, support and skin elasticity

Why important

- As skin ages, it produces less collagen and loses its elasticity

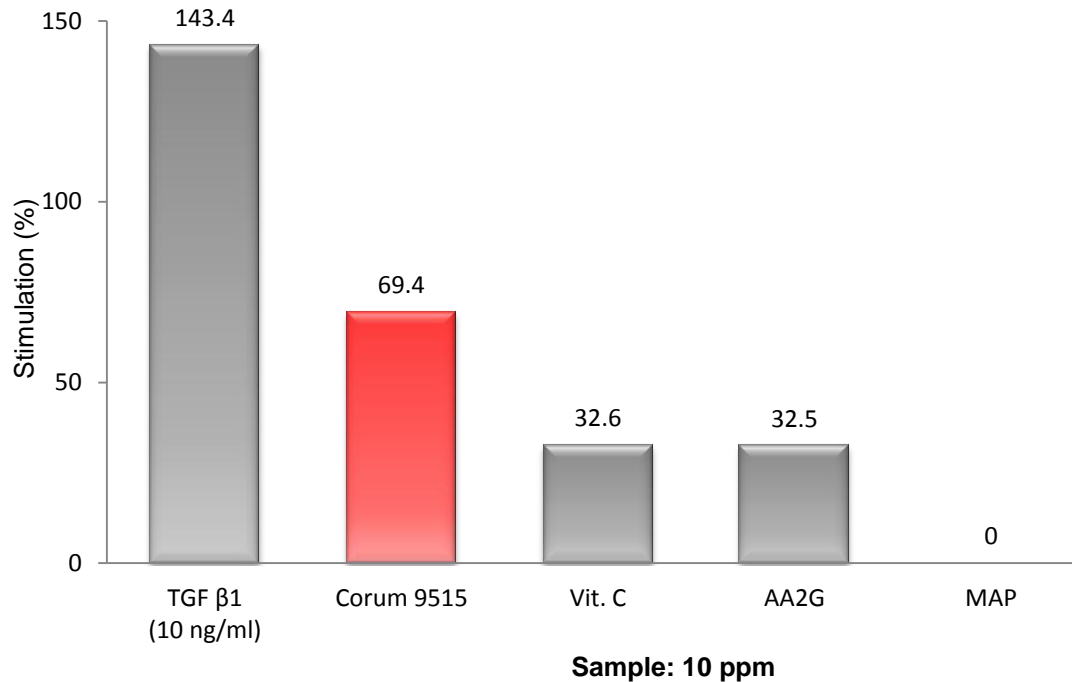


Wrinkle formation
Aged skin

Efficacy Test:

Stimulation of natural collagen synthesis

3T3 cell culture after 72 hours of incubation with sample



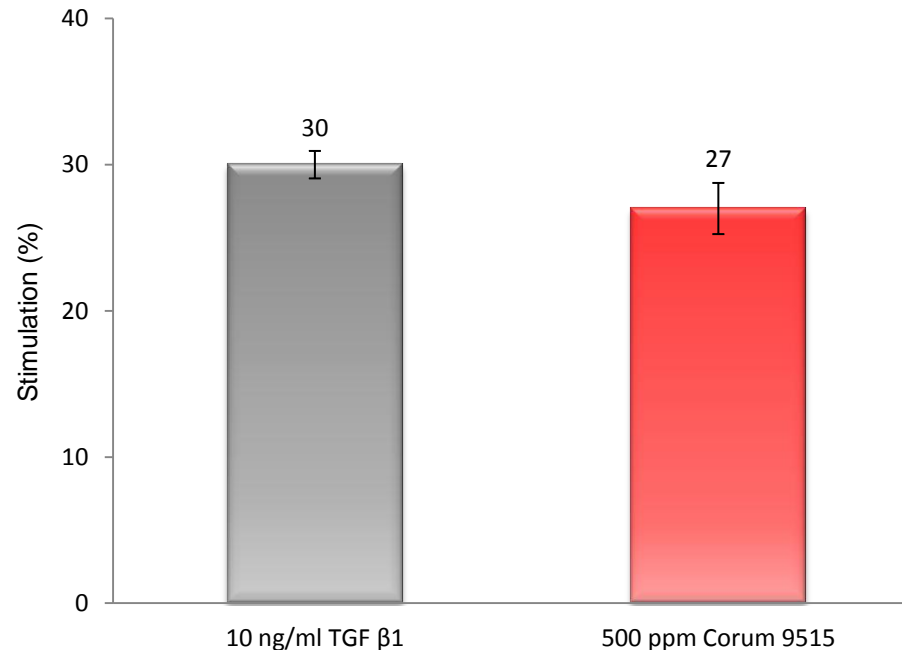
Result: Corum 9515 has the best collagen stimulation effect compared to other Vitamin C derivatives

Efficacy Test:

Stimulation of natural collagen synthesis

Studied by *IDEA*, France
After 48hrs contacts

- TGF β 1 is a very strong collagen synthesis stimulator.
- It is tested with type I collagen



Result: Corum 9515 has a similar effect on collagen synthesis as TGF β 1

Ref: "Activity study of a test item on collagen synthesis from human fibroblast culture", *IDEA*, June, 2008.

CORUM 9515 Efficacy Studies

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- Stimulation of collagen synthesis
- **Radical scavenging effect**
- DNA Protection



Corum 9515 free-radical scavenging mechanism

What are free radicals

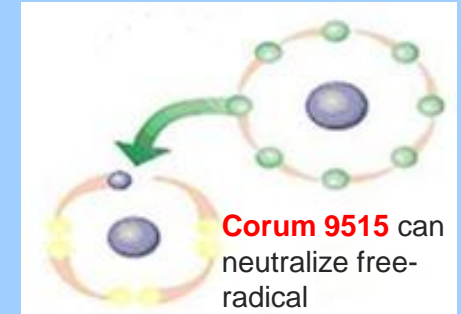
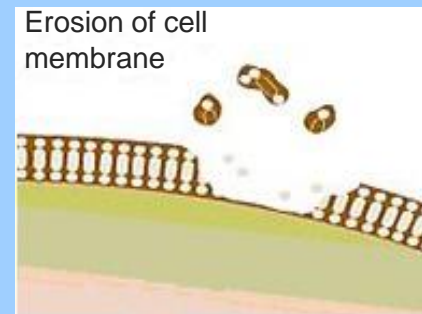
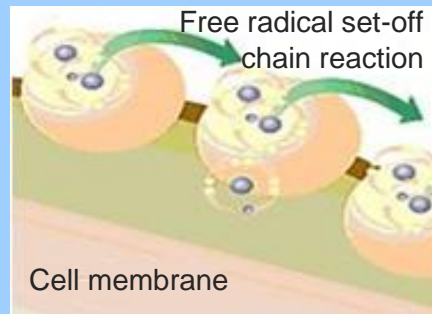
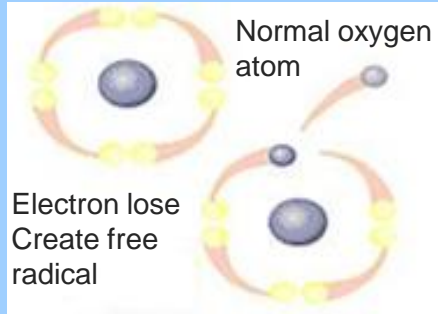
- Atoms, molecules, or ions with unpaired electrons
- Due to its unpaired electrons, they are often highly reactive
- Often causes chain reactions

Cause

- UV rays, stress & environmental pollution etc.

Why important

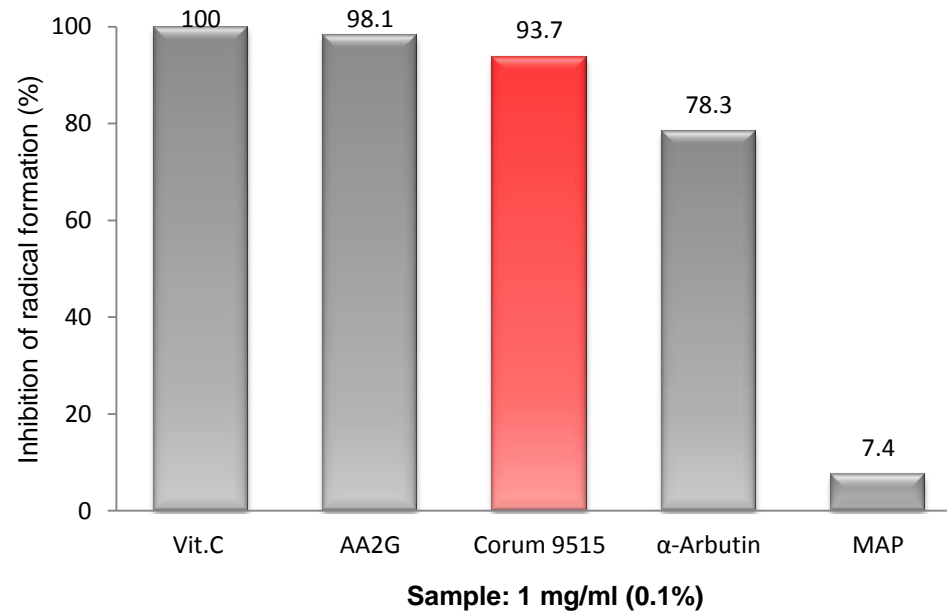
- It can participate in unwanted side reactions resulting in cell damage.
- Many form of cancer are thought to be the result of reactions between free radicals and DNA.



Efficacy Test: Radical scavenging test

DPPH assay

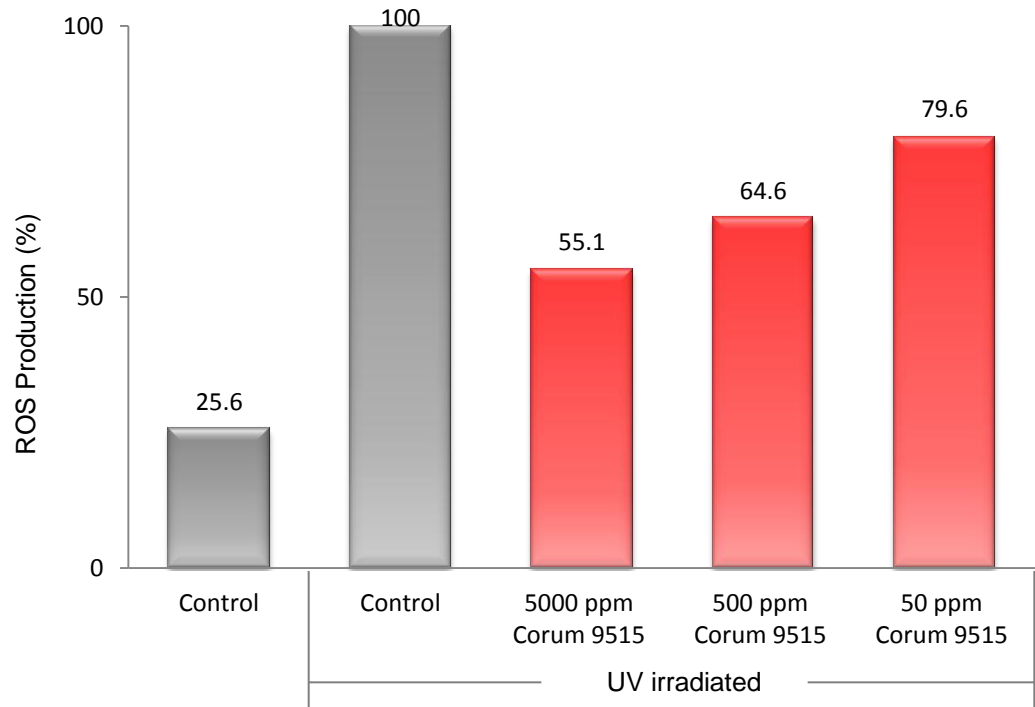
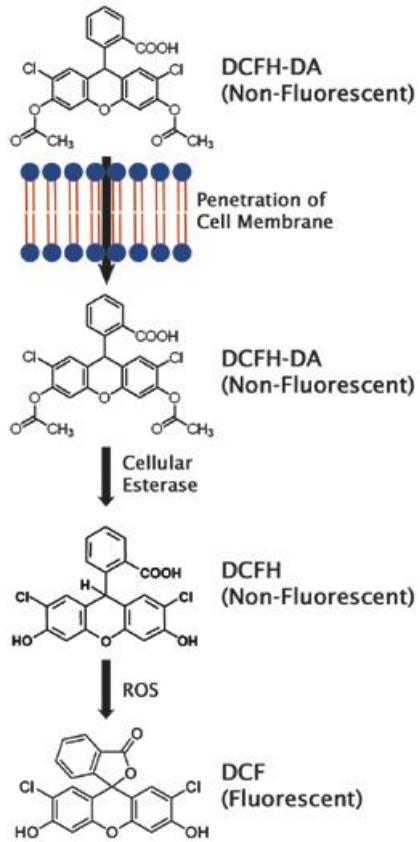
- Other benefits:
 - Anti-oxidant effect (L-ascorbic acid function)
 - Radical Scavenging effect (L-ascorbic acid function)



Efficacy Test: Radical scavenging test

ROS assay after UVB irradiated

Principle



Result: Corum 9515 shows a great ability on ROS inhibition.

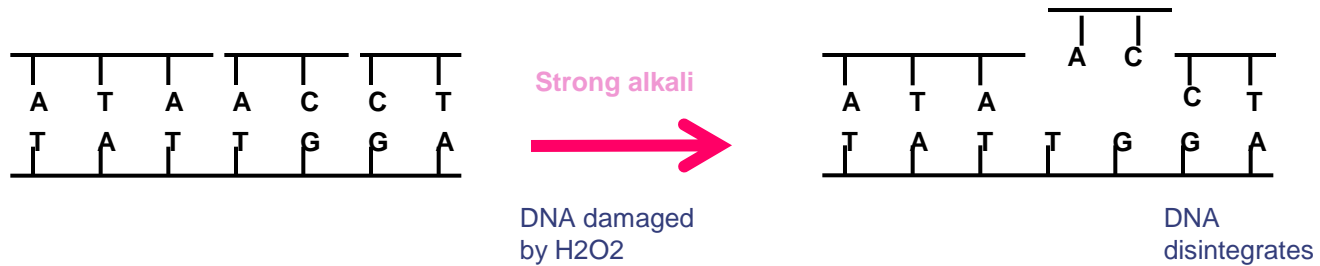
CORUM 9515 Efficacy Studies

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- In-vivo whitening efficacy
- Anti-inflammation test
- Stimulation of collagen synthesis
- Radical scavenging effect
- **DNA Protection**
 - a. **In-vitro, Comet Assay**
 - b. **Ex-vivo**

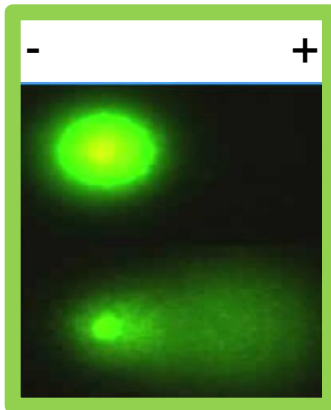
Efficacy Test: DNA Protection

Comet Assay

- **Method: Single Cell Gel Electrophoresis Assay – Comet Assay**
- **Theory:**

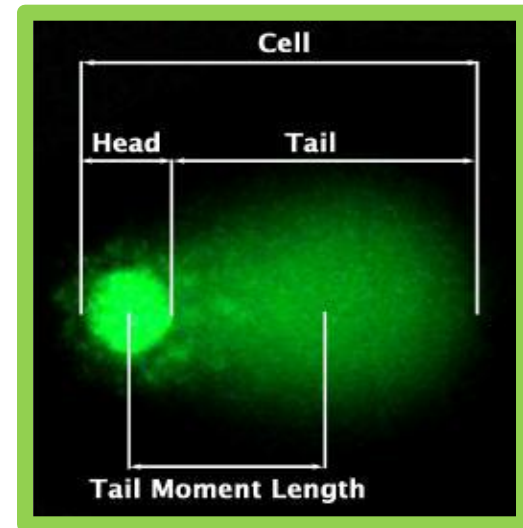


Single cell electrophoresis

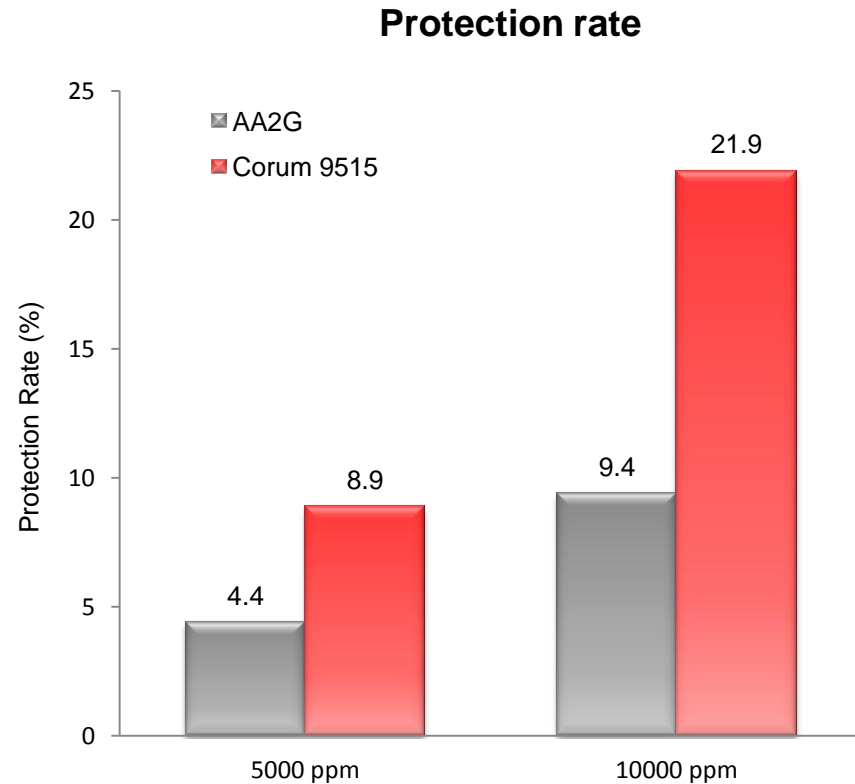
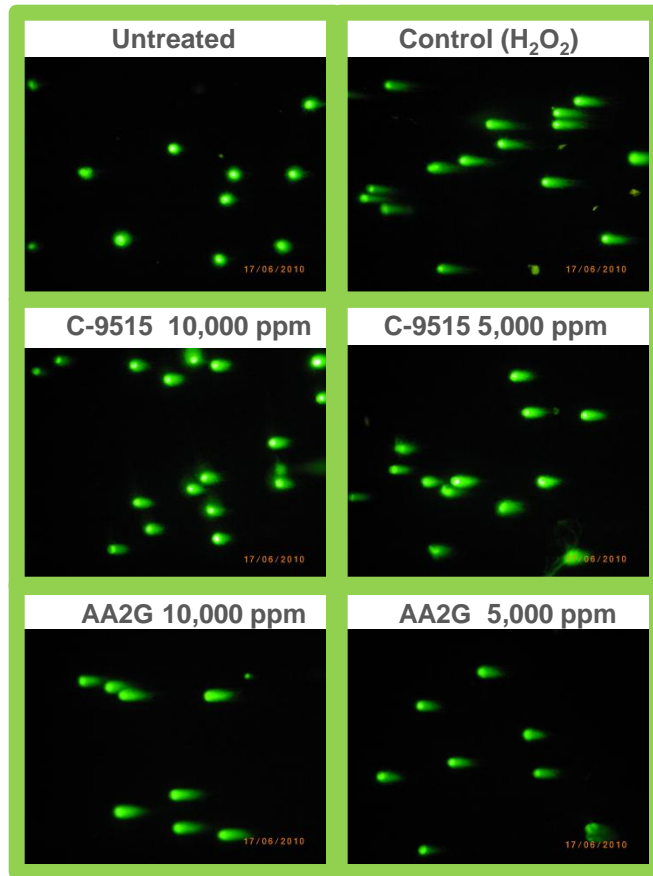


No damage

Serious damage



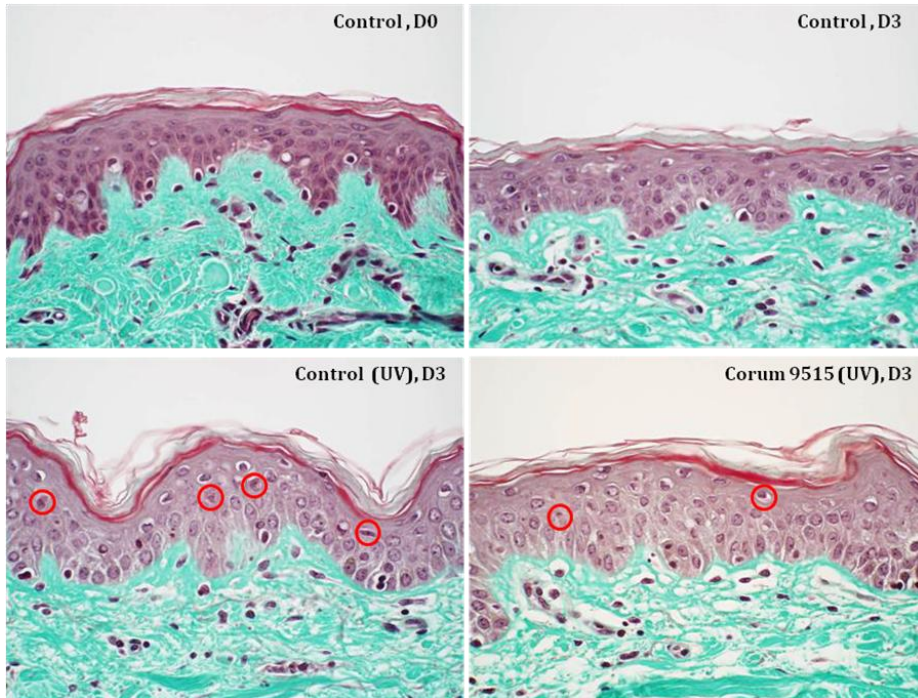
Efficacy Test: DNA Protection Comet Assay



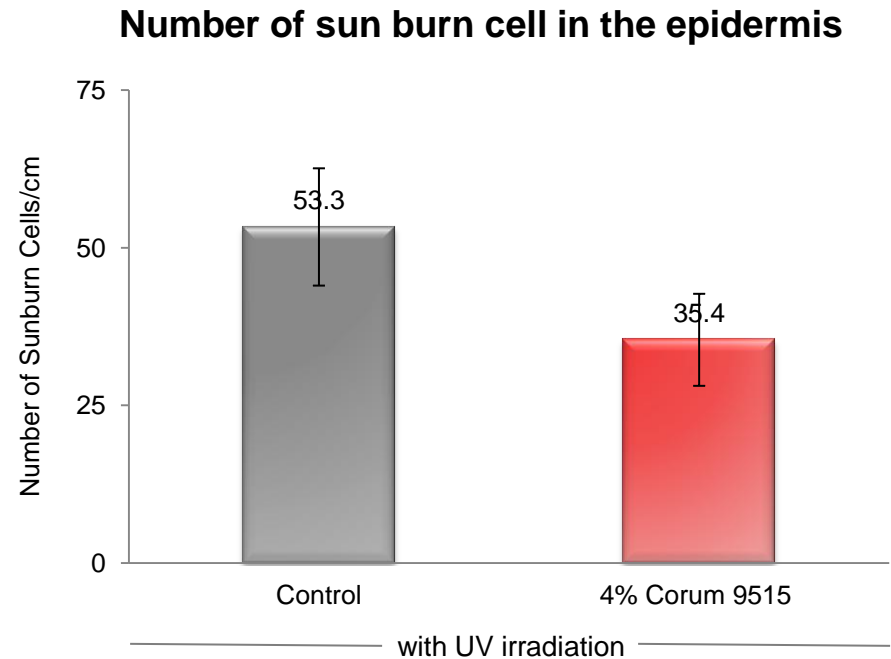
Result: Corum 9515 shows high efficiency on DNA protection activity.

Efficacy Test: DNA Protection after UVB irradiation on human living skin explants

Studied by *BIO-EC*, France



*Sunburn cell are indicated by an circle



Result: Corum 9515 shows high efficiency on DNA protection activity after UVB irradiation.

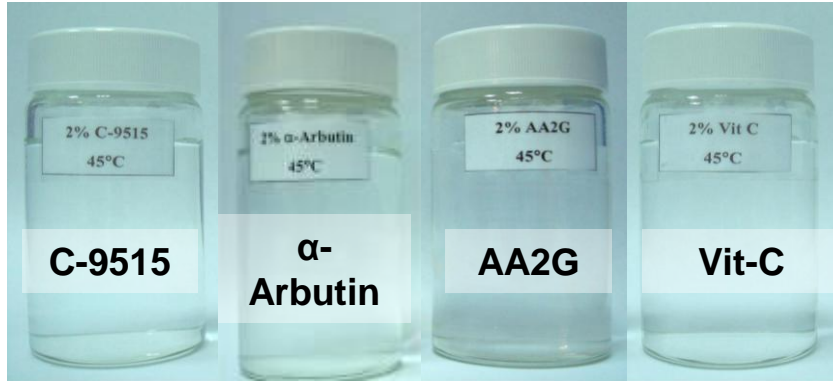
Stability Tests

- **Heat-stability (45°C, 1 month)**
 - color (Transmittance in 440nm)
 - purity (HPLC)
- **Photo-stability (sunlight, 1 month)**
- **Buffer system and pH effect (45 °C , 56 days and RT, 90 days)**

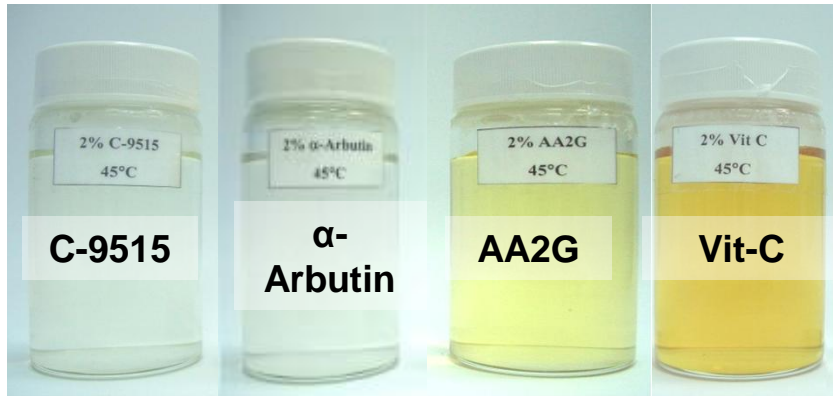


Stability: Heat-Stability

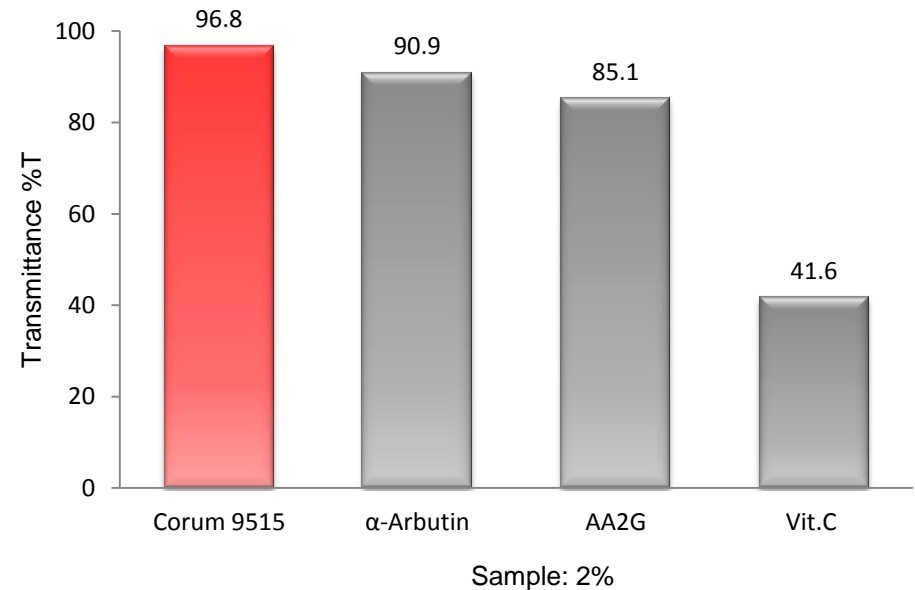
0 month 2% 45 °C without buffer



1 month 2% 45 °C without buffer



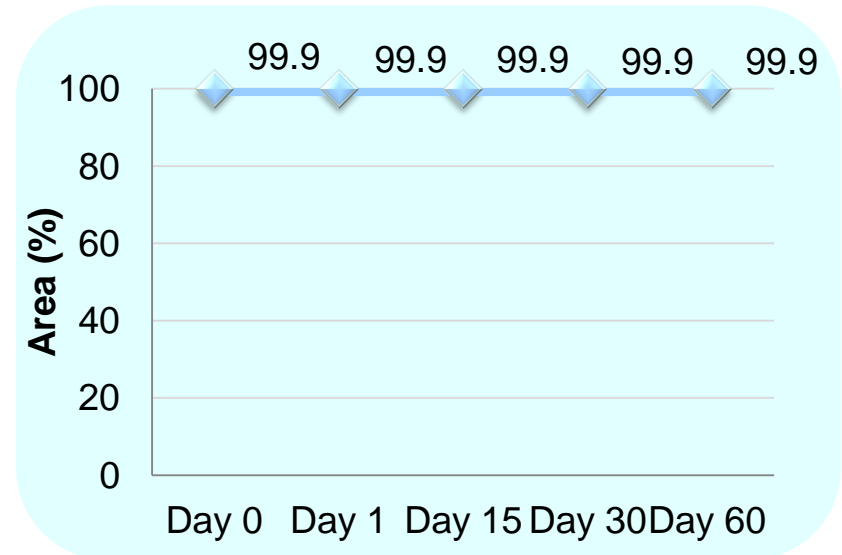
1 Month 45°C Stability
(Transmittance in 440nm)



Stability: Crystalline Powder Heat-Stability

60 Days 45 °C Purity

- HPLC system
- UV detection at 214nm
- Corum 9515 crystalline powder



Result: Corum 9515 crystalline powder remains stable under 45 °C for 60 days

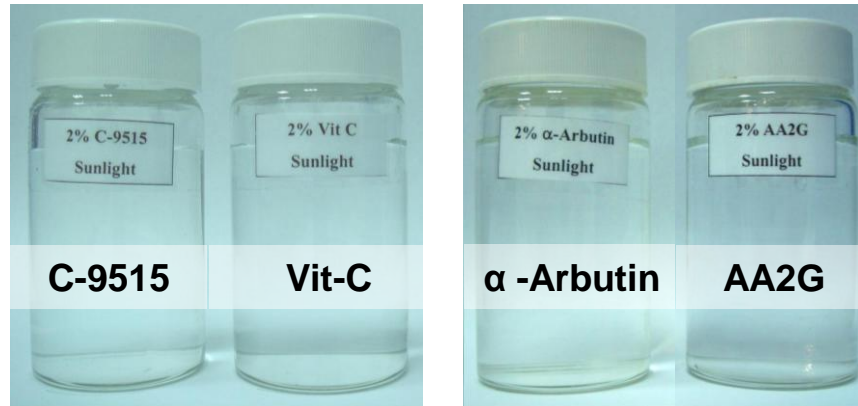
Stability Tests

- Heat-stability (45°C, 1 month)
 - color (Transmittance in 440nm)
 - purity (HPLC)
- Photo-stability (sunlight, 1 month)
- Buffer system and pH effect (45 °C , 56 days and RT, 90 days)

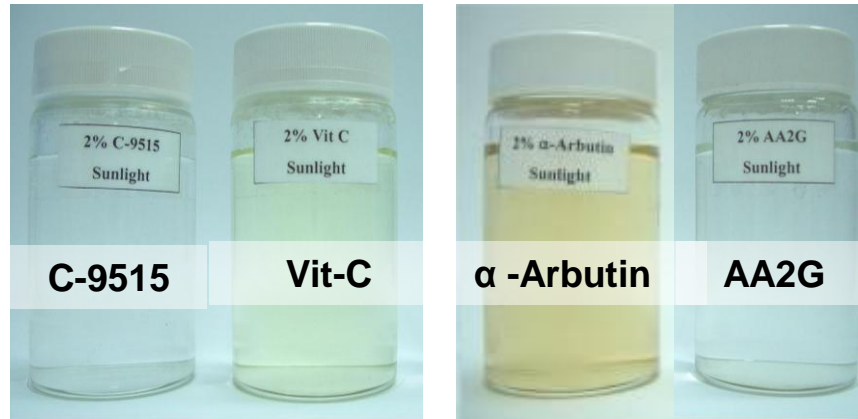


Stability: Corum 9515 Photo-Stability

0 month 2% under sunlight without buffer



1 month 2% under sunlight without buffer



Stability Tests

- Heat-stability (45°C, 1 month)
 - color (Transmittance in 440nm)
 - purity (HPLC)
- Photo-stability (sunlight, 1 month)



- Buffer system and pH effect (45 °C , 56 days and RT, 90 days)

Stability under different pH & buffer

PURITY assay by HPLC

Preparation:

2% buffer solution

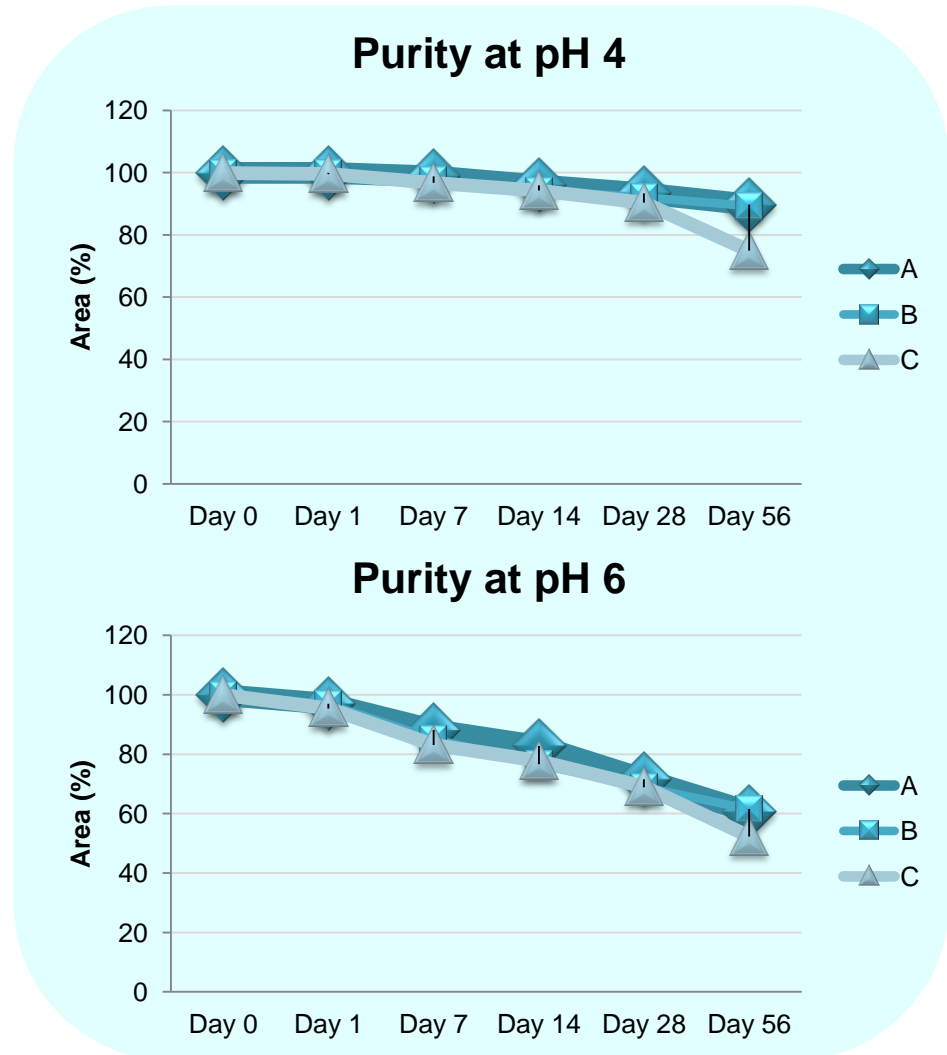
Day: 56 days at 45°C

Buffer system:

A- Sodium Citrate – Citric Acid

B- Na₂HPO₄- Citric Acid

C- NaHCO₃ – Citric Acid



pH Stability under different buffer

pH Stability

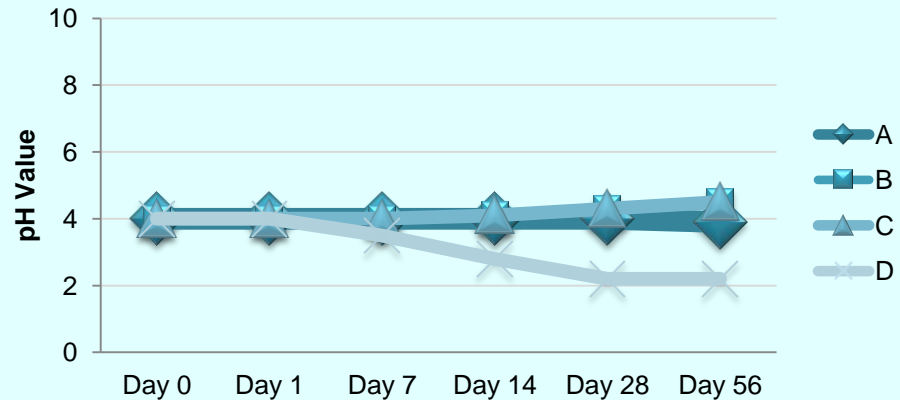
Preparation:
2% buffer solution

Day: 56 days at 45°C

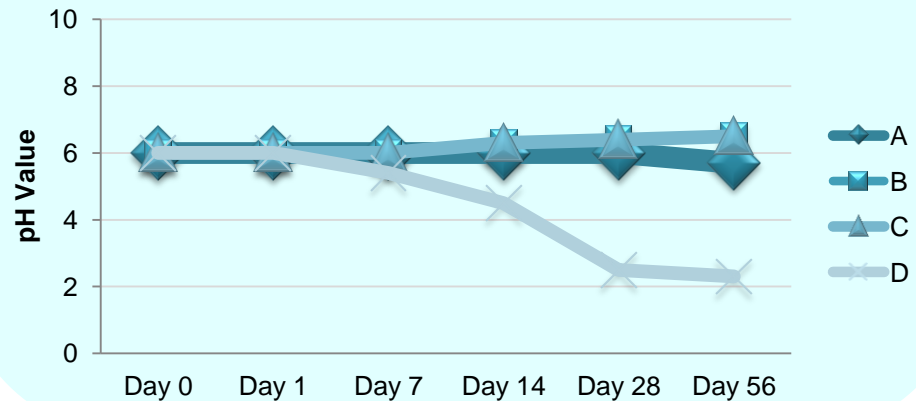
Buffer system:

- A- Sodium Citrate - Citric Acid
- B- Na₂HPO₄ - Citric Acid
- C- NaHCO₃ - Citric Acid
- D- NaOH - Citric acid

Stability of pH value in different buffer system (pH 4)



Stability of pH value in different buffer system (pH 6)



Anti-oxidant lessen color change

Anti-oxidant is recommended to be used in formulation

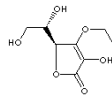
Corum 9515 Ethyl Ascorbic Acid is also a powerful anti-oxidant.

Thus it needs a stronger anti-oxidant to prevent its effect before it reach target area.

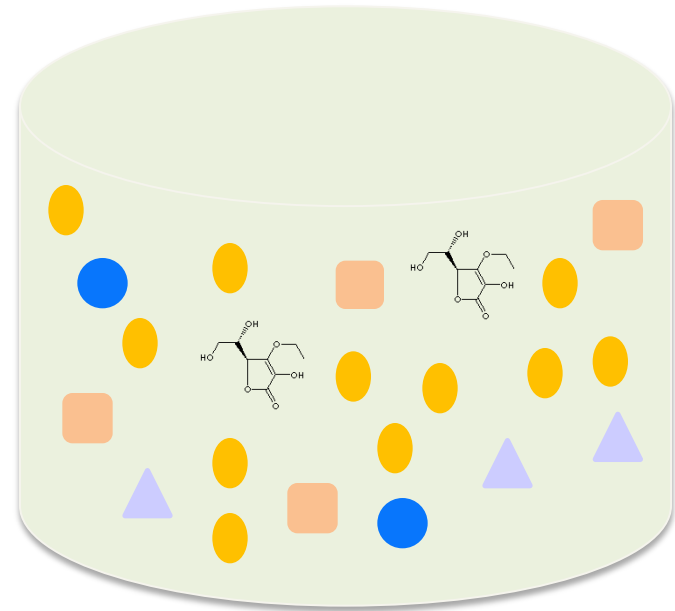
Anti-oxidant



**Corum 9515
(Ethyl Ascorbic Acid)**



**Other
ingredients**



Anti-oxidant lessen color change

45 °C for 6 months



	Control			
C-9515	0%	2%	2%	2%
Sodium bisulfite	0%	0%	0.15%	0.2%

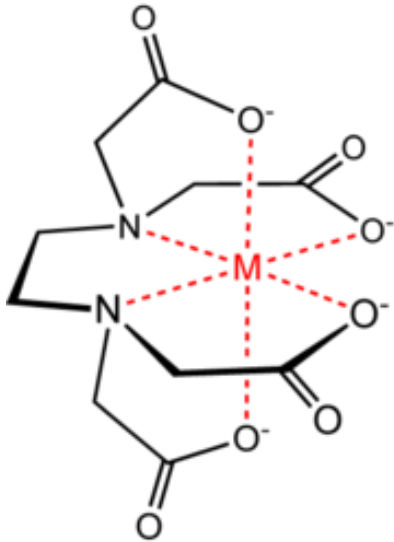
- Sodium bisulfite can lessen color change
- But ! **Too much** sodium bisulfite will accelerate color change

Anti-oxidant lessen color change

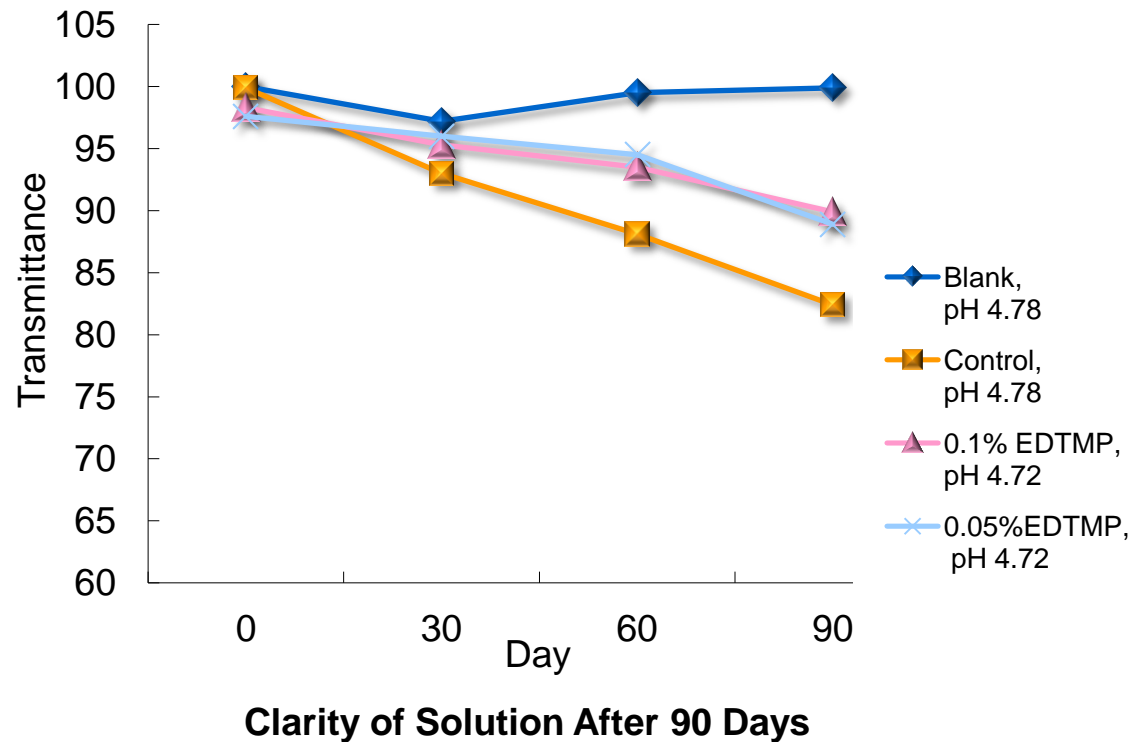
<u>Ingredient</u>	<u>I-1</u>	<u>I-2</u>	<u>I-3</u>	<u>I-4</u>
A Emulium Delta	7.00	7.00	7.00	7.00
A Myristyl Myristate	2.00	2.00	2.00	2.00
A Diethylhexyl Sebacate	6.00	6.00	6.00	6.00
A 2-Octyldodecyl Myristate	6.00	6.00	6.00	6.00
B Water	To 100.00	To 100.00	To 100.00	To 100.00
B Avicel PC591(5%)	40.00	40.00	40.00	40.00
C Water	10.00	10.00	10.00	10.00
C Citric Acid	0.65	0.65	0.65	0.65
C Sodium Citrate	1.30	1.30	1.30	1.30
D Corum 9515	0.00	2.00	2.00	2.00
D Water	0.00	4.00	4.00	4.00
D Sodium Bisulfite	0.00	0.00	0.15	0.20
E Transcutilol CG	5.00	5.00	5.00	5.00
F Optiphen MIT	0.10	0.10	0.10	0.10
pH Value(3min)—D0	4.81	4.82	4.77	4.80
Viscosity(#4/12rpm//30sec) —D3	24600	26700	22500	20400
pH Value(3min)—6 months	4.94	4.93	4.86	4.31

Minimize Color Change - Chelating Agent

Metal Ions can promote oxidation. Chelating agents work to trap and inactivate these metal ions with the formation of chelating complexes in the solution



EDTMP was found to minimize oxidation remarkably in solutions containing Corum 9515 according to our experiments.



Corum 9515 Toxicological Information

1. Skin Irritancy Test by *IDEA, France*

2% Corum 9515 on the external face of the arm maintained over 48 hours with the help of a semi-occlusive patch.

Corum 9515 is found to be non-irritant after 48 hours semi-occlusive patch test.

2. Cytotoxicity Test by *Evic, France*

Cytotoxicity test on Corum 9515 diluted with 10% distilled water.

Corum 9515 diluted at 10 % with distilled water was judged negligible.

3. AMES Test by *Vivotecnia, Spain*

Corum 9515 were found to be non mutagenic and non pro-mutagenic.

Corum 9515 Summary



- **effective** and **stable** skin lightening agent
- Balance the skin tone
- Reduce dark spot
- Increase collagen synthesis
- Excellent anti-oxidation properties
- Scavenge radical
- DNA protection

