



New Generation  
Vitamin C Derivative

**CORUM 9515**

3-O-Ethyl Ascorbic Acid

**AS POTENT AS VITAMIN C  
WITH BETTER STABILITY**

# CORUM 9515

INCI: 3- O – Ethyl ascorbic acid

Also known as:

3- O – ethyl ascorbic ether

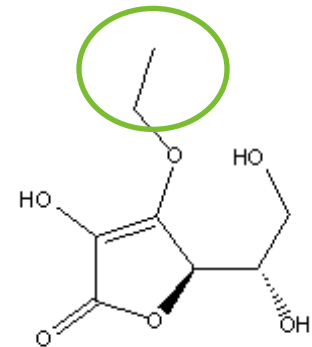
3- O – ethyl ascorbic acid

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

Anti-photoaging

Collagen synthesis

Reversing auto-oxidation

Radical scavenging

DNA protection

Easy to penetrate the epidermis

# CORUM 9515 Efficacy Studies

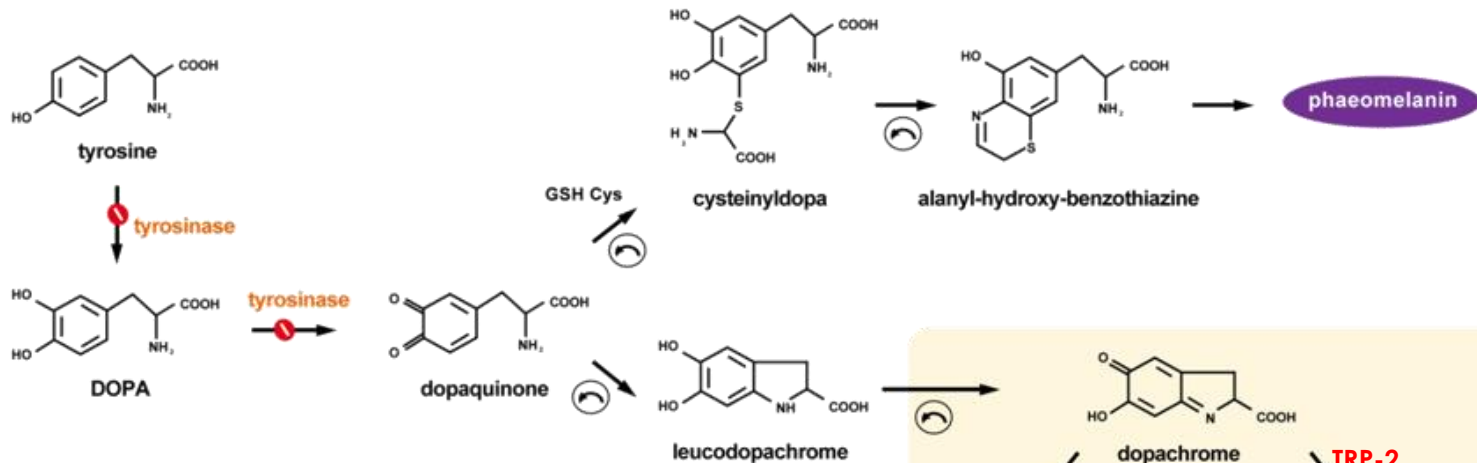
- ***In-vitro* tyrosinase inhibition**
- Reducing activity
- *In-vitro* whitening activity
- *Ex-vivo* melanin assay
- *In-vivo* whitening efficacy
- Anti-photoaging  
(Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile



**CORUM**

Advance through Knowledge

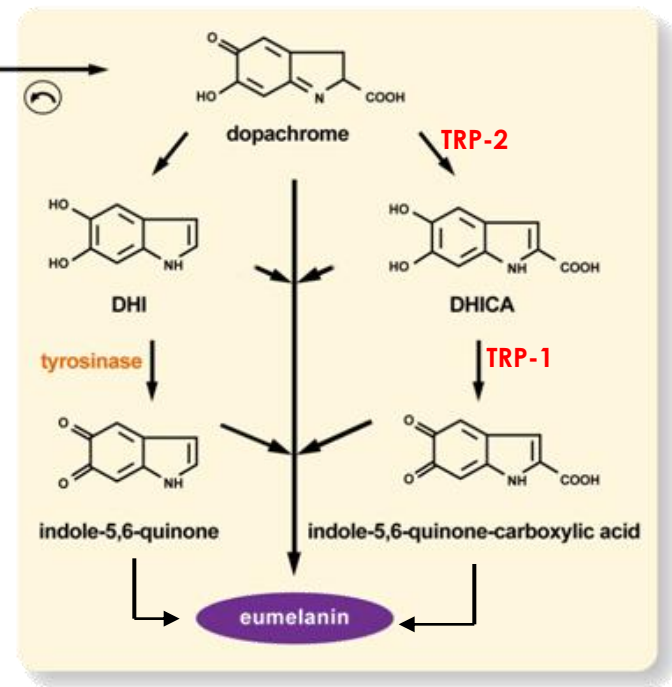
# CORUM 9515 Whitening Mechanism



Three melanin-generating enzymes:

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

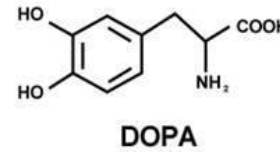
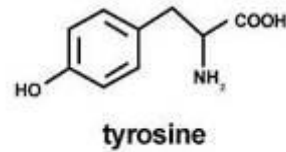
- Inhibit the reaction center  $\text{Cu}^{+2}$  ion of tyrosinase
- Inhibit the activity of TRP-2
- Reduce oxidized melanin pigments



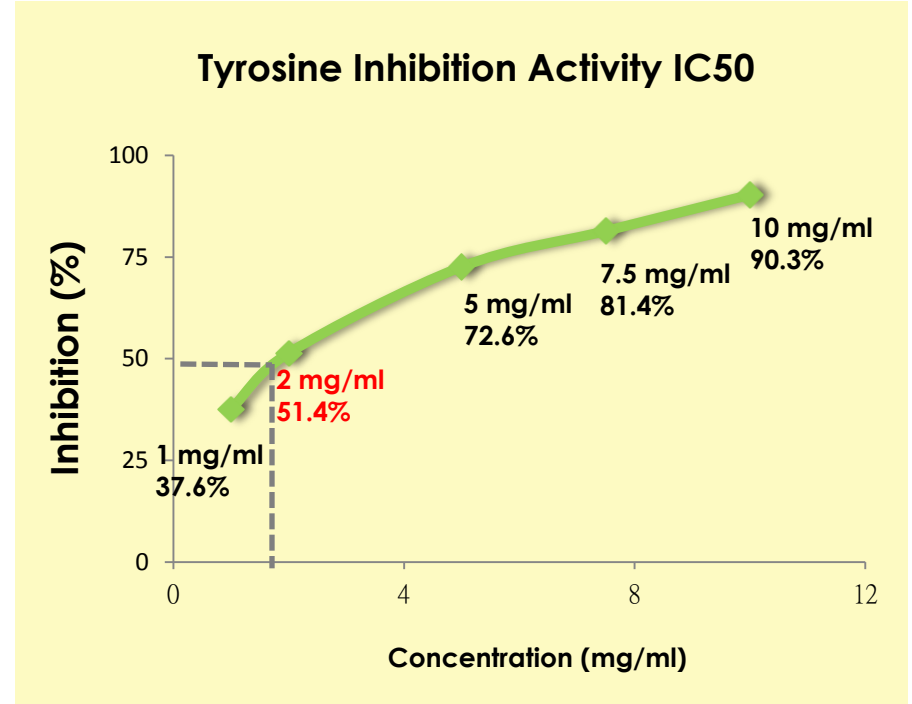
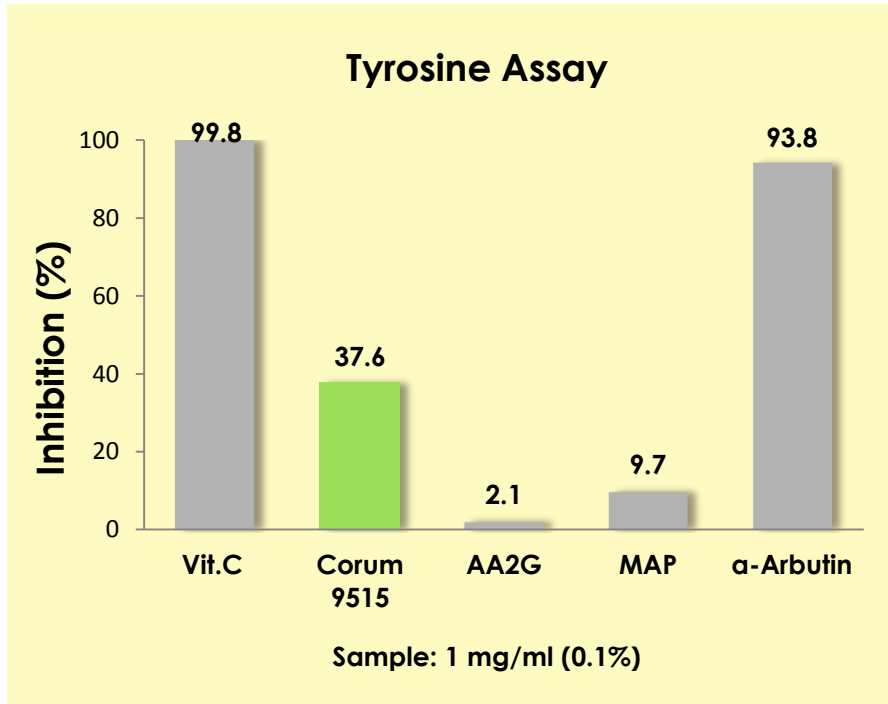
Ref: Briganti, S et al. (2003) Chemical and Instrumental Approaches to Treat Hyperpigmentation Pigment Cell Res 16:101-110.

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

Reaction:



Enzyme level

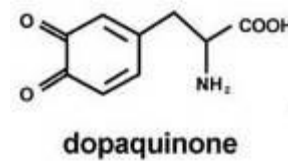
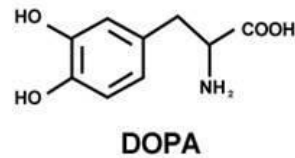


**0.1 % CORUM 9515 can inhibit tyrosinase activity up to 37.6 %.**

**2 mg/mL of CORUM 9515 can reach 50% inhibition.**

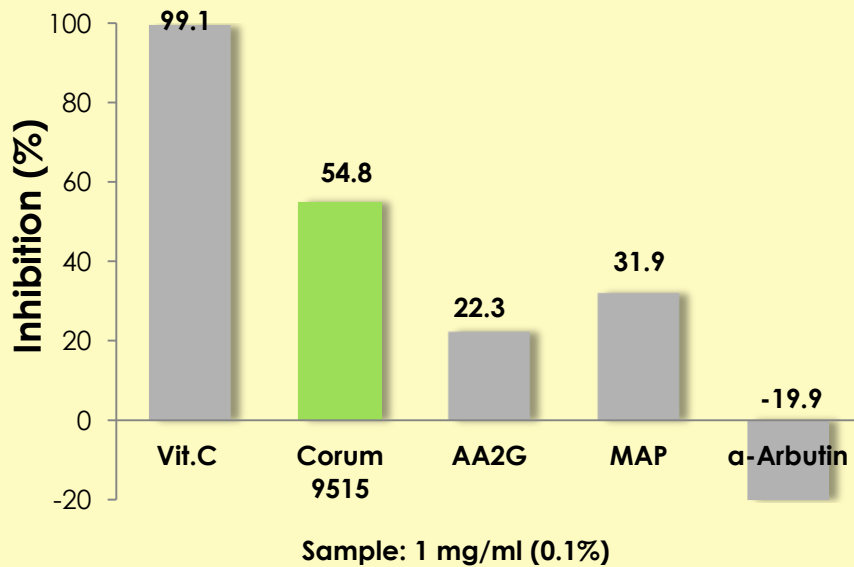
# Efficacy Test: *In-vitro* tyrosinase (II) inhibition

Reaction:

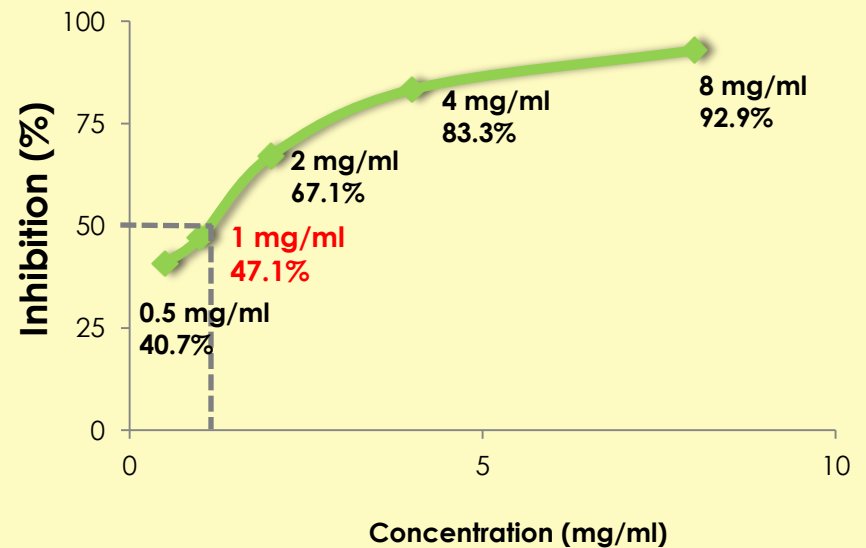


Enzyme level

### DOPA Assay



### Tyrosine Inhibition Activity IC50



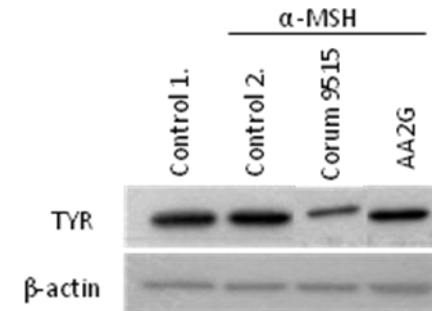
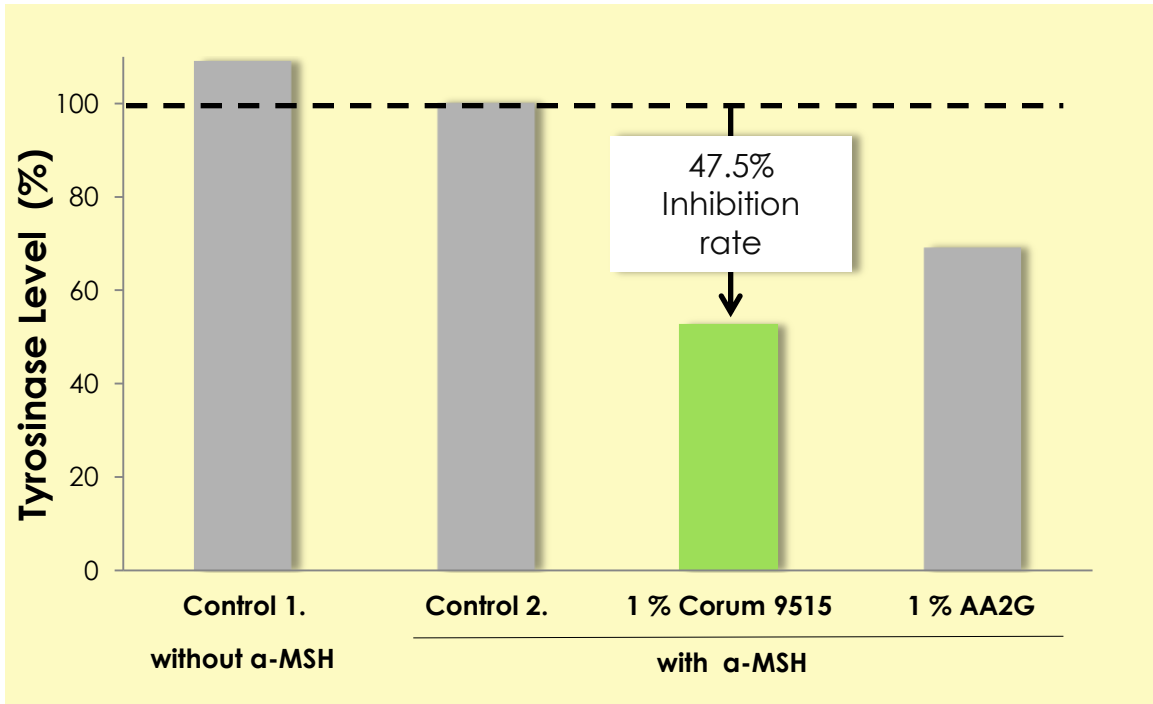
**0.1 % Corum 9515 can inhibit tyrosinase activity up to 54.8%.**

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

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

Protein level

## Tyrosinase inhibition



Test:

- Western blot – **Tyrosinase**
- In-vitro melanogenesis inhibition (with α-MSH)

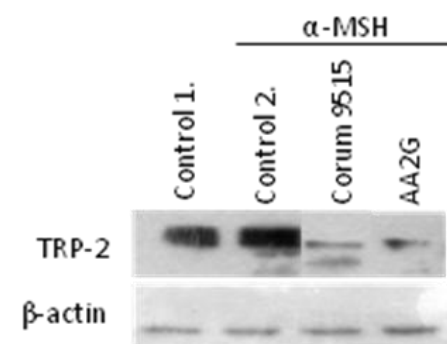
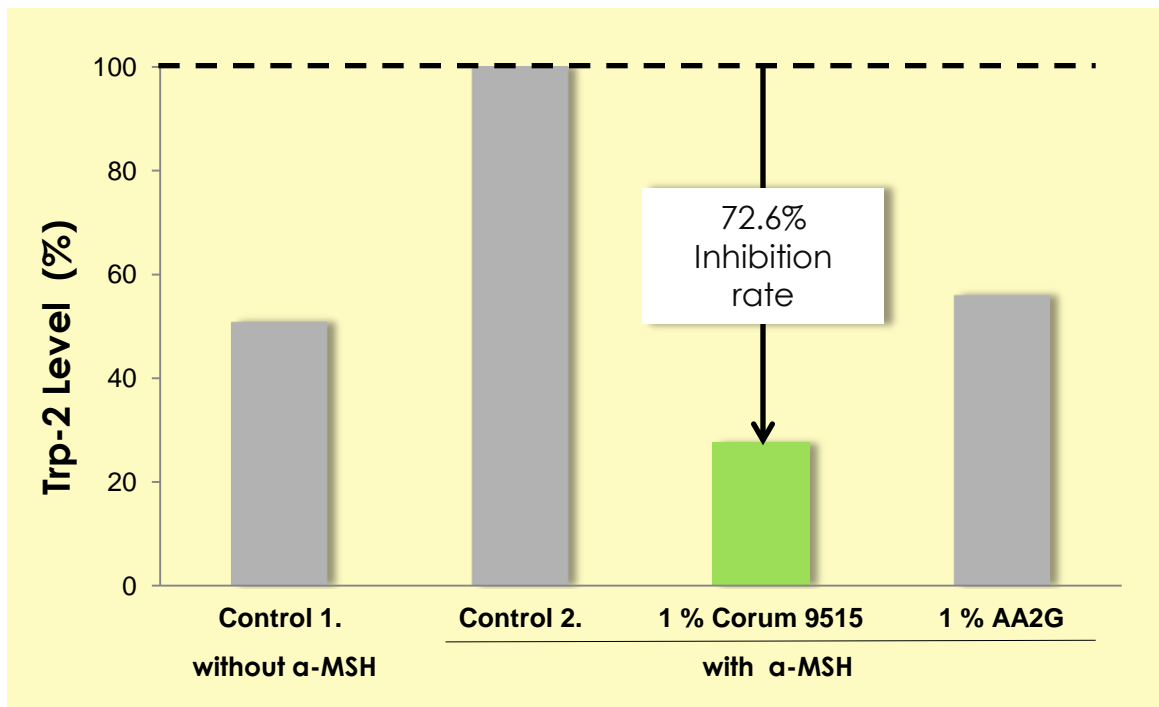
**Result : 1 % Corum 9515 can inhibit tyrosinase activity up to 47.5 % in protein level.**



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

## Trp-2 inhibition

Protein level



Tests:

- Western blot – **Trp-2**
- *In-vitro* melanogenesis inhibition (with α-MSH)

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

# CORUM 9515 Efficacy Studies

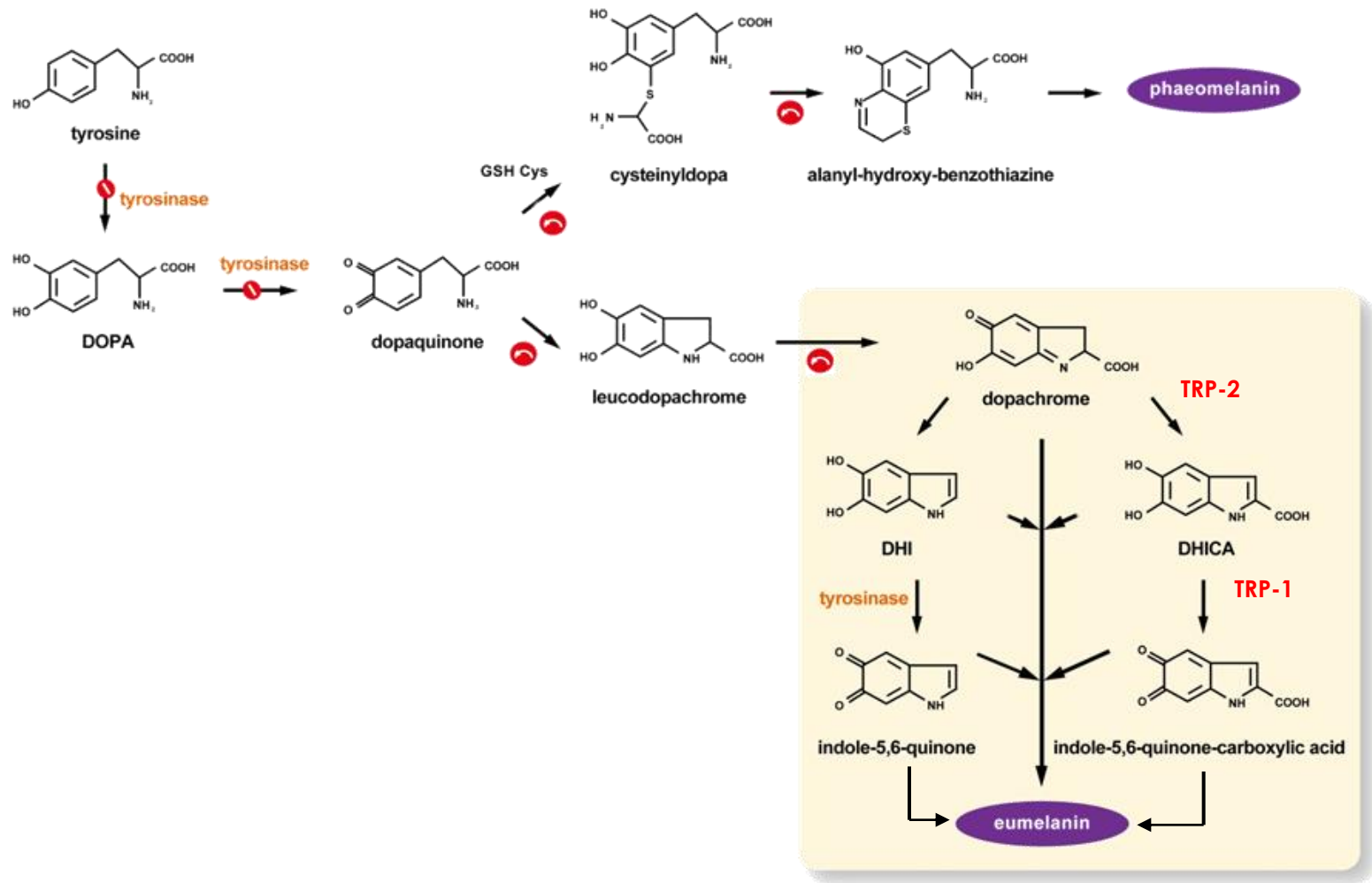
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**CORUM**

Advance through Knowledge

# Efficacy Test: Ferric Reducing Ability

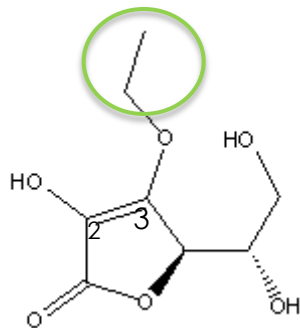
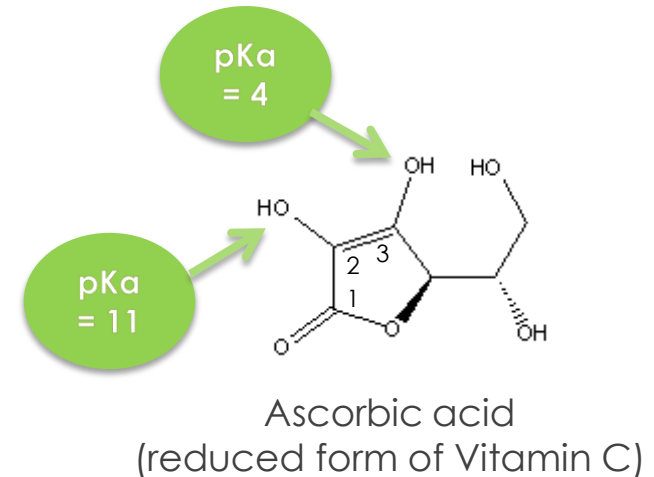


Ref: Briganti, S et al. (2003) *Chemical and Instrumental Approaches to Treat Hyperpigmentation* Pigment Cell Res 16:101–110.

# Efficacy Test: Ferric Reducing Ability

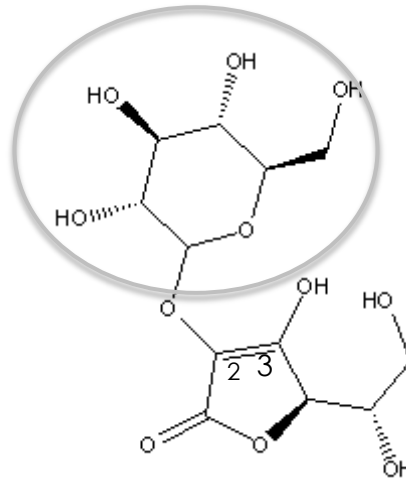
■ pKa value greater - give electron easier  
- better reducing ability

■ **2' carbon** without ethyl group bonded  
has **better reducing ability**



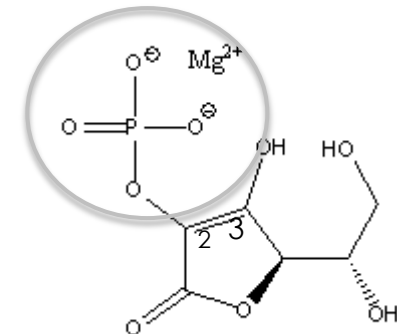
**Ethyl Ascorbic Acid**

MW: 204.18



**AA2G**

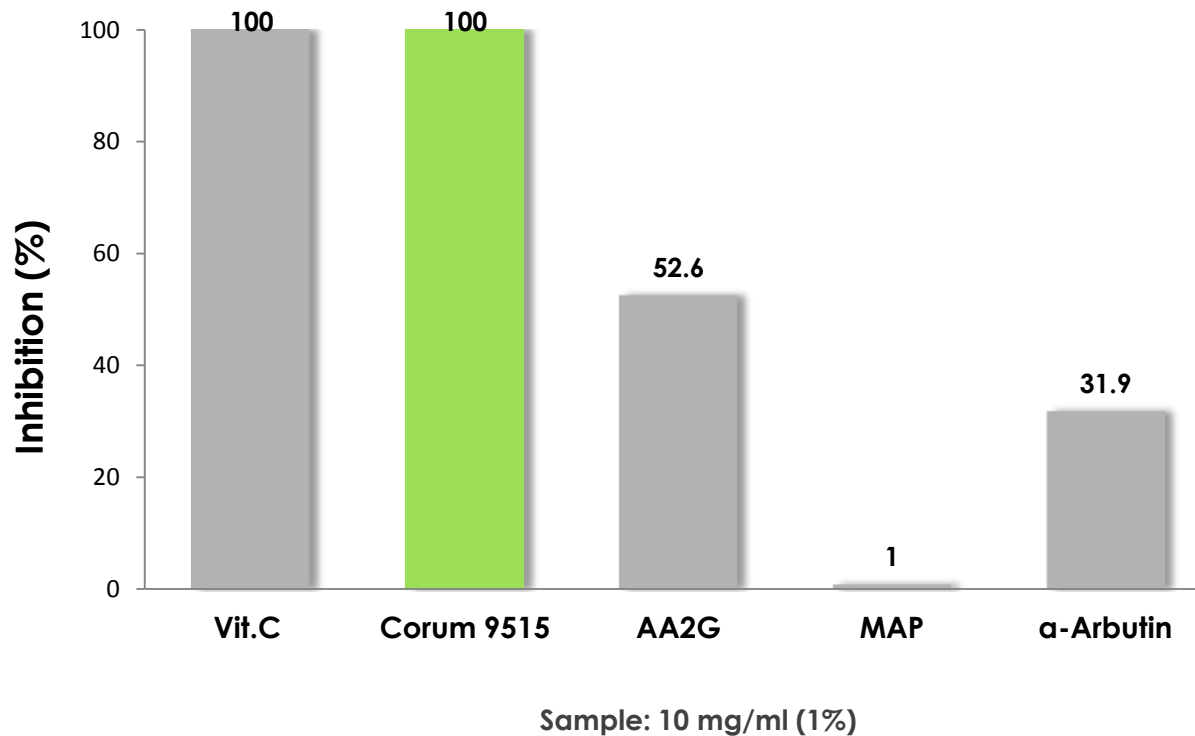
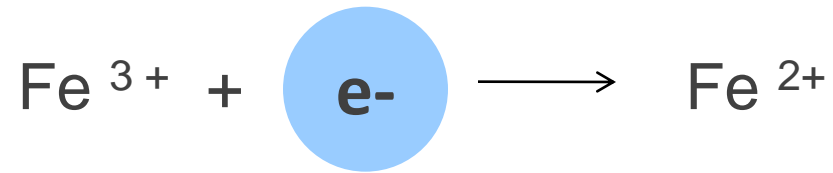
MW: 338.26



**MAP**

MW: 759.22

# Efficacy Test: Ferric Reducing Ability



# CORUM 9515 Efficacy Studies

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**CORUM**

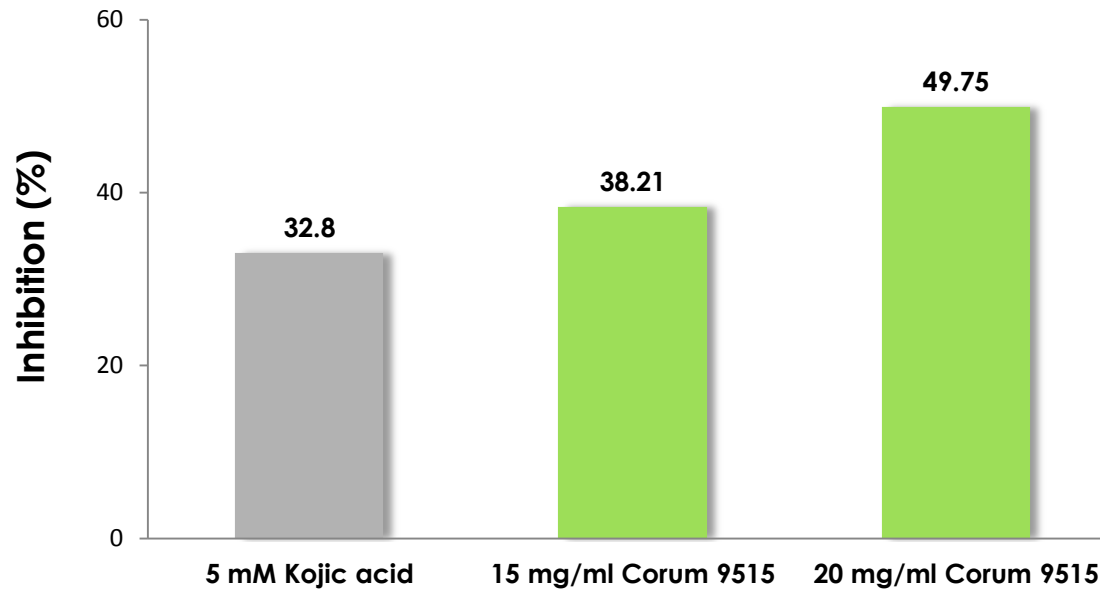
Advance through Knowledge

# Efficacy Test: *In-vitro* whitening activity study - **melanin assessment**

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

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**CORUM**

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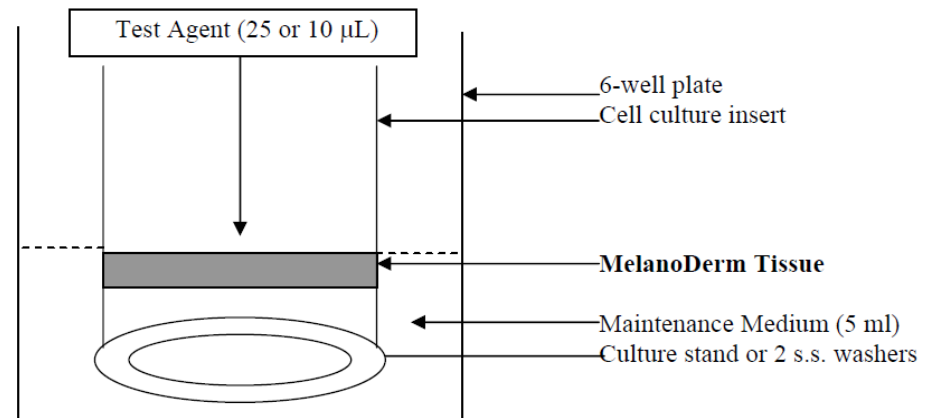
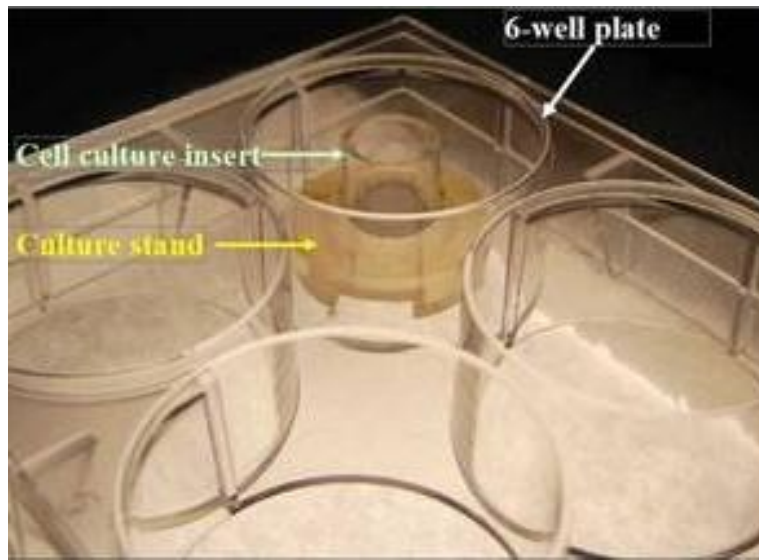
# Efficacy Test: *Ex-vivo* Melanin Assay

## MelanoDerm™

by BioInnovation Laboratories, Inc (USA)

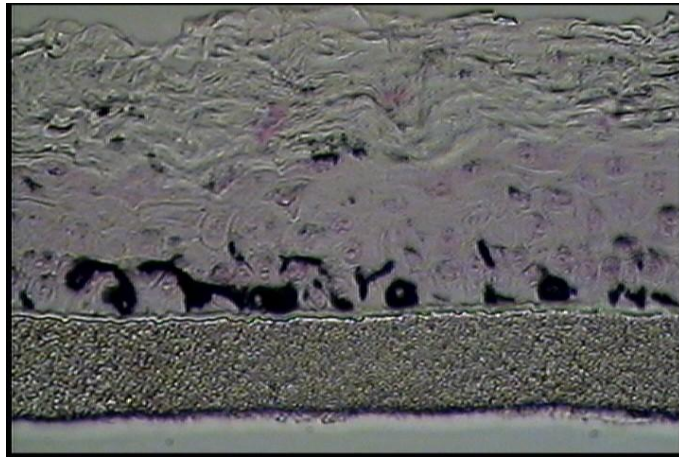
### Purpose

- 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



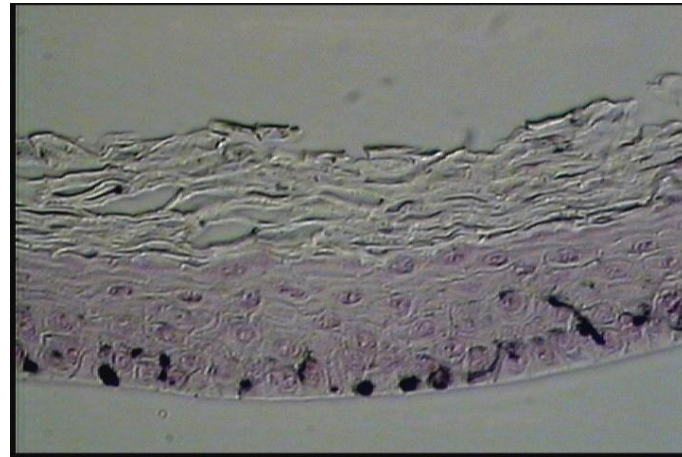
# Efficacy Test: Ex-vivo Melanin Assay

Day 0

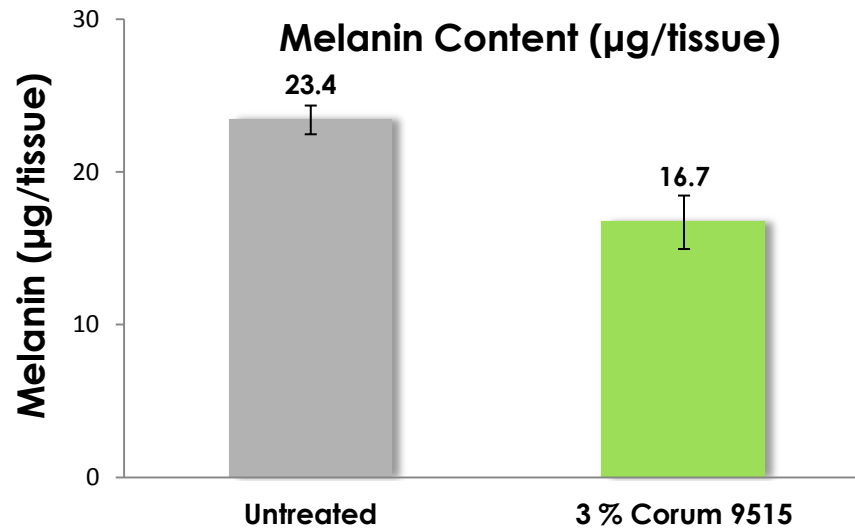


Untreated Tissue

Day 9



3 % Corum 9515



# CORUM 9515 Efficacy Studies

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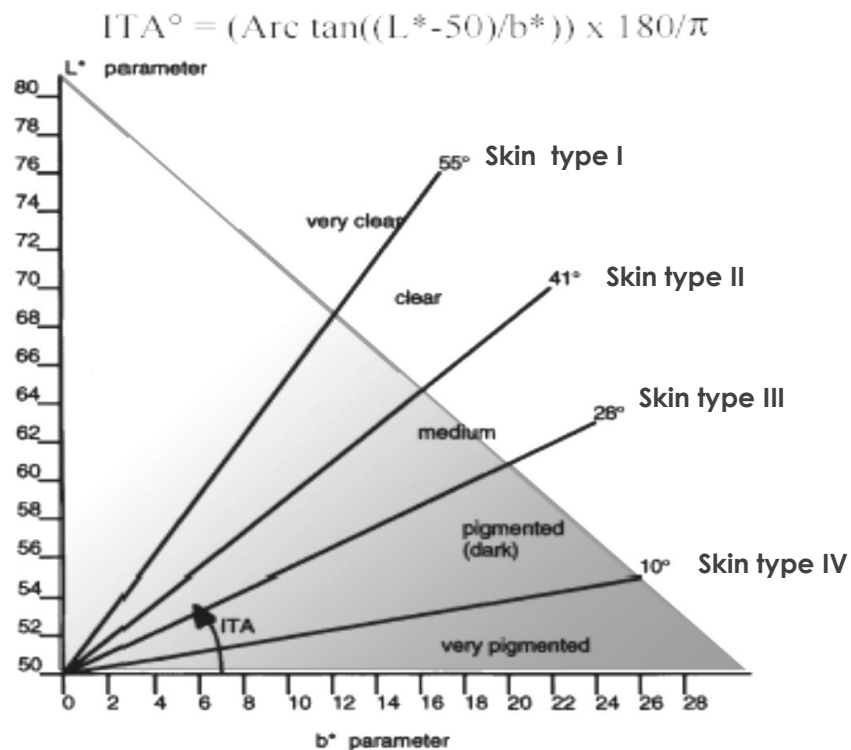
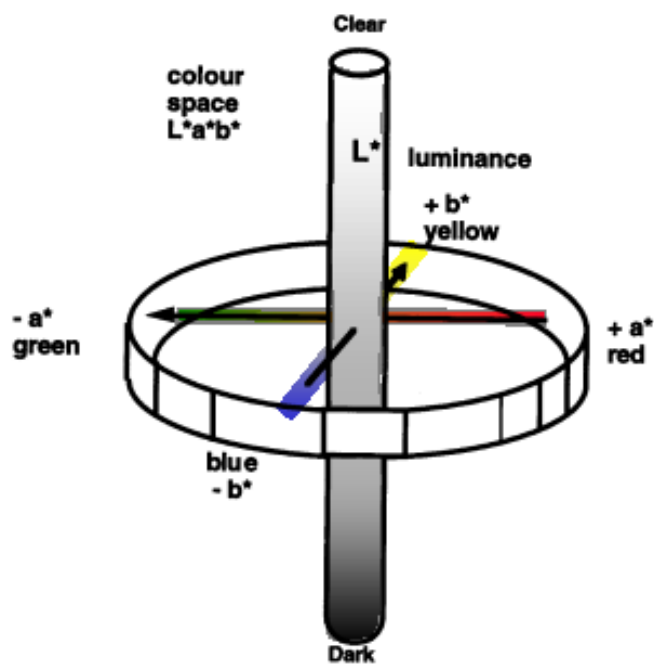
**CORUM**

Advance through Knowledge

# Efficacy Test: *In-vivo* whitening efficacy

by SPINCONTROL

Method : Chromametric Analysis



# Efficacy Test: *In-vivo* whitening efficacy

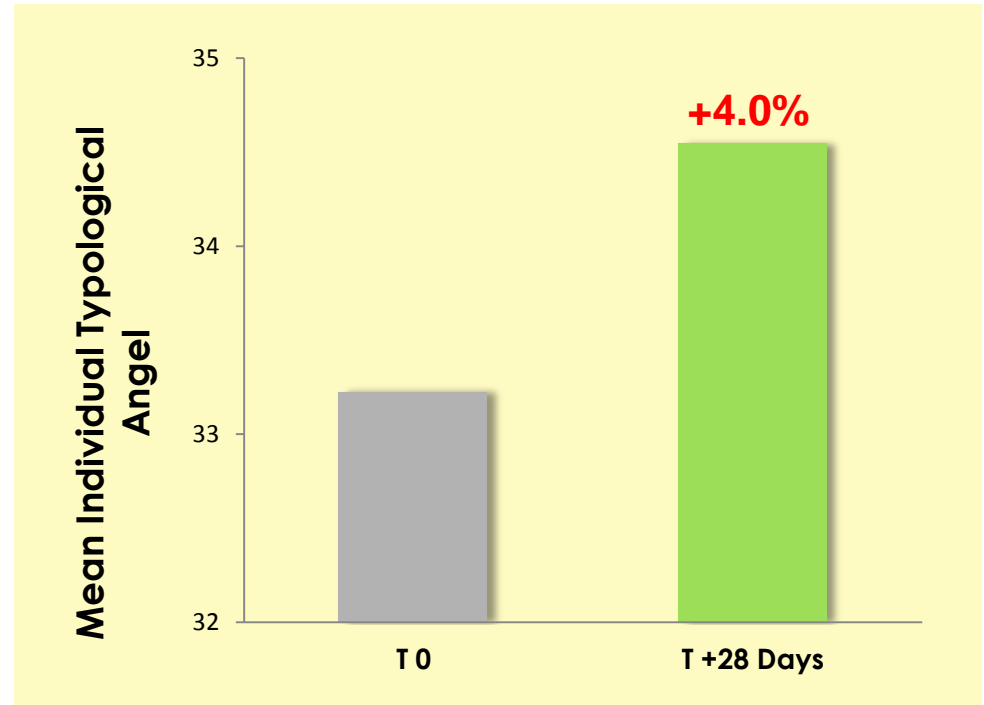
by SPINCONTROL

## Volunteers:

- 20 healthy Asian
- Female
- 25-40 years old
- Skin type III

## Method:

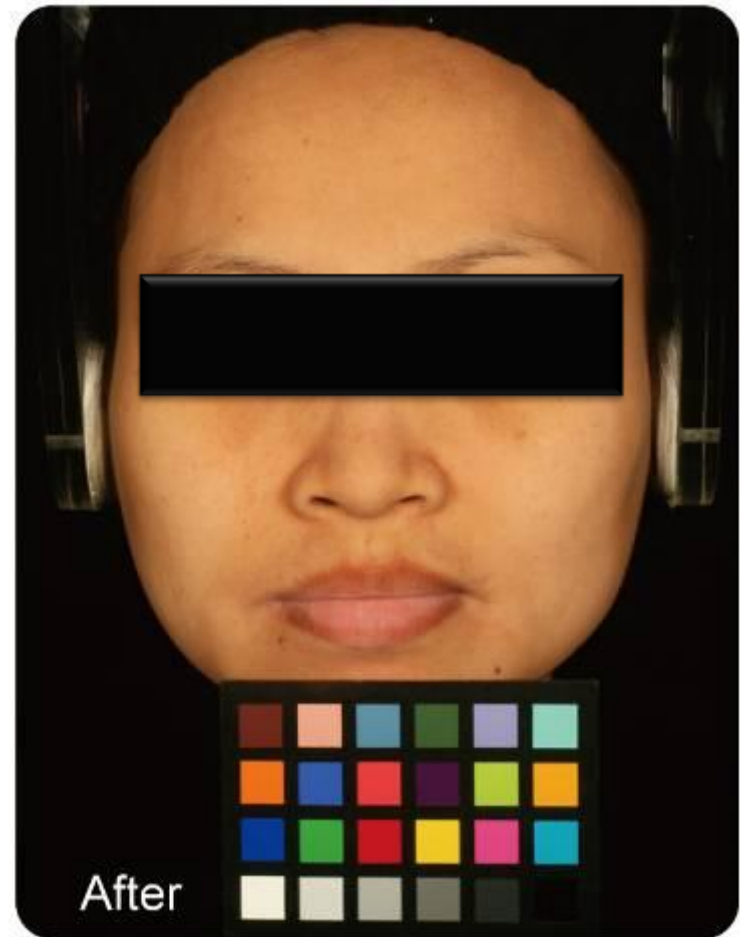
- Chromametry (CR-300)
- 2% Corum 9515 cream



**Result: Significant increase on mean ITA**

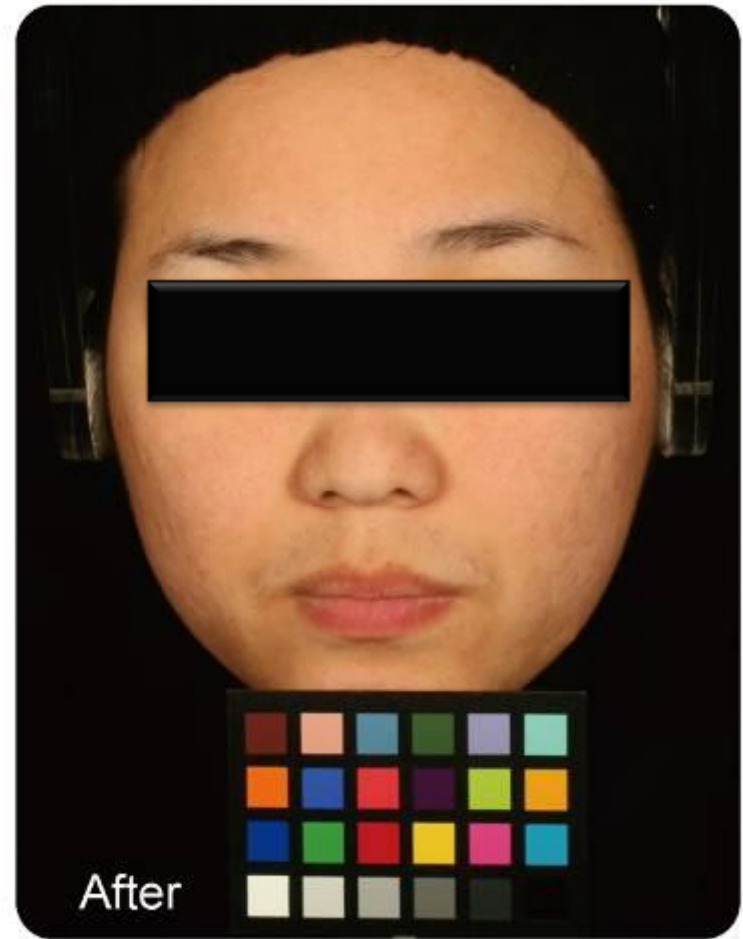
Ref: "In vivo evaluation of the efficacy of one whitening lotion in healthy Asian subjects by chromatography", Spincontrol Asia, November, 2007.

# Efficacy Test: *In-vivo* whitening efficacy



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# Efficacy Test: *In-vivo* whitening efficacy



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# CORUM 9515 Efficacy Studies

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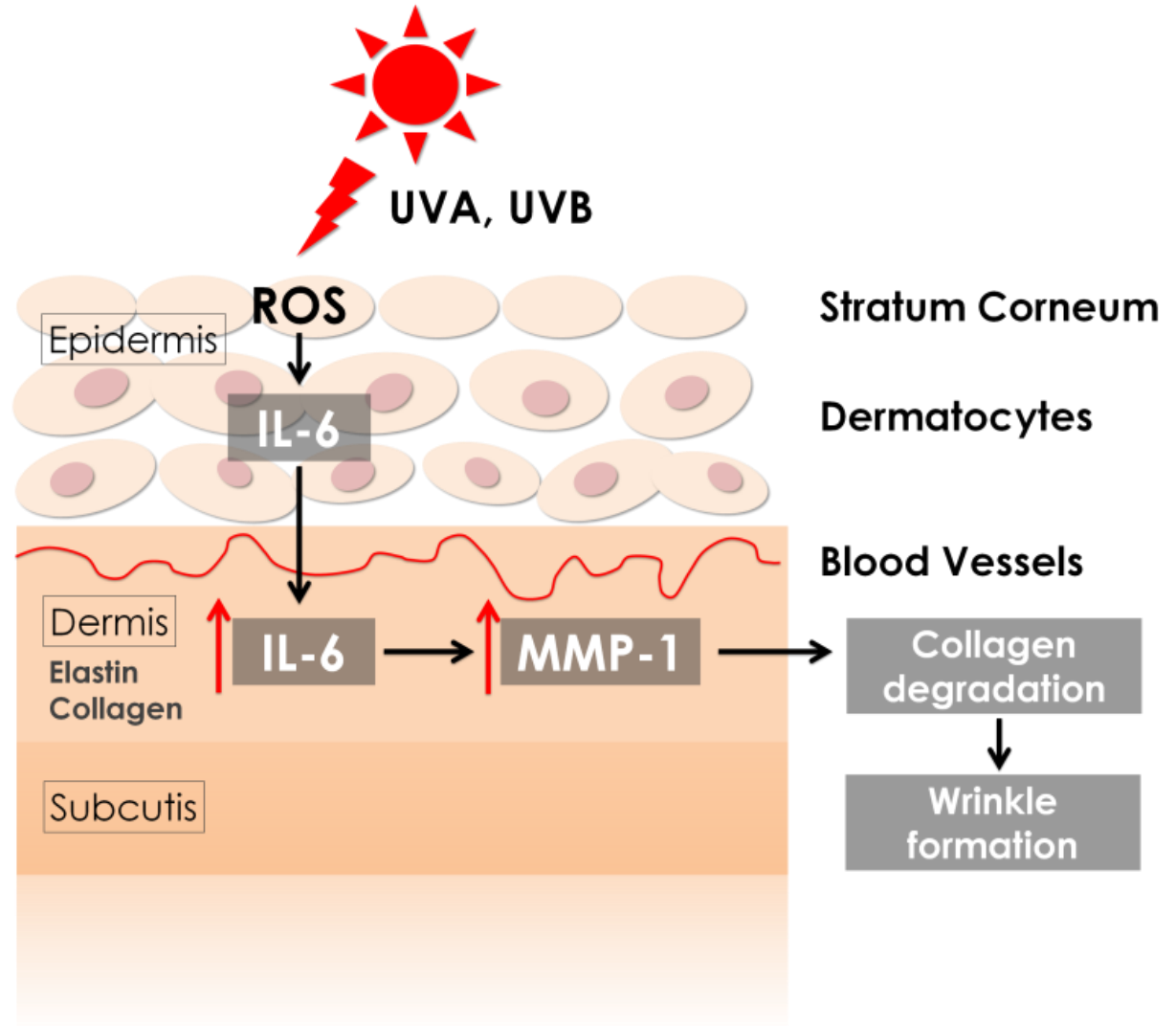
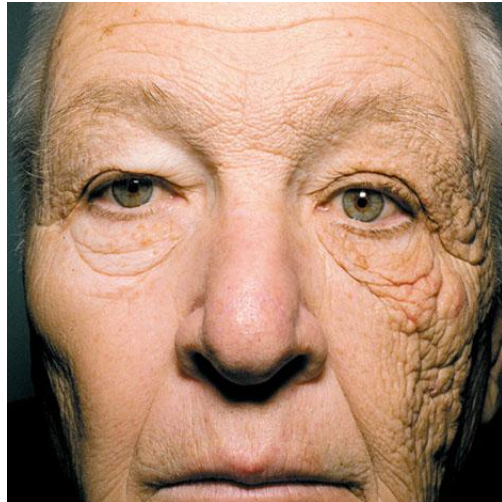


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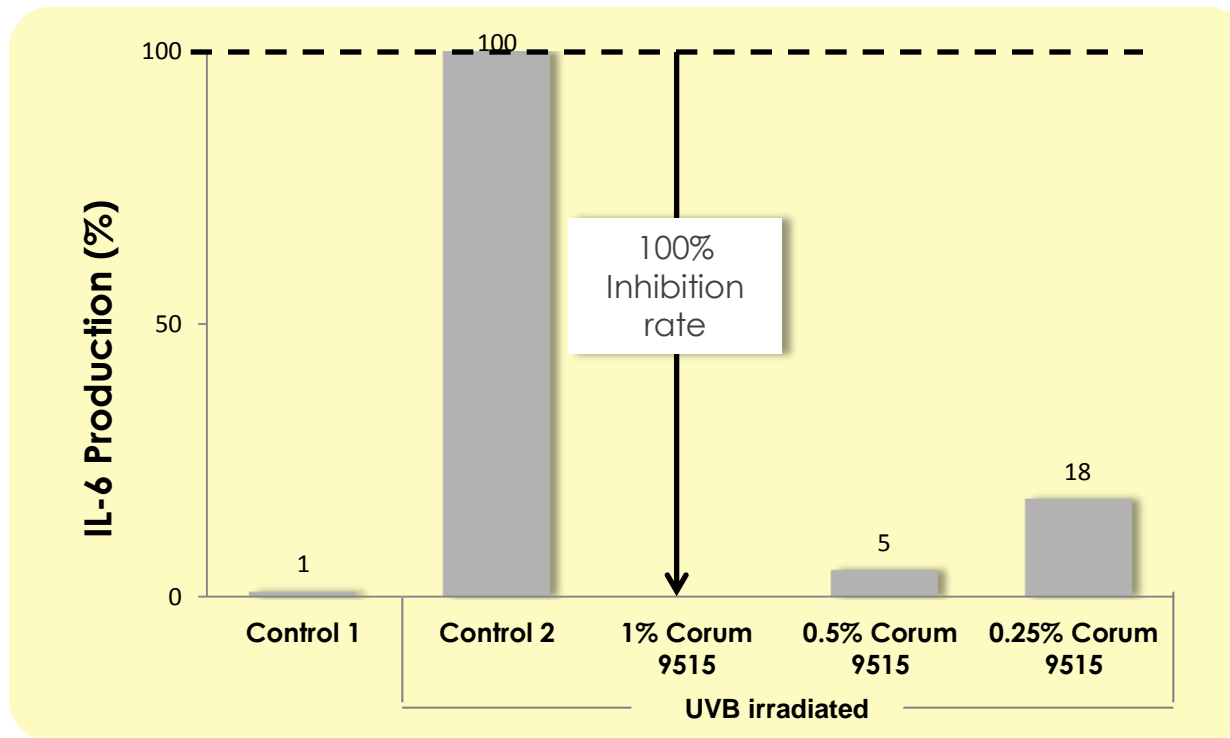
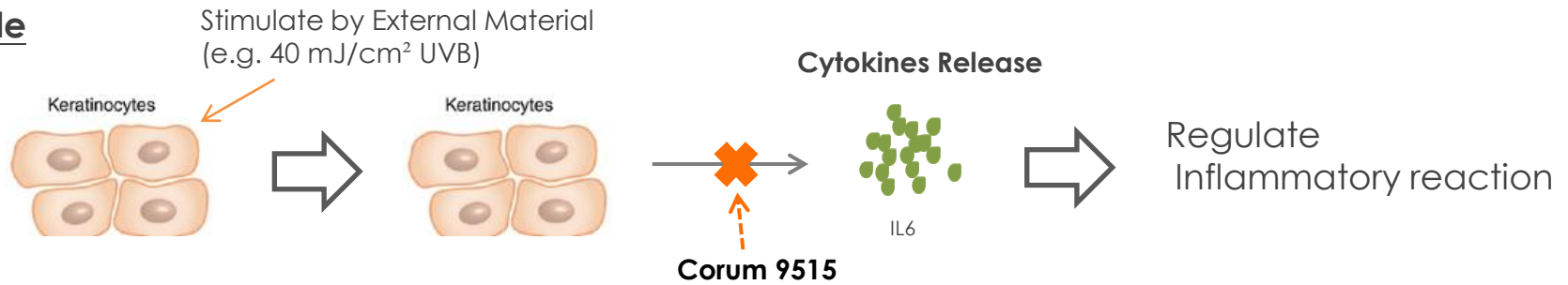


# Mechanism of Photoaging



# Efficacy Test: Anti-inflammation IL-6 test

## Principle



# CORUM 9515 Efficacy Studies

- *In-vitro* tyrosinase inhibition
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- *In-vitro* whitening activity
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(Anti-inflammation test)
- **Stimulation of collagen synthesis**
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- Skin penetration profile



**CORUM**

Advance through Knowledge

# 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

## Functions

- Impart strength, support and skin elasticity

## Why important

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

**Wrinkle formation + Aged skin**

Abundant collagen



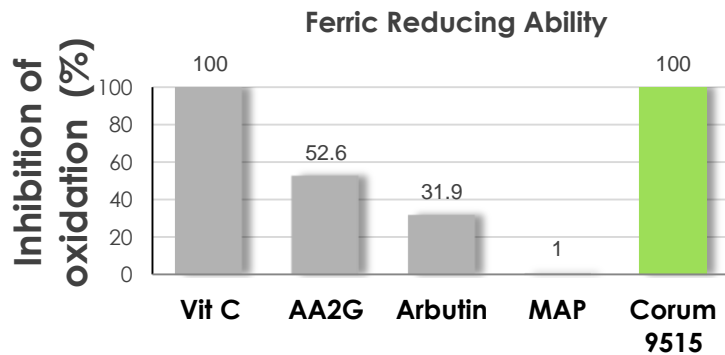
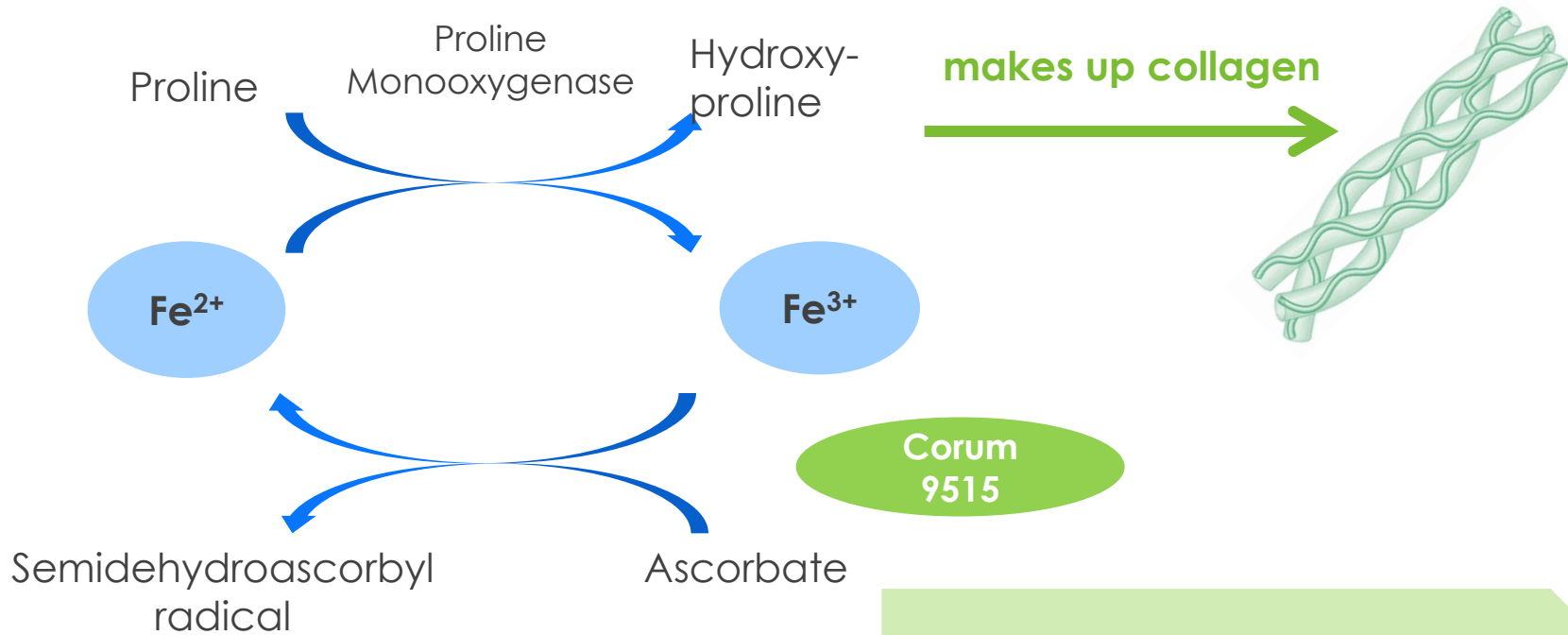
Epidermis

Dermis

Less collagen



# The role of ascorbic acid in collagen synthesis

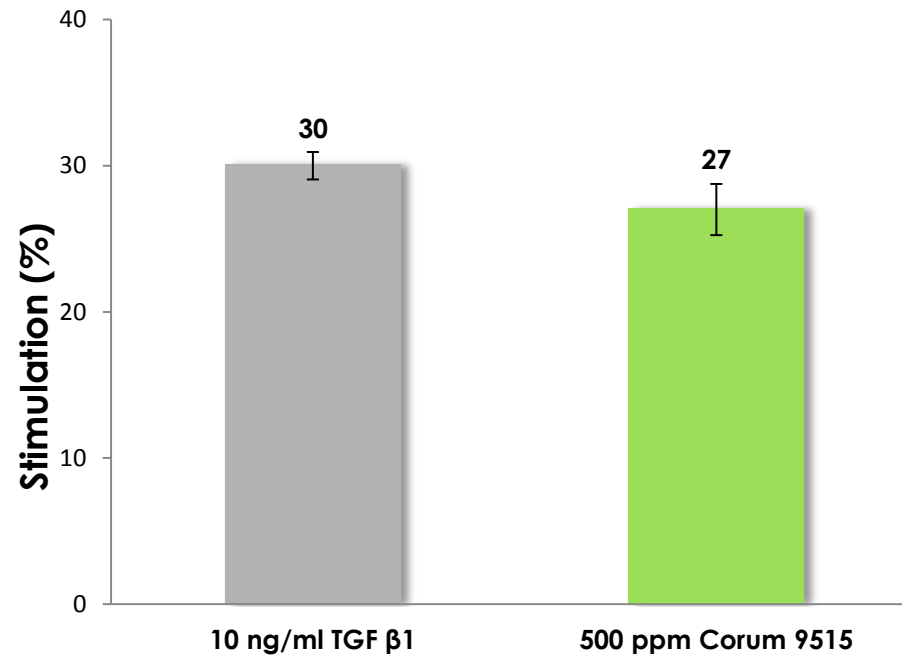


- Proline residues convert into hydroxyproline during the synthesis of collagen
- Hydroxylation requires Vitamin C

# Efficacy Test: Stimulation of natural collagen synthesis

by *IDEA*, France  
After 48hrs contacts

- TGF  $\beta$ 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  $\beta$ 1**

# CORUM 9515 Efficacy Studies

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**CORUM**

Advance through Knowledge

# Corum 9515 Free-Radical Scavenging Mechanism

## What are free radicals

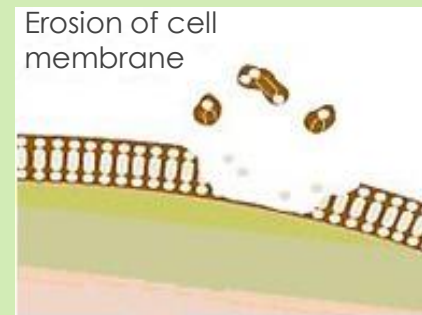
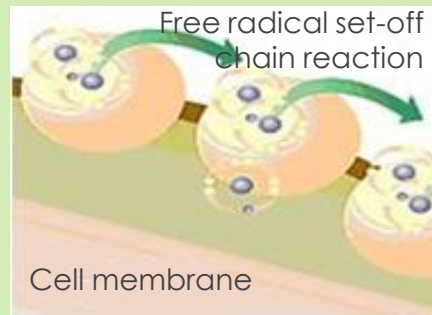
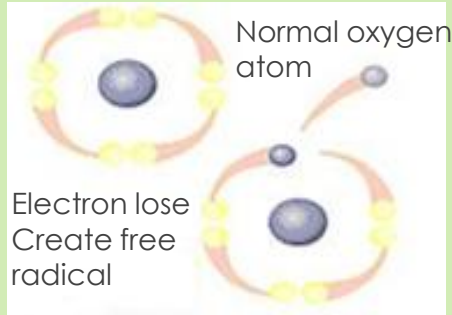
- Atoms, molecules, or ions with unpaired electrons
- Due to its unpaired electrons, they are often highly reactive
- Often causes chain reactions

## 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

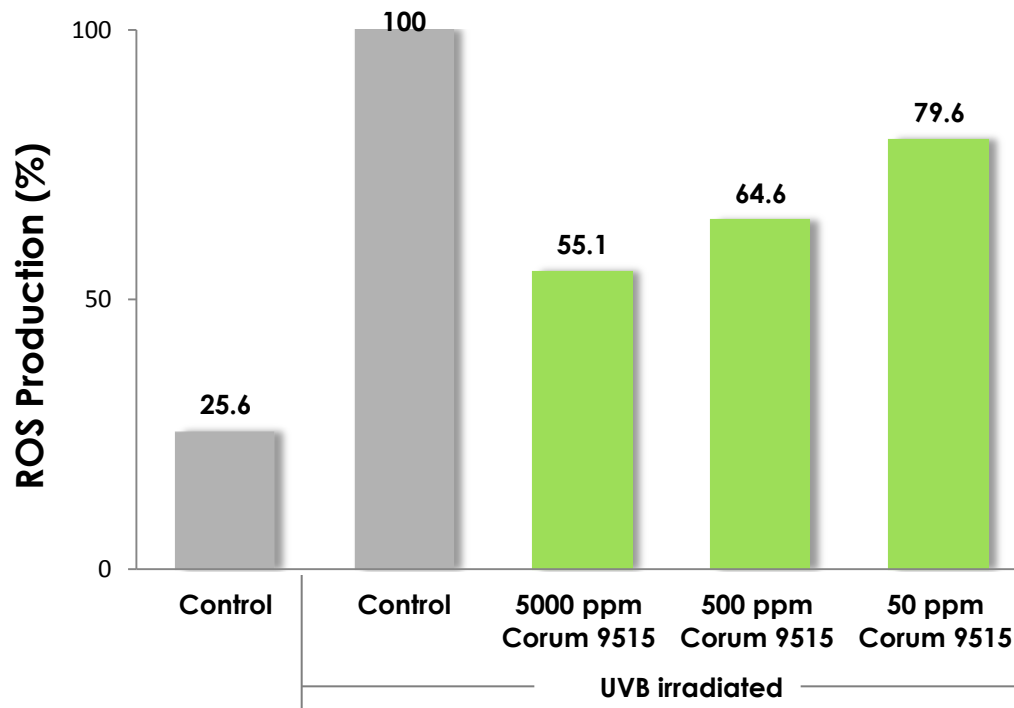
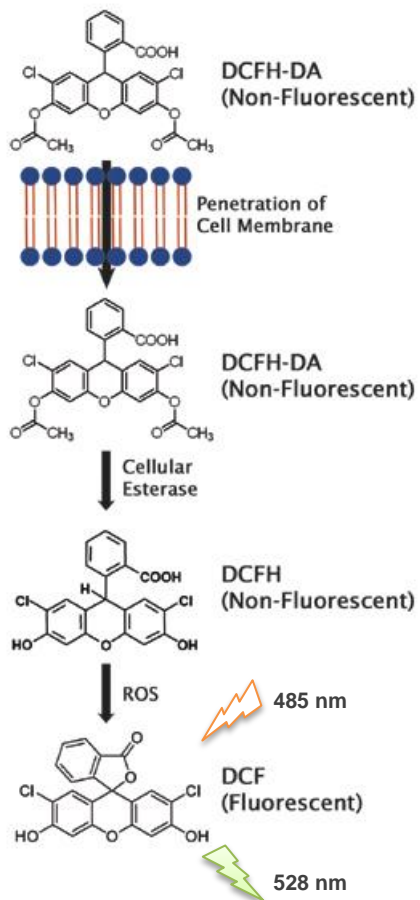




# Efficacy Test: Radical scavenging test

## ROS assay after UVB irradiated

### Principle



**CORUM 9515 shows great ability on ROS inhibition.**

# CORUM 9515 Efficacy Studies

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- **DNA protection**
  - a. *In-vitro*, Comet Assay
  - b. *Ex-vivo*
- Skin penetration profile



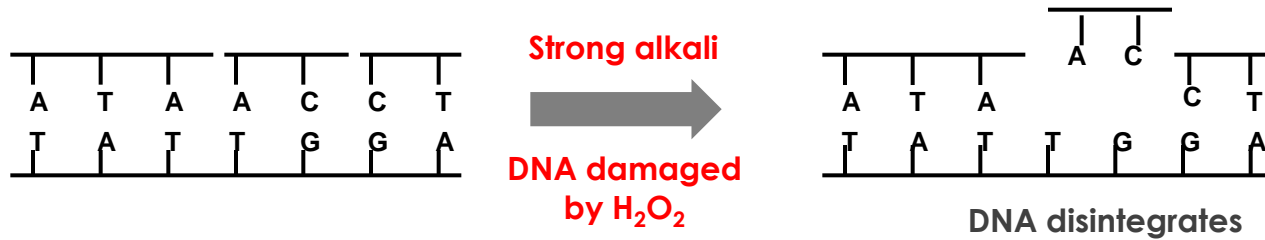
**CORUM**

Advance through Knowledge

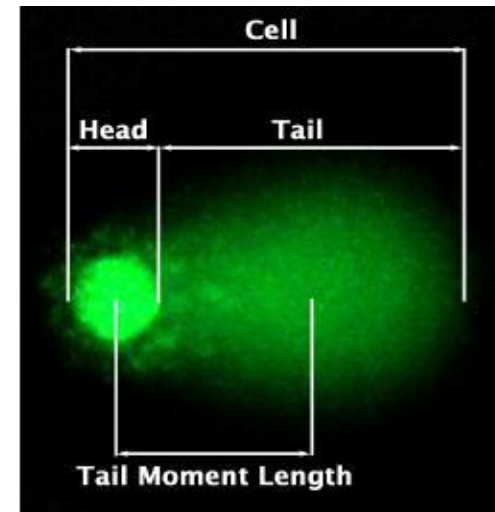
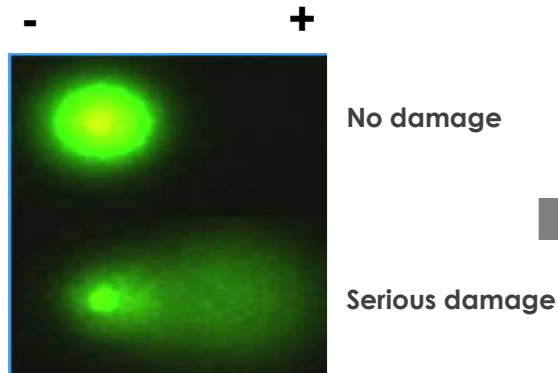
# Efficacy Test: DNA Protection

## Comet Assay

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

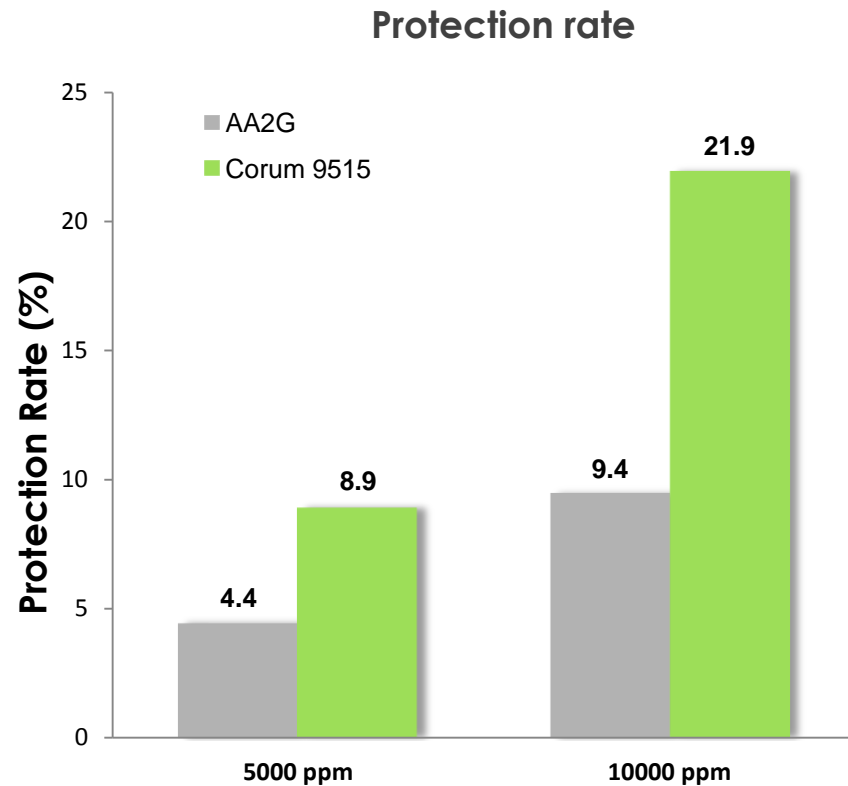
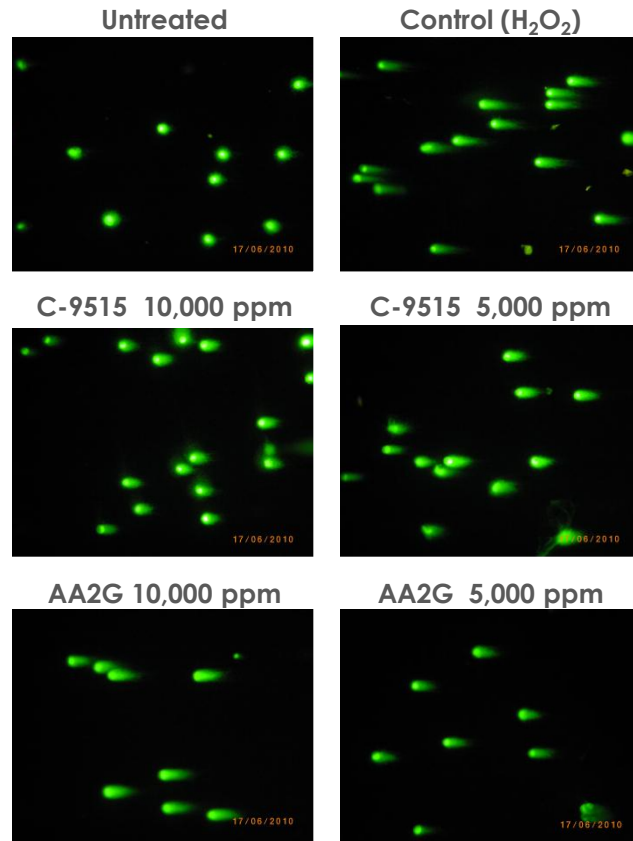


Single cell electrophoresis



# Efficacy Test: DNA Protection

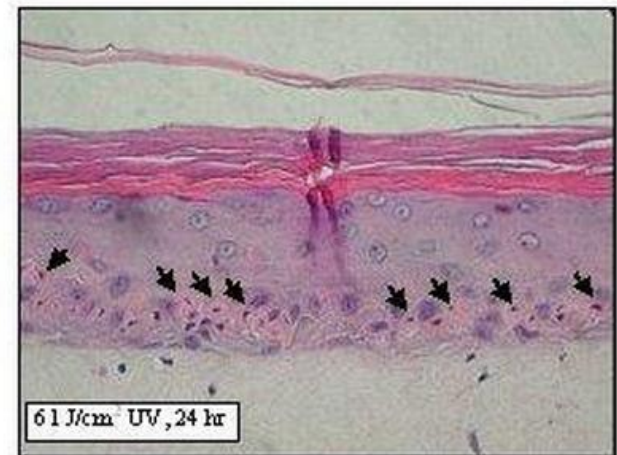
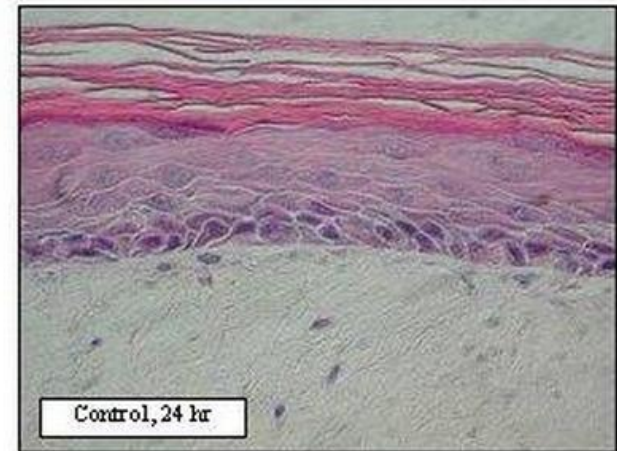
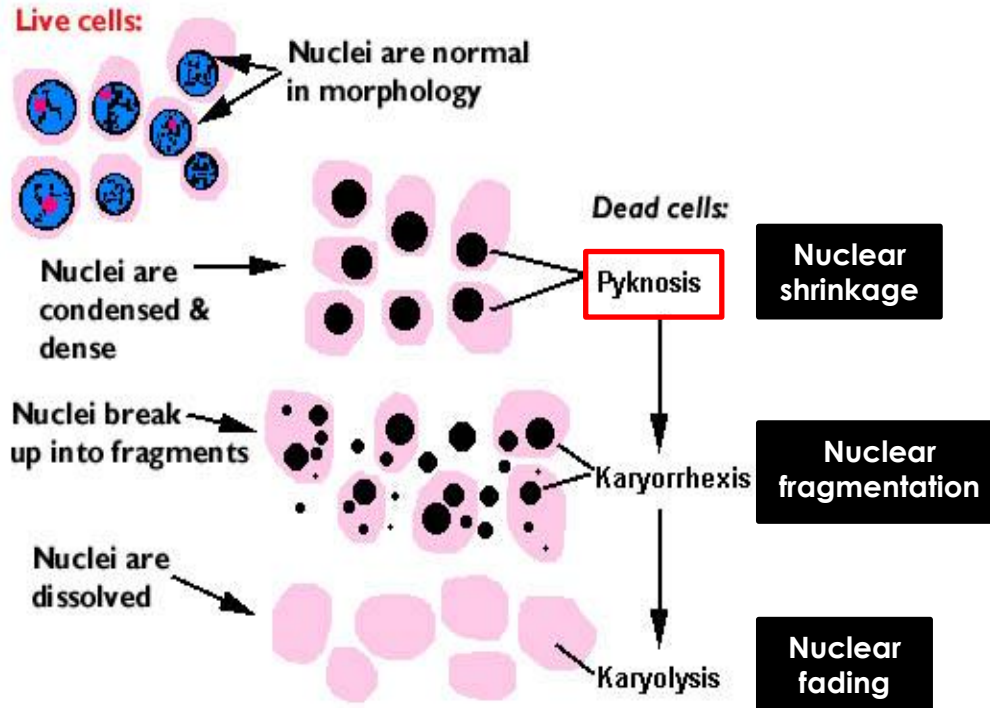
## Comet Assay



**Result: CORUM 9515 shows high efficiency on DNA protection activity.**

# What are Sun Burn Cells?

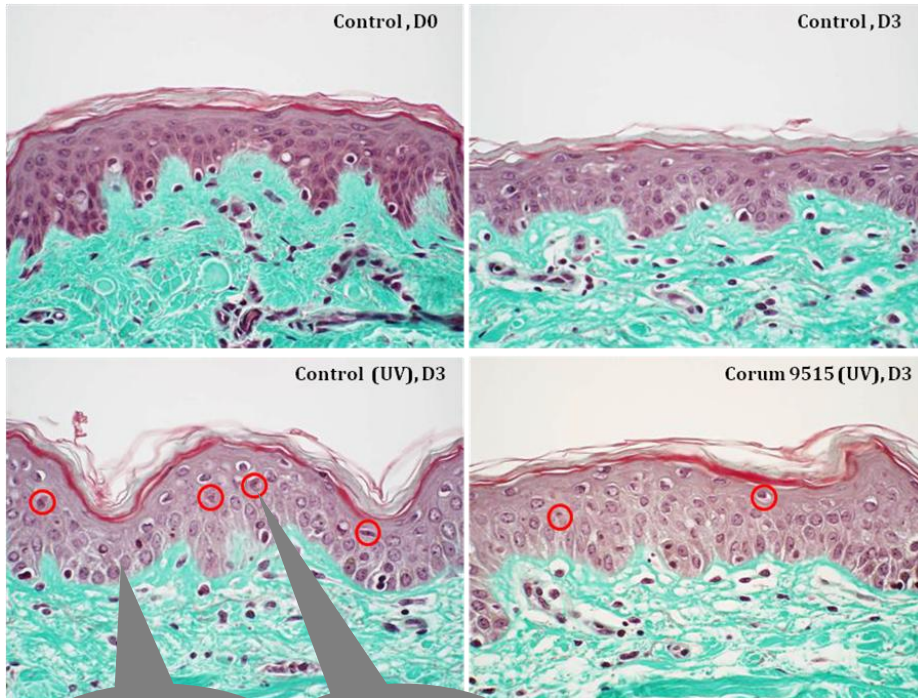
**Sunburn cells (SBC)** are keratinocytes **undergoing apoptosis** as a **protective mechanism** against the carcinogenic effects of UVB irradiation which irreversibly damages their DNA or other chromophores.



Sun burn cells are indicated by arrow.

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

by BIO-EC, France



Spongiosis is slight in the basal layers

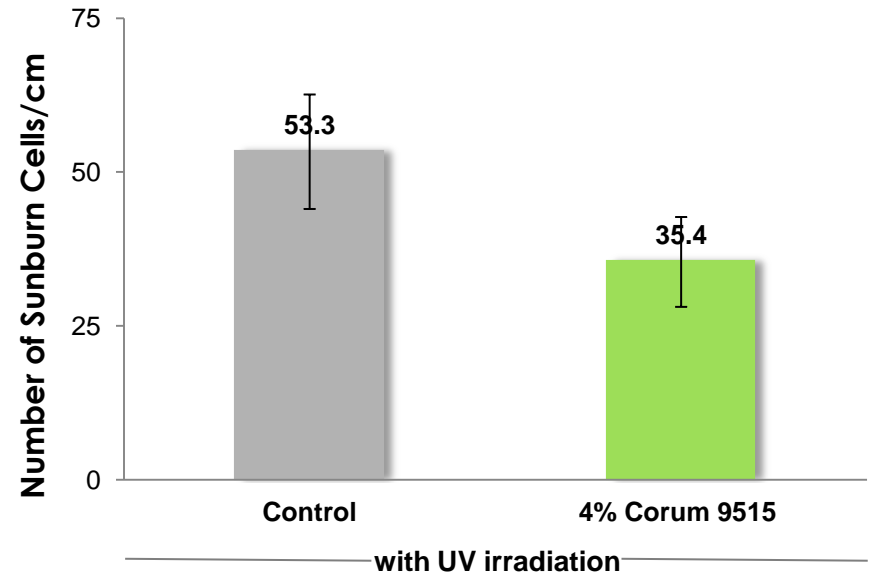
Some cells with picnotic nuclei.

\*Sunburn cell are indicated by circles

## Explants preparation:

- From a 45-year-old Caucasian woman
- 12 skin explants of an average diameter of 11 mm ( $\pm 1$ mm)

## Number of sun burn cell in the epidermis



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

# CORUM 9515 Efficacy Studies

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# Skin Penetration Profile

## Human Epidermis Model EPISKIN® : *In Vitro* Skin Penetration Test

*in vitro* reconstructed human epidermis which is similar to the *in vivo* human epidermis.

✂ Episkin is a skin model as alternatives to animal testing.

### Applications:

#### ■ Percutaneous absorption

■ *In vitro* skin corrosion

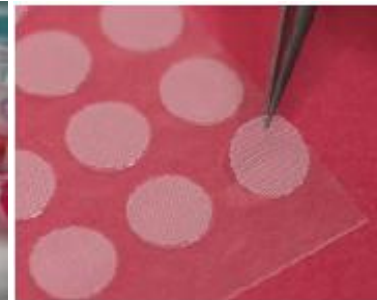
■ *In vitro* skin irritation

■ Pharmacology /Toxicology

### Test materials:

■ 2 % solution

■ 2 % Whitening cream

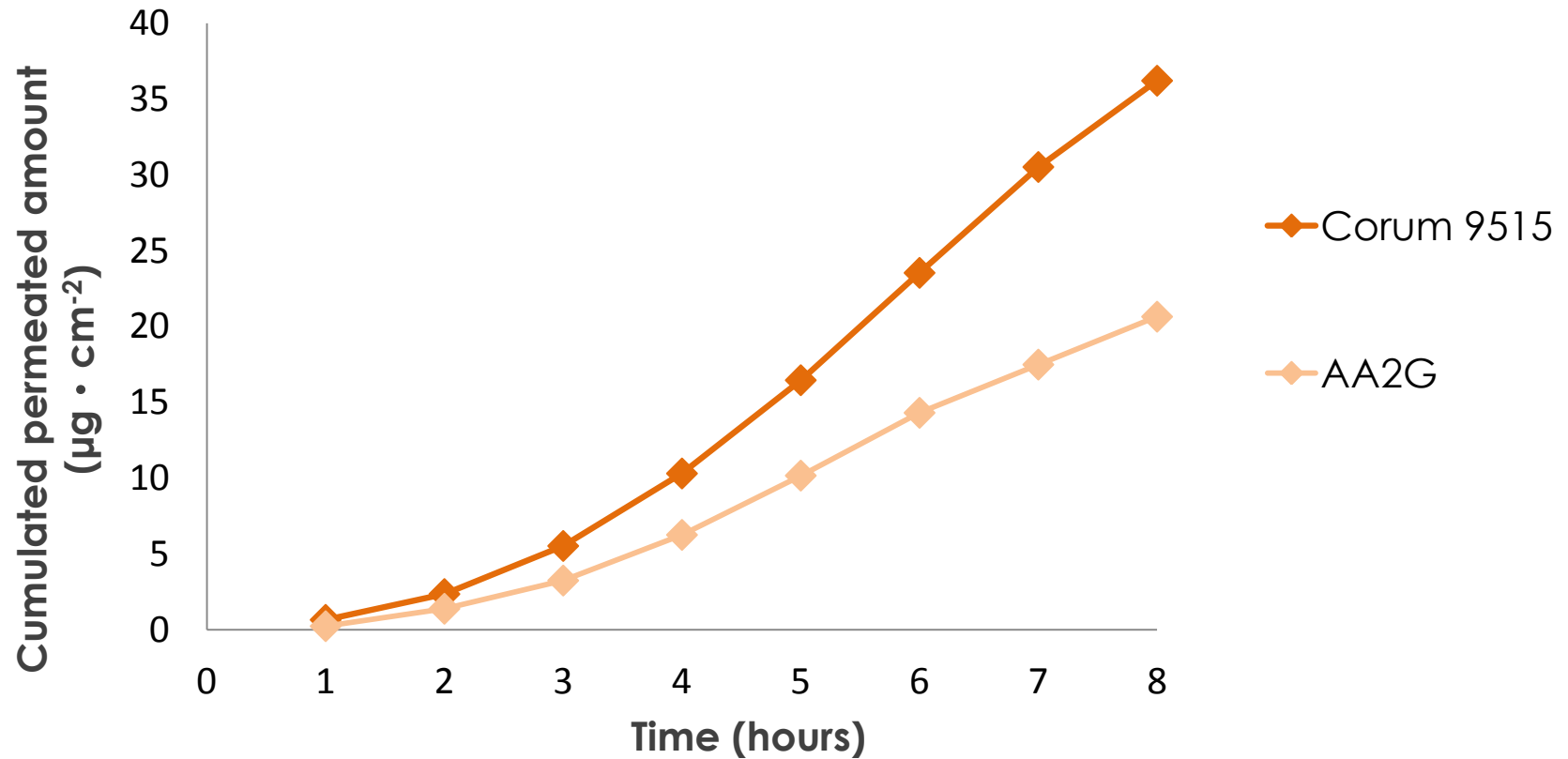




# Skin Penetration Profile

## Human Epidermis Model EPISKIN® : *In Vitro* Skin Penetration Test Cumulated permeated amount

Test materials: 2 % solution

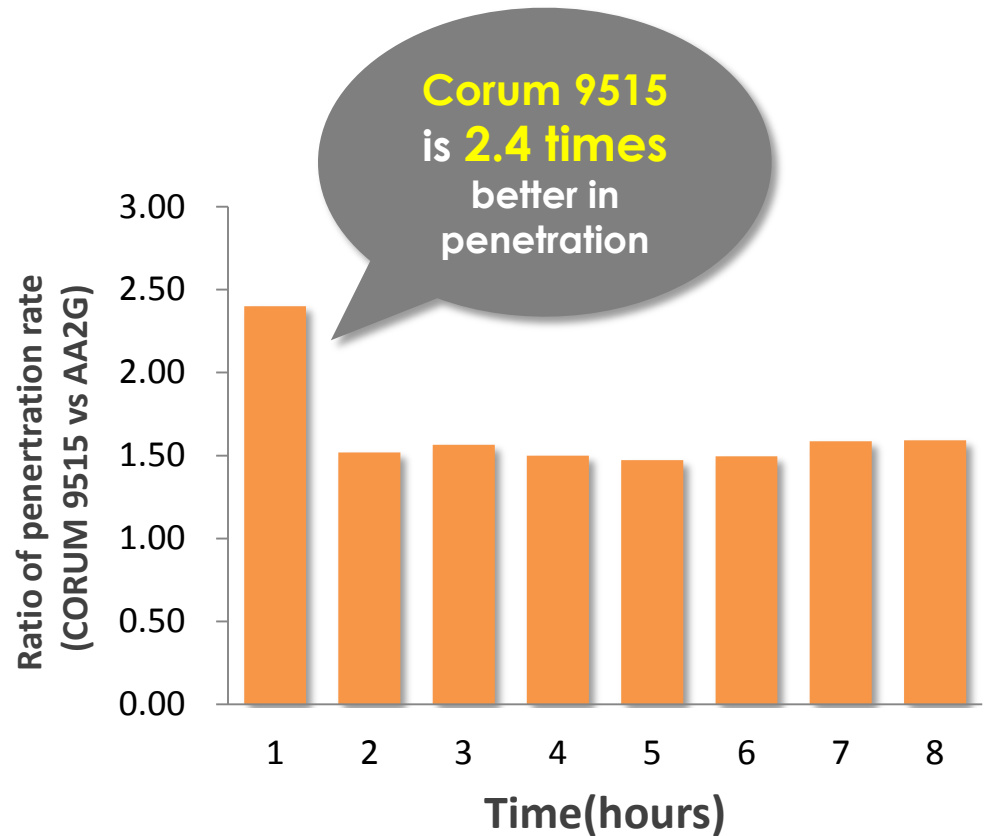
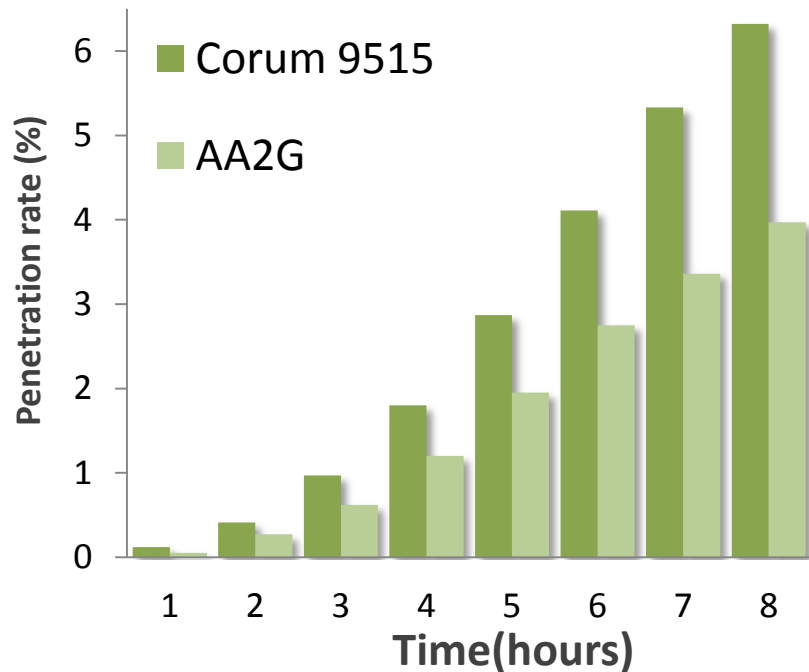


**Result: CORUM 9515 shows better skin penetration ability compared with AA2G.**

# Skin Penetration Profile

## Human Epidermis Model EPISKIN® : *In Vitro* Skin Penetration Test Penetration rate • Ratio (Corum 9515 vs. AA2G)

Test materials: 2 % solution



Corum 9515  
is 2.4 times  
better in  
penetration

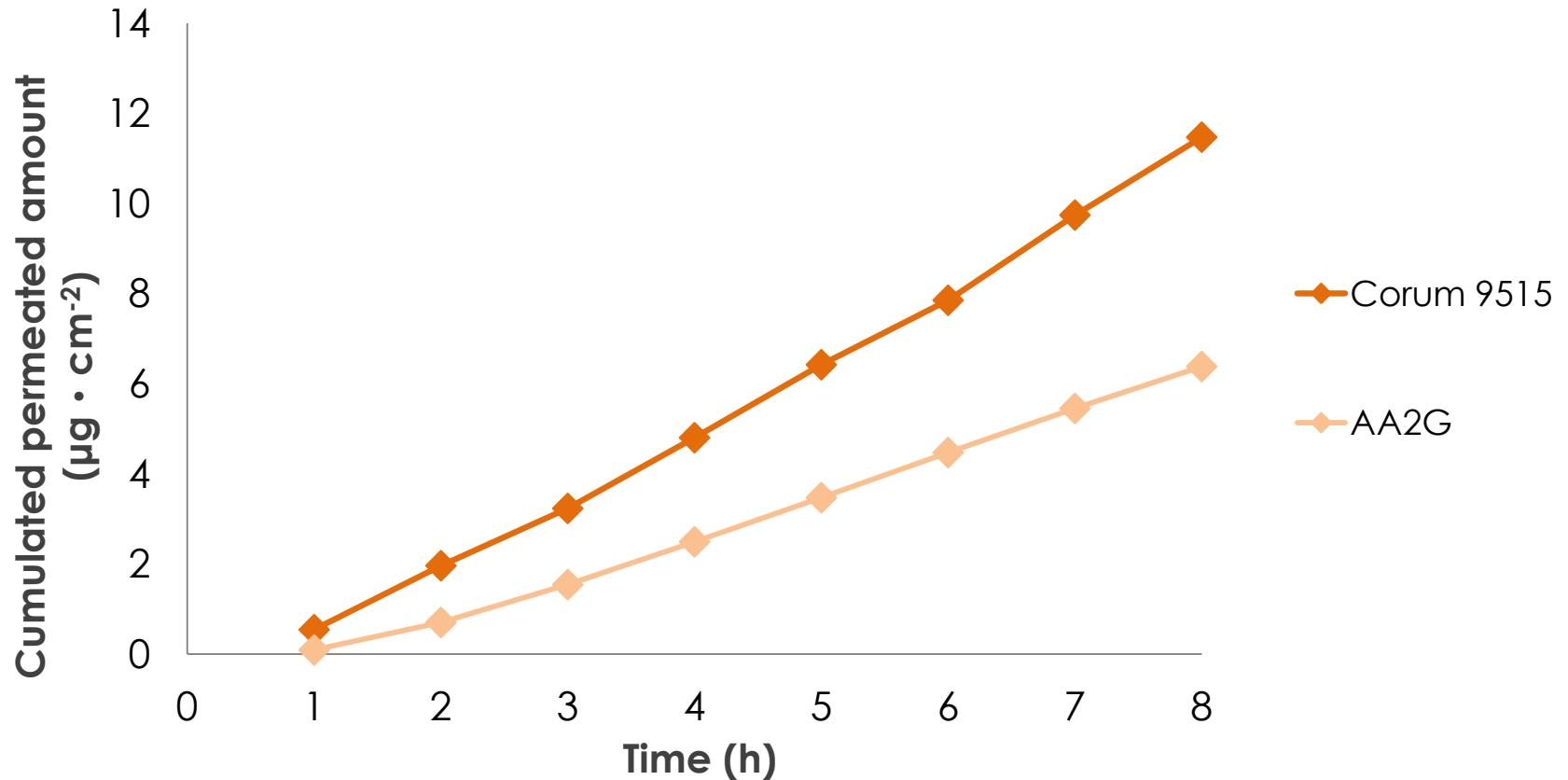
**Result: CORUM 9515 shows better skin penetration ability compared with AA2G.**

# Skin Penetration Profile

Human Epidermis Model EPISKIN® : *In Vitro* Skin Penetration Test

Cumulated permeated amount

Test materials: 2 % Cream

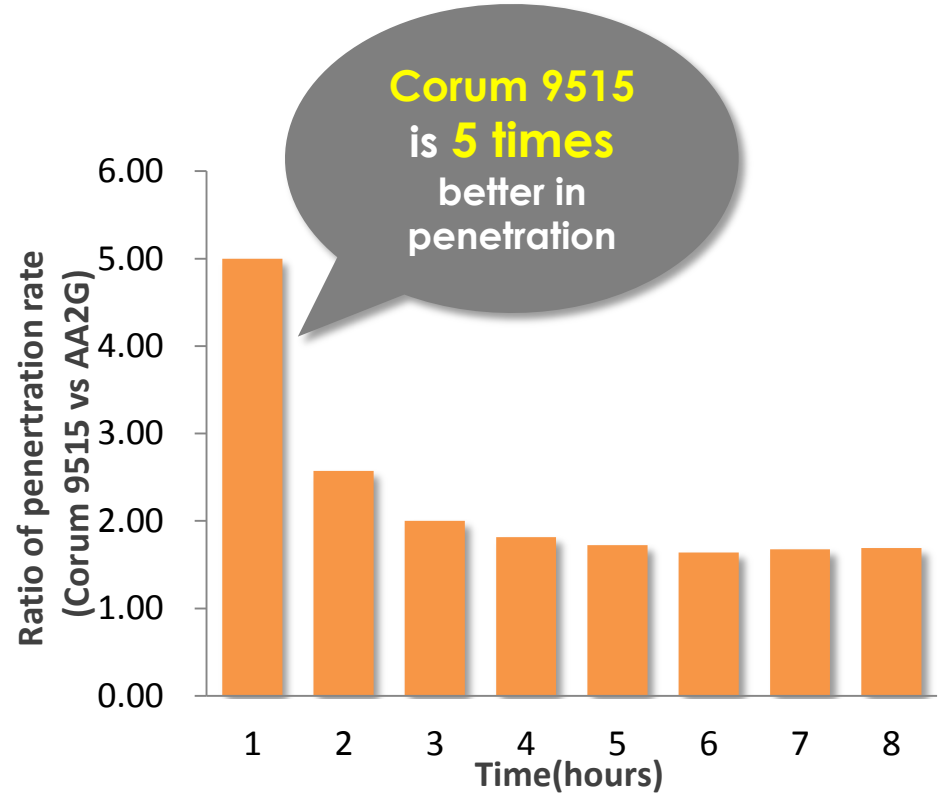
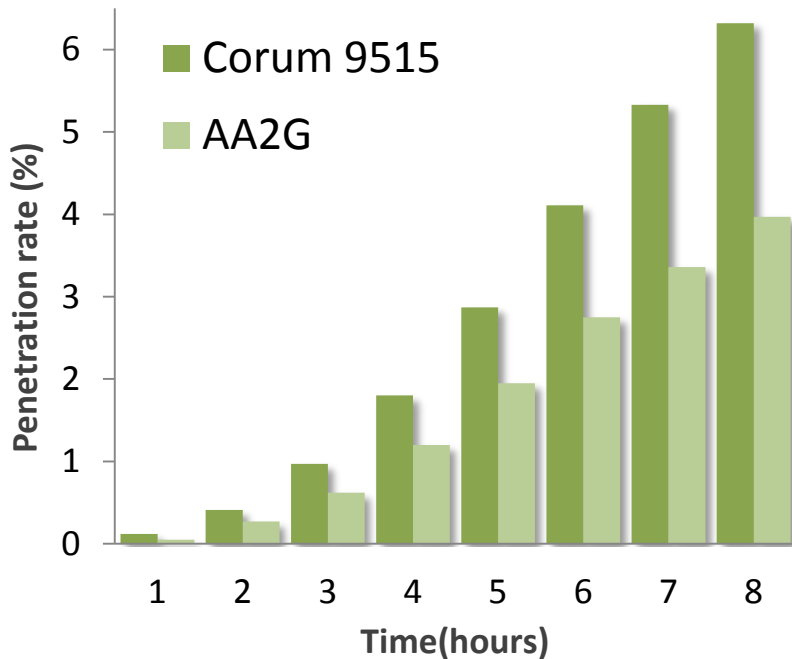


Result: CORUM 9515 shows better skin penetration ability compared with AA2G.

# Skin Penetration Profile

## Human Epidermis Model EPISKIN® : *In Vitro* Skin Penetration Test Penetration rate • Ratio (Corum 9515 vs. AA2G)

Test materials: 2 % Cream



**Result: CORUM 9515 shows better skin penetration ability compared with AA2G.**

# Stability Tests

- **Heat-stability (45 °C , 1 month)**

- color (Transmittance in 440nm )
- purity (HPLC)

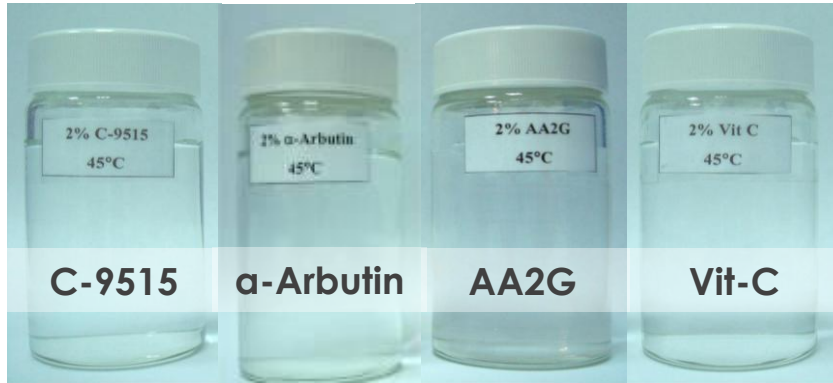
- **Photo-stability (sunlight, 1 month)**

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

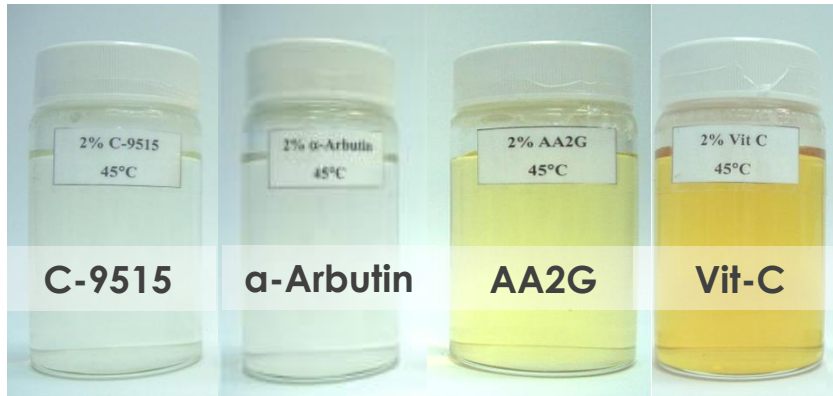


# Stability: Heat-Stability

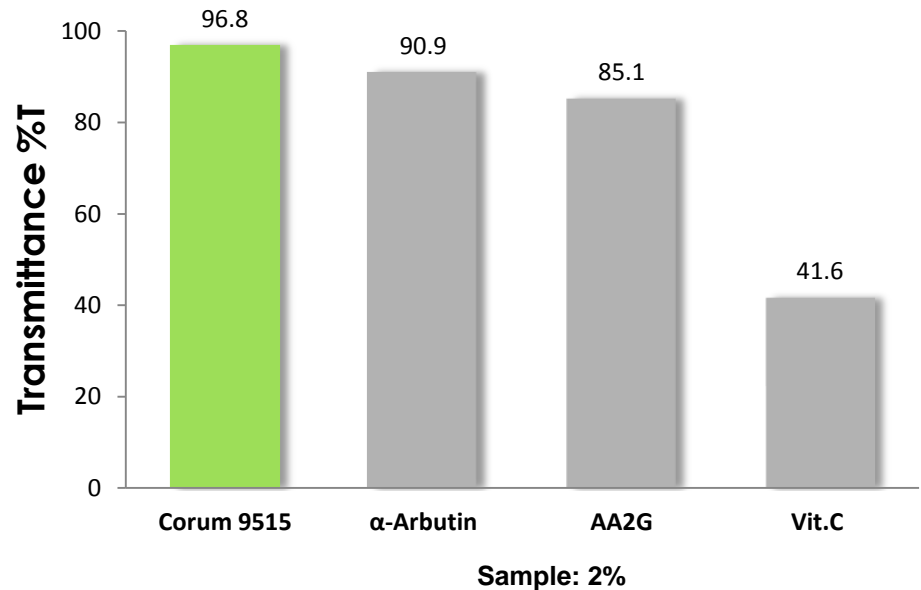
0 month 2% 45°C without buffer



1 month 2% 45°C without buffer



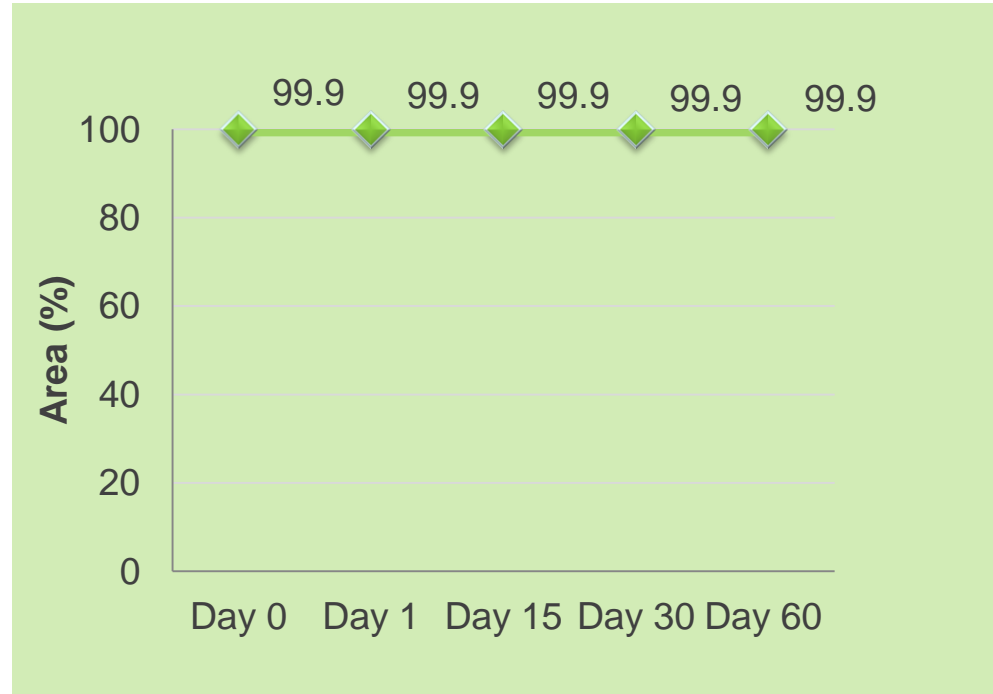
1 Month 45 °C Stability  
(Transmittance at 440nm )



# 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)**

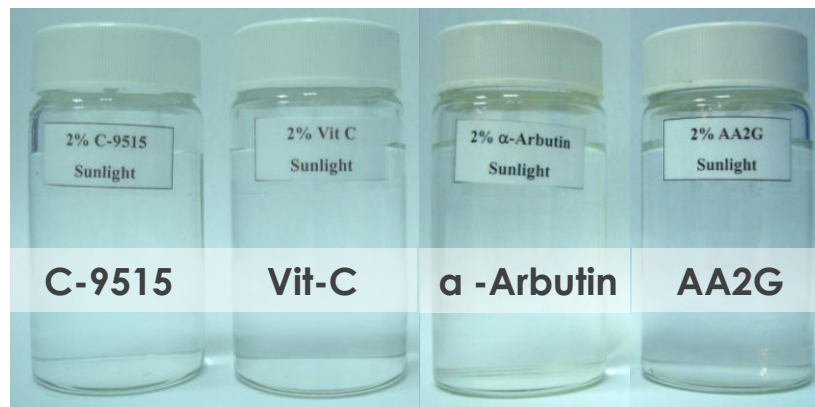
- **Buffer system and pH effect (45 °C, 56 days and RT, 90 days)**



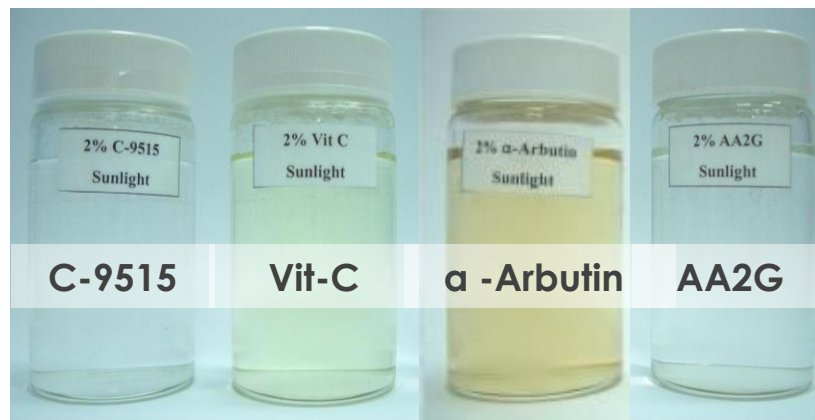


# Stability: CORUM 9515 Photo-Stability

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

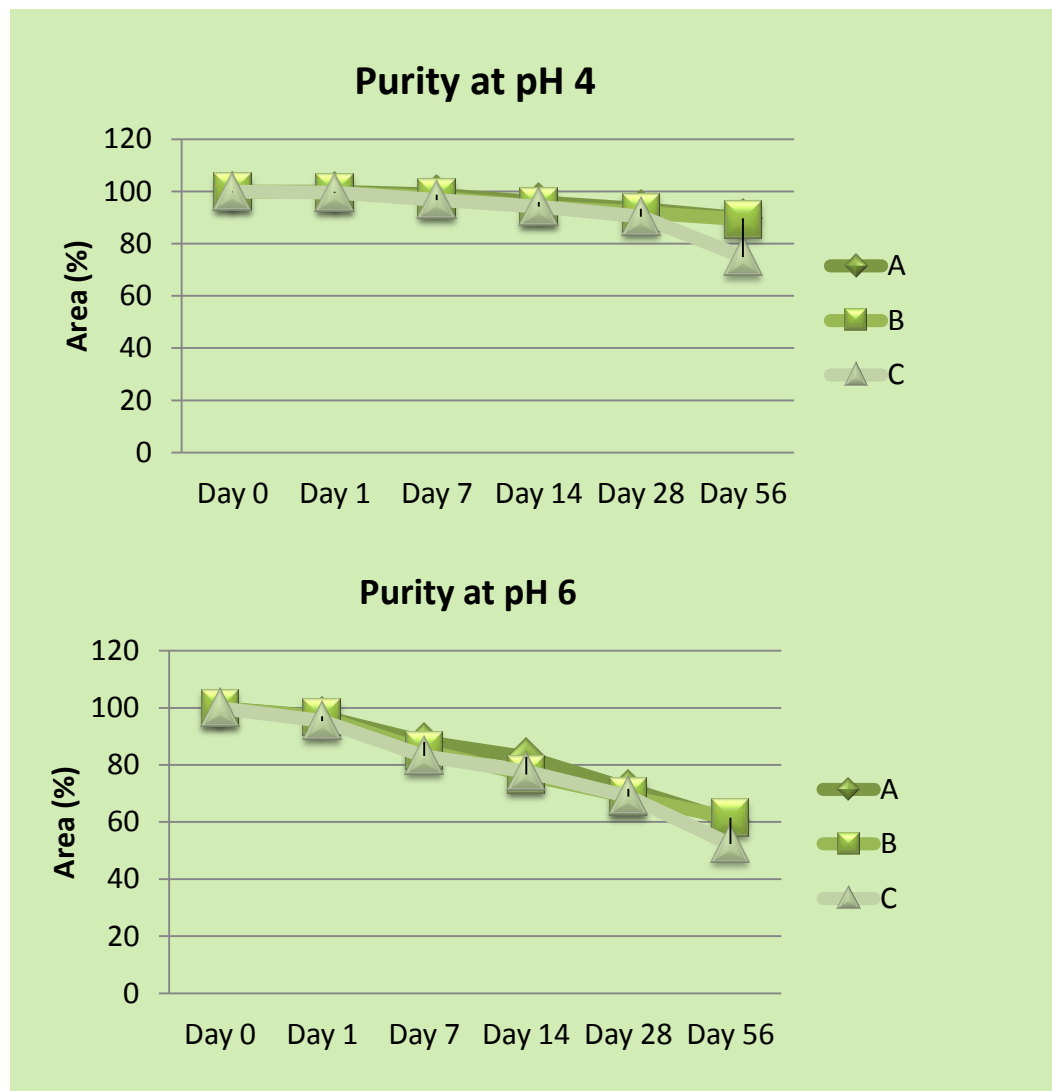
Day: 56 days at 45 °C

### Buffer system:

A - Sodium Citrate – Citric Acid

B - Na<sub>2</sub>HPO<sub>4</sub>- Citric Acid

C - NaHCO<sub>3</sub> – Citric Acid



# pH Stability under different buffer

## pH Stability

### Preparation:

2% buffer solution

Day: 56 days at 45 °C

### Buffer system:

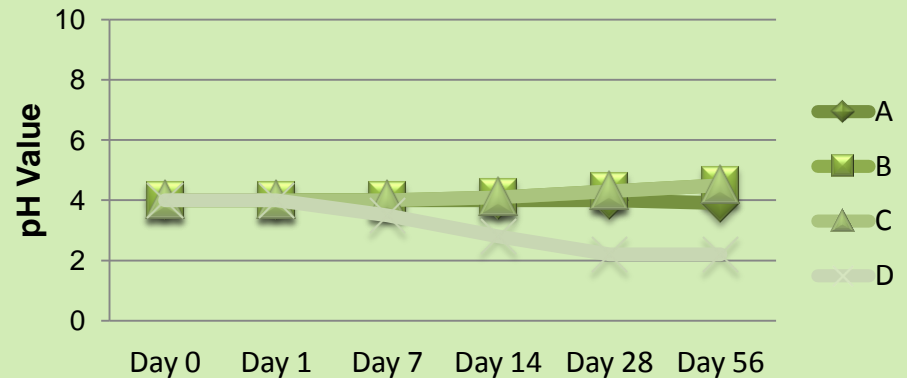
A - Sodium Citrate - Citric Acid

B - Na<sub>2</sub>HPO<sub>4</sub> - Citric Acid

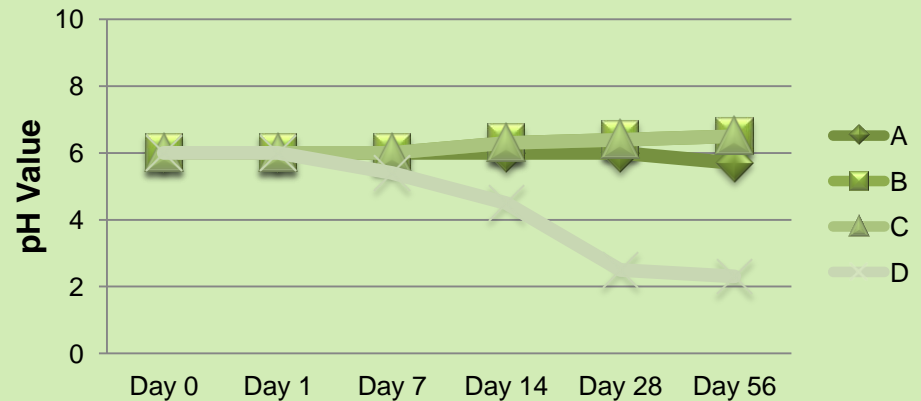
C - NaHCO<sub>3</sub> - Citric Acid

D - NaOH - Citric acid

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



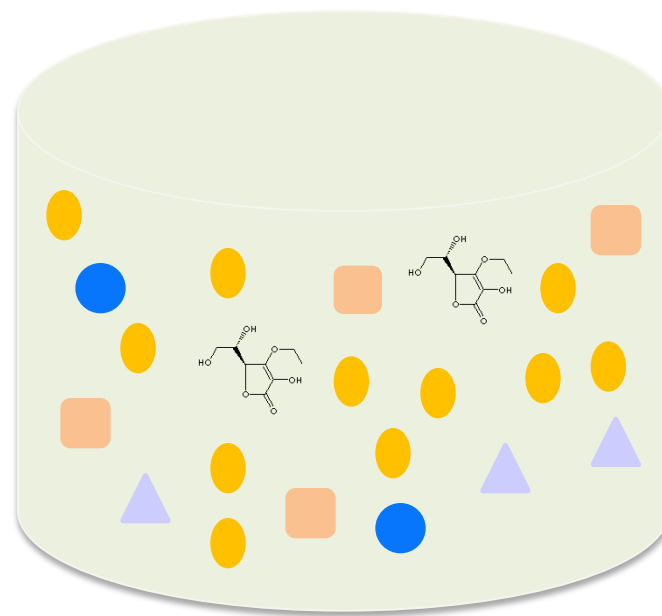
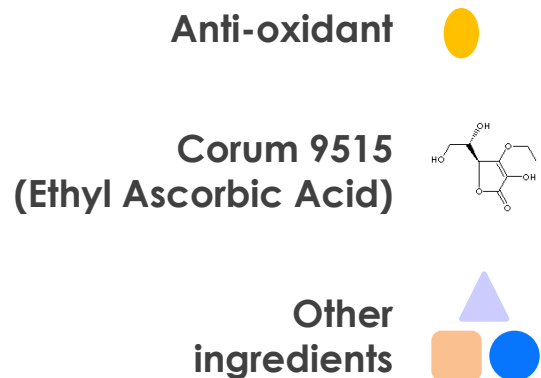
Stability of pH value in different buffer system  
(pH 6)



# Anti-oxidant lessen color change

Corum 9515 Ethyl Ascorbic Acid is a powerful anti-oxidant

Thus a **stronger Anti-oxidant** is recommended to be used in formulation → prevent losing Corum 9515's effect before it reaches its target area



# Anti-oxidant lessen color change

45°C for 6 months

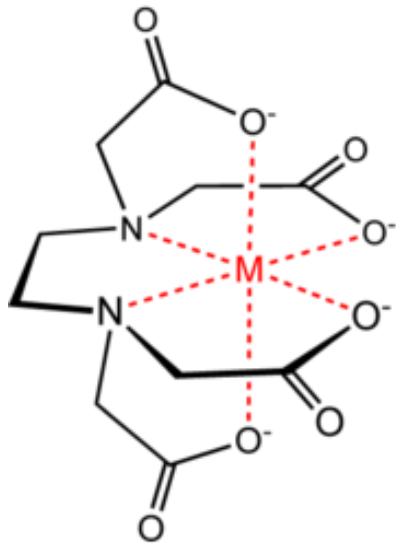


	Control			
C-9515	0%	2%	2%	2%
Sodium bisulfite	0%	0%	0.15%	0.2%

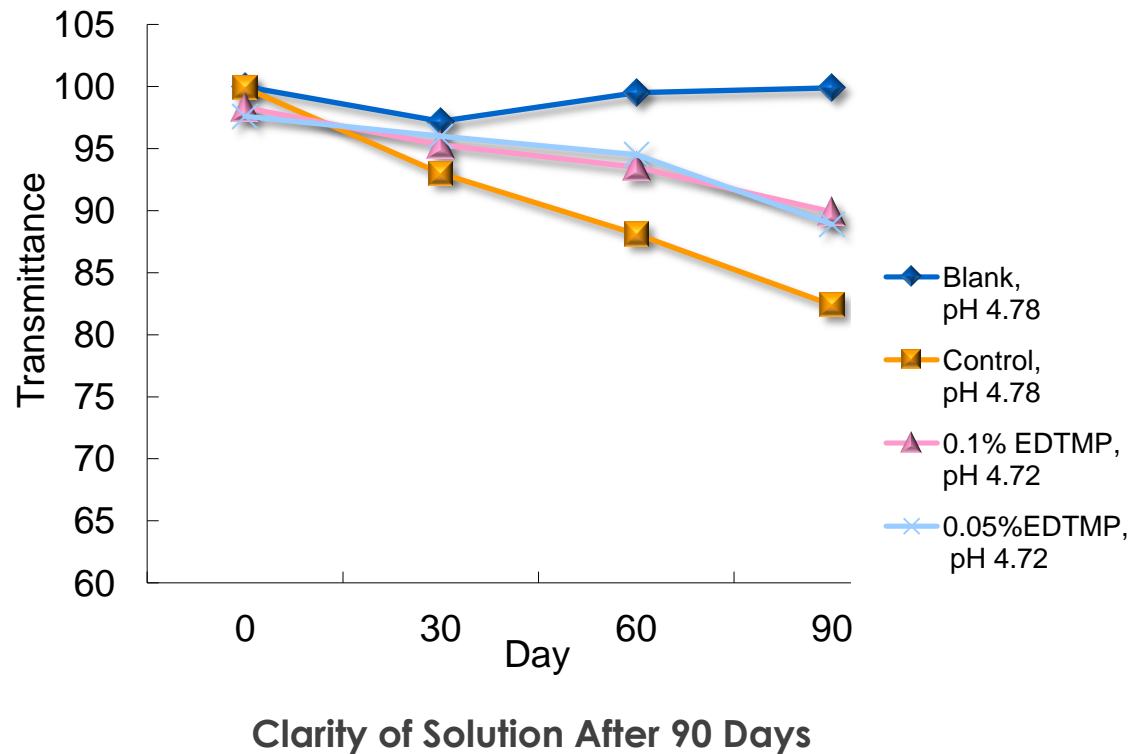
- Sodium bisulfite can lessen color change.
- But ! **Too much** sodium bisulfite will accelerate color change.

# Minimize Color Change - Chelating Agent

**Metal ions** promotes 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

## ■ 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.

## ■ 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.

## ■ 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
- Prevent photoaging
- Increase collagen synthesis
- Excellent anti-oxidation properties
- Scavenge radical
- DNA protection





**Thank you**  
for your attention!