NV ASCORBIC ACID

NANO VETORES® GROUP

Vitamin C

in its most active and stable form.

Active ingredient: Ascorbic Acid

Characteristics

Features

Aspect: White to cream milky liquid
Suggested usage concentration: 2% - 10% or at the formulator's discretion up to 60%
pH stability: Less than 4.0. Maximum efficiency with pH less than 3.0

Benefits

- Evens skin tone
- Antioxidant
- Stimulates collagen synthesis
- Improves skin texture and firmness
- Prevents skin aging
- Fights against expression lines
- Hydration

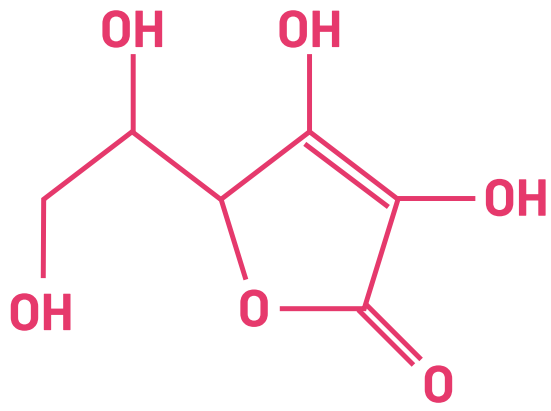
Usage

Emulsions in general with pH less than 4.0.

IN Harmony NANOVETORES

11.05.2021 - Rev.: 04

NV Ascorbic Acid has the active ingredient ascorbic acid encapsulated in polymeric particles with particle diameter larger than 200 nm. The technology developed by Nanovetores allows greater stability against heat and light as well as protection against oxidation of vitamin C in its most active form, ascorbic acid, ensuring benefits associated with its use, such as even skin tone, smoothing blemishes, improved hydration, texture and skin firmness, as well as helping to reduce expression lines. NV Ascorbic Acid can be used daily, providing, in continuous applications, its main benefits.



Chemical name: L - Ascorbic acid CAS: 50-81-7
Molar mass: 176,12 g/mol

Figure 1. Chemical information and molecular structure of ascorbic acid.

100%

of the study participants stated that they have perceived skin improvements in the following aspects:*

- **Firmness;**
- **Skin Rejuvenation;**
- **Texture (Smoothing);**
- **Hydration (Softening and Moisturizing);**
- **Skin Tone (Uniformity);**
- **Skin Strenght.**

94%

of the study participants perceived wrinkles reduction.

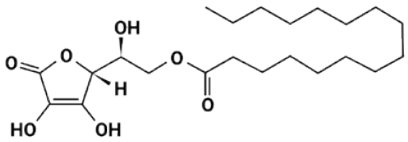
*TEST REPORT NVT226-19-F
*TEST REPORT NVT226-19-H

Description

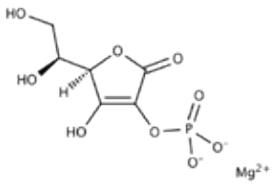
Vitamin C, or ascorbic acid, is a water-soluble and thermolabile vitamin widely used in cosmetic and skin care products, as it presents significant physiological effects on the skin, such as inhibition of melanogenesis, stimulation of collagen synthesis and antioxidant action, establishing a protective film and helping prevent skin aging. Thus, it acts through different action mechanisms, providing beneficial effects on aesthetic treatments designed to combat the signs of skin aging.[1,2]

Ascorbic acid is the most active form of vitamin C but the most unstable due to its difficulty for stabilization (Figure 1).[2,3]

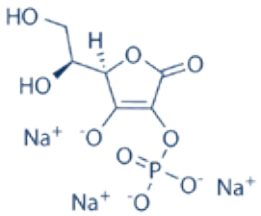
Several more stable derivatives were developed, and their structures are represented in Figure 2. Ascorbyl palmitate, for example, has only one branch and although it penetrates the skin, it is limited in its conversion to L-ascorbic acid, the active form of vitamin C. In turn, magnesium ascorbyl phosphate is not able to go through the stratum corneum. Sodium ascorbyl phosphate has two substitutions and ascorbyl tetraisopalmitate has four substitutions in its chemical structure. The greater the number of substituted radicals, the lower the vitamin C activity of ascorbic acid derivatives. So, in terms of effectiveness, ascorbic acid is the most effective form of vitamin C and ascorbyl tetraisopalmitate the least effective.[3,4]



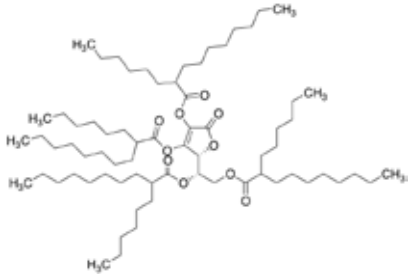
ASCORBYL PALMITATE
Chemical name: L-Ascorbyl 6-palmitate CAS: 137-66-6
Molar mass: 414,5 g /mol



MAGNESIUM ASCORBYL PHOSPHATE
Chemical name: L-Ascorbic acid 2-phosphate sesquimagnesium CAS: 113170-55-1
Molar mass: 289,5 g/mol



SODIUM ASCORBYL PHOSPHATE
Chemical name: Sodium L-Ascorbyl-2-Phosphate CAS: 66170-10-3
Molar mass: 322,05 g / mol



ASCORBYL TETRAISOPALMITATE
Chemical name: Ascorbyl tetra-2-hexyldecanoate CAS: 183476-82-6
Molar mass: 1129,8 g/mol

Figure 2. Information and chemical structures of the main ascorbic acid derivatives.

Table 1 shows some characteristics of the different forms of Vitamin C, as we can see there is an inversely proportional relation between the molar mass and the ascorbate percentage, that is, the lower the molar mass of the molecule, the greater the ascorbate percentage, thus, Ascorbic Acid is the most active molecule of Vitamin C. The level of ascorbate in ascorbic acid and its derivatives can be better visualized in Figure 3.

Table 1. Characteristics of different forms of Vitamin C.

Compound name	Molar mass	Ascorbate %	1g of the compound has X g of Ascorbate
Ascorbic Acid	176,12	100	1
Magnesium Ascorbyl Phosphate	289,5	60,8	0,608
Sodium Ascorbyl Phosphate	322,05	54,7	0,547
Ascorbyl Palmitate	414,5	42,5	0,425
Ascorbyl Tetraisopalmitate	1129,8	15,6	0,156

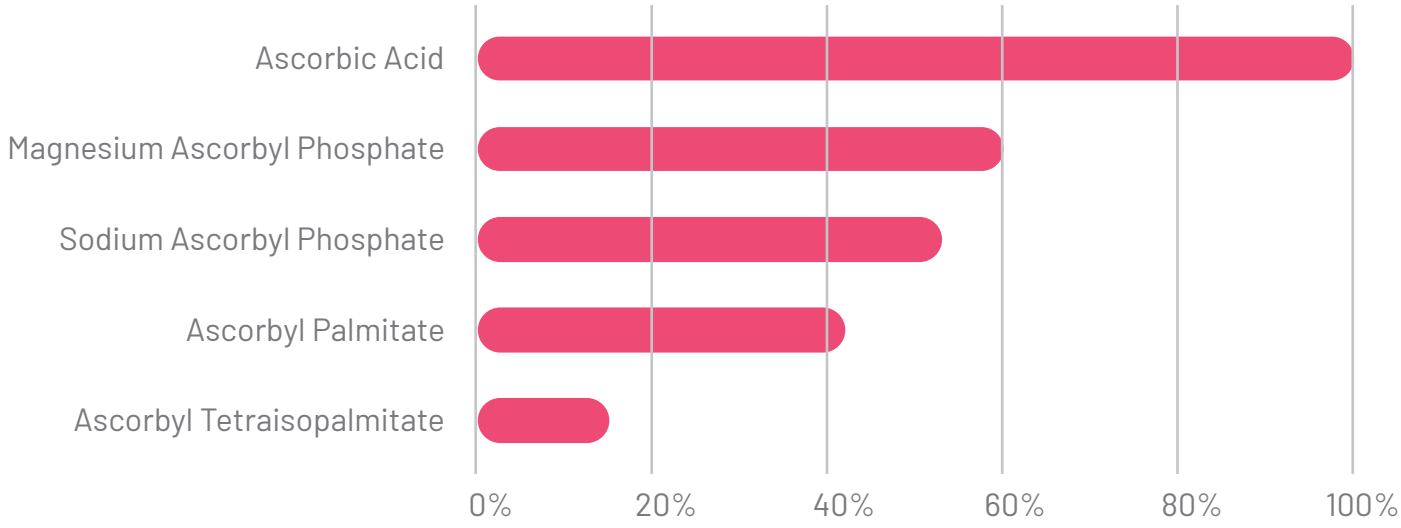


Figure 3. Ascorbate concentration among the different Vitamin C forms.

Mechanism of Action

Vitamin C has antioxidant, depigmenting, and photo rejuvenation activity, it also reduces the signs of photoaging, improves the epidermis texture, and fight wrinkles [5]. It acts througth different ways, providing beneficial effects on aesthetic treatments designed to combat the signs of skin aging.

Ascorbic acid acts on the synthesis of the supporting dibers (collagen), whin are essential to ensure skin firmness. By improving the synthesis, it delays on the most noticeable signs in skin aging**(Figure 4)**.

Vitamin C reduces the production of melanin pigmets via inhibition of the tyrosinase enzyme, which is the main regulator of the formation reactions of these pigments; therefore, it has been suggested as a depigmenting agent[6].

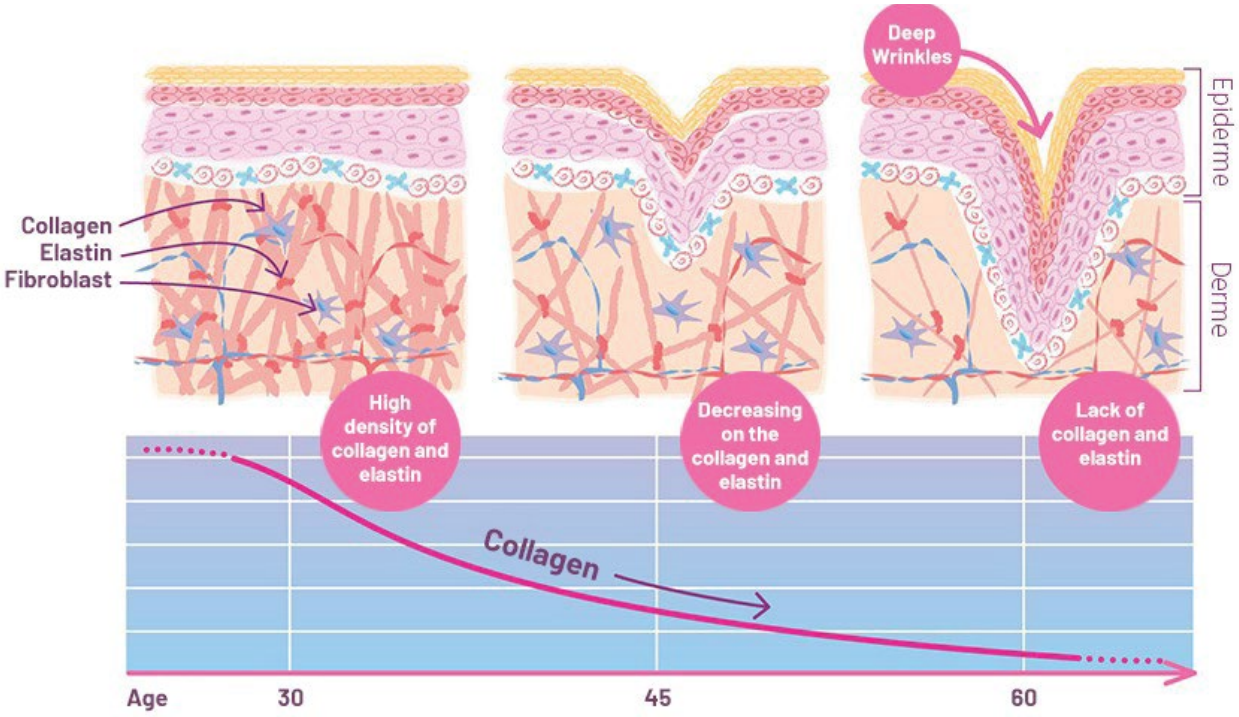


Figure 4: Observed damage during skin aging.

Vitamin C occurs naturally in two forms, the readuced form, L-ascrbic acid (AA) and the oxidized form also known as L-dehydroascorbic acid (ADA) [7]. Both L-ascorbic acid and L-dehydroascorbic acid are physiologically active, because in the ADA is easily reduced and retained as AA intracellular tissues.[8]

L-ascorbic acid is a white, odorless crystalline solid, highly polar, soluble in alcohol and aqueous solutions; however, it is insoluble in low-polarity solvents[9]. In its solid form, AA is stable to exporsure to air, light and temperature for a long period, whereas in aqueous solutions its stability is intrinsically related to the storage conditions and composition of the formulation[7]. AA is easily oxidezed reversibly to ADA. The biological activity of Vitamin C is lost when ADA is hydrolyzed, which results in an irreversible process, where the carbon ring is opened to form 2,3 - diketogulonic acid (ADG) and from there the formation of other inactive products that provide the solution a brownish color[9,10].

Figure 5 represents the oxidation of L-ascorbic acid and the subsequent hydrolysis of dehydroascorbic acid to 2,3 - diketogulonic acid[8].

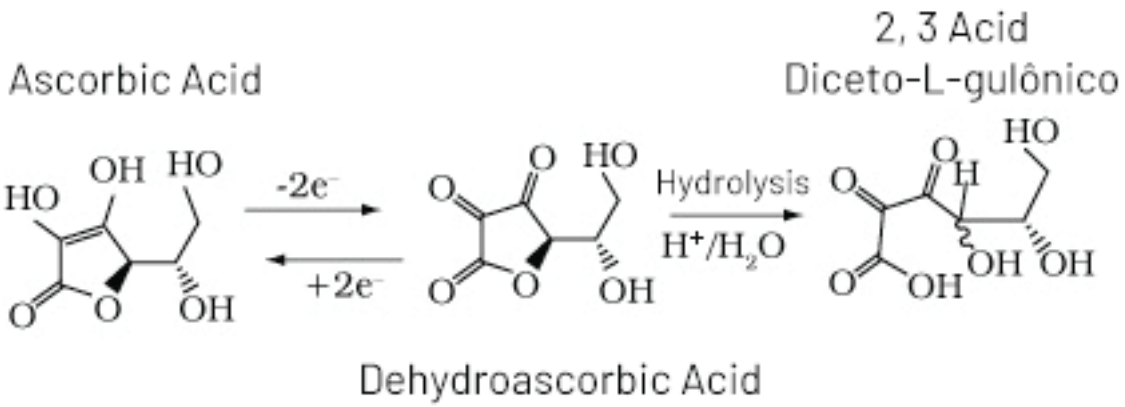


Figure 5: Process of oxidation of ascorbic acid to dehydroascorbic acid and its hydrolysis to 2,3-diketogulonic acid.

L-dehydroascorbic acid has 80% of the bioactivity of L-ascorbic acid[11]. ADA in solution is colorless and extremely labila, being rapidly hydrolyzed to 2,3-diketogulonic acid. Once the lactone ring in the ADA breaks, the reaction beacomes irreversible and the new formed compund, ADG, has no biological activity [8].

The bownish color, characteristic of aqueous solutions containing vitamin C, is due to the degradation of dehydroascorbic acid, since this acid rapidly hydrolyzes into aqueous solutions forming 2,3-diketogulonic acid. The decomposition of ADG gives rise to different brown-colored pigments, which in turn add color to the vitamin c solutions[10].

Therefore, the brown tone observed in the free active ingredient after 38 days of stability indicates the occurrence of hydrolysis and the loss of effectiveness of ascorbic acid. It is concluded that the use of Nanovetores encapsulation technology provides greater stability and permeation, providing the development of effective products based on ascorbic acid.

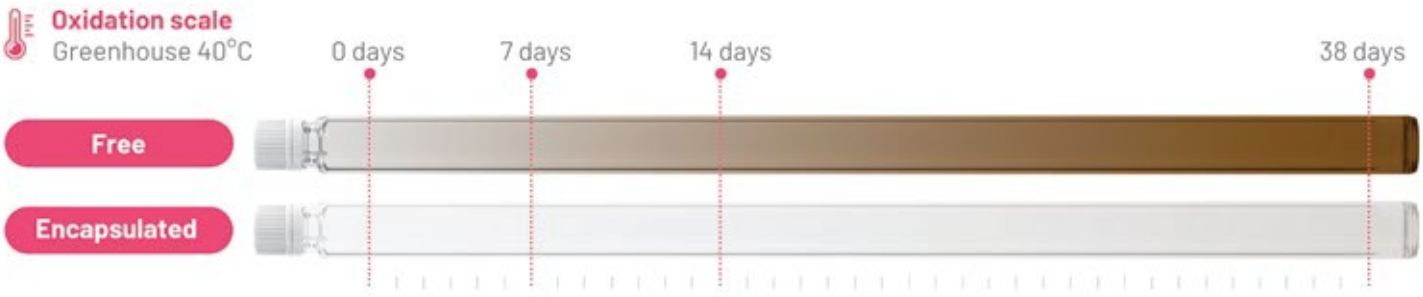


Figure 6: Brownish pigment formation indicating the ascorbic acid degradation.

Physical-Chemical and Organoleptic Analysis

In a 90-day stability test, free encapsulated ascorbic acid active ingredient samples were stored under dark conditions (room temperature at ± 25ºC), light and greenhouse at 40ºC. The encapsulated active ingredient showed change in color after 60 days at 40°C, while the free active ingredient started to change after 12 days at the same temperature.

When exposed to sunlight, the encapsulated active ingredient showed no yellowing, while the free active ingredient began to change color with 24 days of stability.

In the dark, the encapsulated active ingredient did not change its color until the end of stability, while the free active ingredient began to yellow with 38 days of stability.

It is possible to conclude that in the encapsulated form there was a greater preservation of its properties, significantly reducing oxidation, while the free active in 38 days at 40°C has a brownish color due to the decomposition of the DKG, which results in an irreversible process that nullifies the activity of ascorbic acid, as shown in

Figure 7. The active ingredient ascorbic acid in its encapsulated and free form after 38 days under dark (room temperature), light conditions and greenhouse at 40"C.

Chemical Stability Assay

Theoretical Information

Feedstock: NV Ascorbic Acid

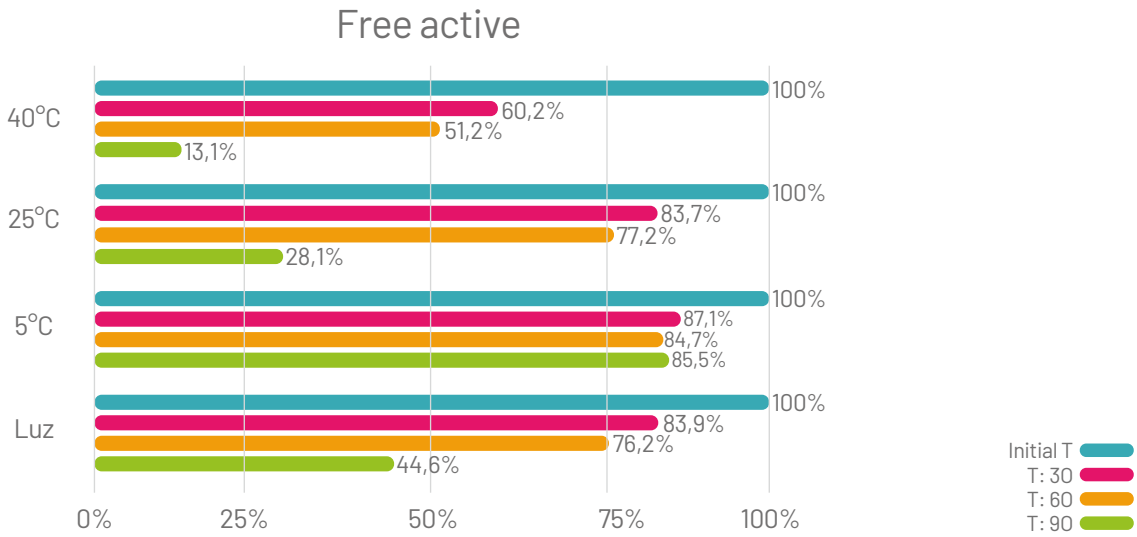
Analyzed Active: Ascorbic acid

Analysis conditions: 5°C, 25°C, 40°C and Sunlight

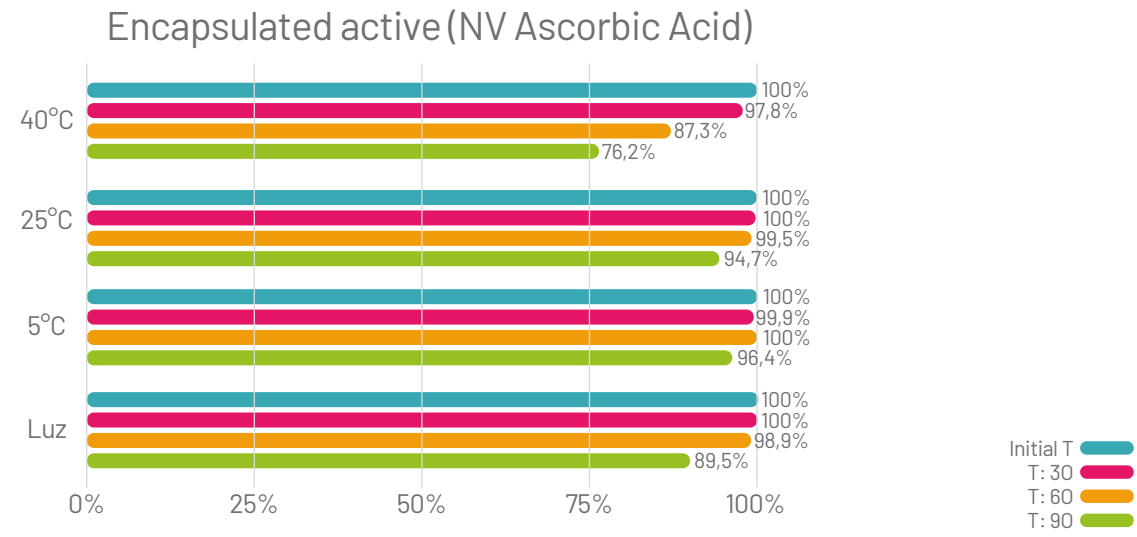
Technique: HPLC (High Performance Liquid Chromatography)

Results

Variation of the active concentration in the feedstock considering the initial time (Initial T) as 100%.(Graph 1 and 2)



Graph 1: Analysis of ascorbic acid content by HPLC, in sample of free ascorbic acid exposed to the conditions of 5°C, 25°C, 40°C and sun light, in times 0 day,30 days, 60 days and 90 days.



Graph 2: Analysis of ascorbic acid content by HPLC, in sample of NV Ascorbic Acid exposed to the conditions of 5°C, 25°C, 40°C and sun light, in times 0 day,30 days, 60 days and 90 days.

Conclusion

The comparative graphs show the results of the ascorbic acid content between the samples of free and encapsulated actives in the period of 90 days and exposed in different conditions (Sunlight, 5°C, 25°C and 40°C). Through the HPLC tests and after comparative analysis between the results in the time of 90 days at 40°C, it was verified that the encapsulated active had 76.2% of ascorbic acid content compared to 13.1% of the free active, therefore the results prove the 500% increase in stability, preserving all the properties and benefits of ascorbic acid in the long term.

NV Ascorbic Acid Permeation Test

Applied Technique: UV-VIS Spectrometry.

Methodology

The methods were used to check skin absorption/ permeation, and dermal distribution of molecules and/or products can be divided into two categories: in vivo and in vitro. In vitro methods using skin are well established and provide essential pharmacokinetic information. In this context, we can highlight the OECD Test Guideline 428 that describes experimental parameters of the Franz diffusion cell model in human skin.

To analyzing the samples received, it was decided to use the UV-VIS spectrometry technique. This technique has broad application in the food, pharmaceutical and scientific research industry. The molecular absorption spectrophotometry technique in UV-VIS is widely used in the analysis of ascorbic acid and its derivatives. Initially, a wavelength scan of 190 to 800 nm of a solution containing ascorbic acid was performed to verify the wavelength of greatest absorbance by the analyte.

After the wavelength of greatest absorption was selected, a calibration curve was built with solutions containing ascorbic acid in concentrations of 0.2; 0.5; 0.1; 1.5 and 2.0 ppm.

Finally, the samples containing ascorbic acid with unknown concentration had their absorbance measured. After ading all the solutions and samples, data were analyzed. (Figure 8)

Permeation increase of 22% of the encapsulated activein relation to the free active

Concentration of Ascorbic Acid permeated in the skin after 8 hours of application of the encapsulated and free active ingredients.

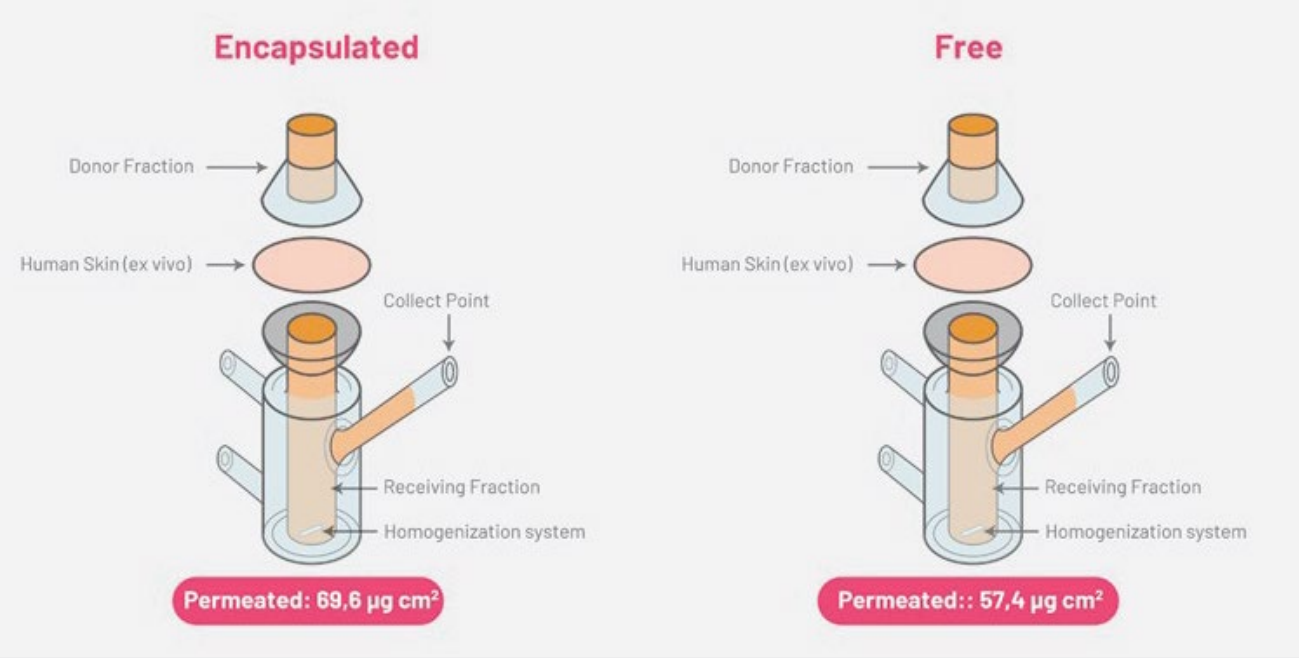


Figure 8: Schematic Drawing of Permeation in Franz Cell.

in vitro comparative study of the antioxidant action potential

Samples: CREME COM NV ASCORBIC ACID 10% and BENCHMARK Product with 10% pure acid ascorbic declared content; Positive control: 10% of Trolox in PBS; Control group: cell culture medium.

Objective: Evaluate and compare the samples potencial in reducing free radicals production using cell cultures of human fibroblast cells from the dermis after 24h.

Methodology: In this study, it was used human fibroblasts cells from the dermis, maintained in cultivation with DMEM (Dulbecco's Modified Eagle's Medium) with the addition of supplements, in a greenhouse at 37C° and 5C° of CO^2 for 24h. The presence of free radicals was analyzed using the CM-H2DCFDA probe with excitation wavelength of 495nm and emission of 525nm .

The actives oxidation potential can be determined by the reduction of the quantity of free radicals, reactive oxygen species, produced by the cells. The samples were evaluated during the 24h period and the results obtained after the application are compared to the control group results (baseline cell state of free radicals production). The TROLOX reagent (positive control) indicates the success in the used methodology, as it already has standardized results.

As it can be observed in **Graph 3**, the CREME COM NV ASCORBIC ACID 10%, presented reduction of 64,2% (±1,18) in free radicals production while the BENCHMARK presented reduction of only 35,9% (±4,52). That is, the CREME COM NV ASCORBIC ACID 10% presented 1,8x more potent results When compared to the Benchmark. The superior results of CREME COM NV ASCORBIC ACID 10% could be showcased, even though the product has a much lower ascorbic acid content than the one declared by the Benchmark. These results indicate that the encapsulation system of NV ASCORBIC ACID makes possible an effective active delivery in its action target, with greater ascorbic acid protection, which makes it more potent in the combat of free radicals.

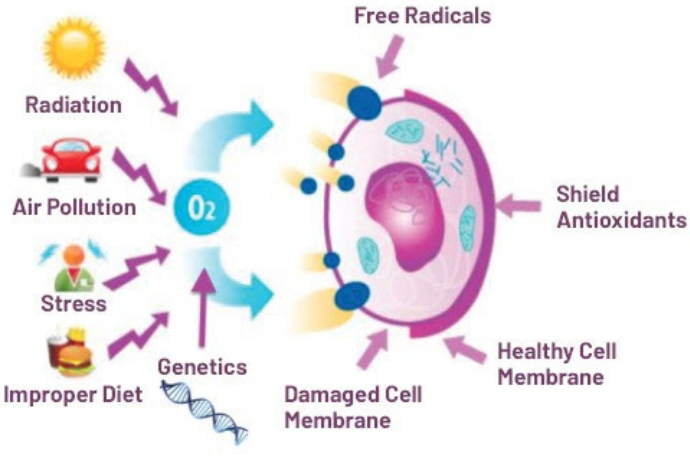
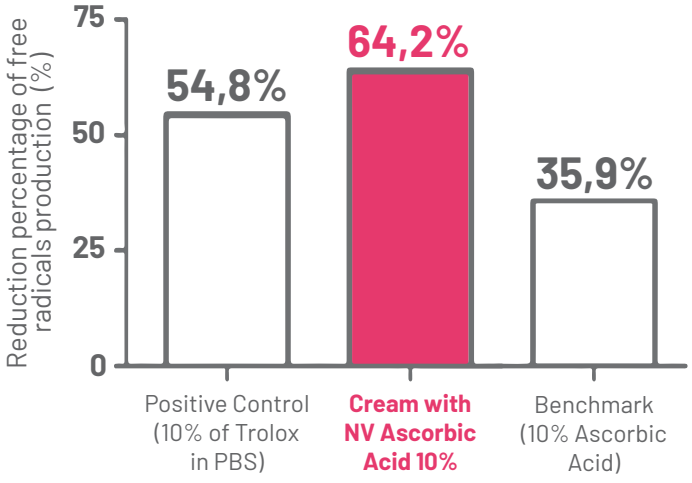


Figure 9: Stress factors related to free radicals.



Graph 3: Reduction percentage of free radicals production after 24 hours compared to the control group.

Effectiveness Test

Assessment of product effectiveness in the reduction of wrinkles and expression lines as well as uniformization of skin tone through subjective analysis by perceived effectiveness.

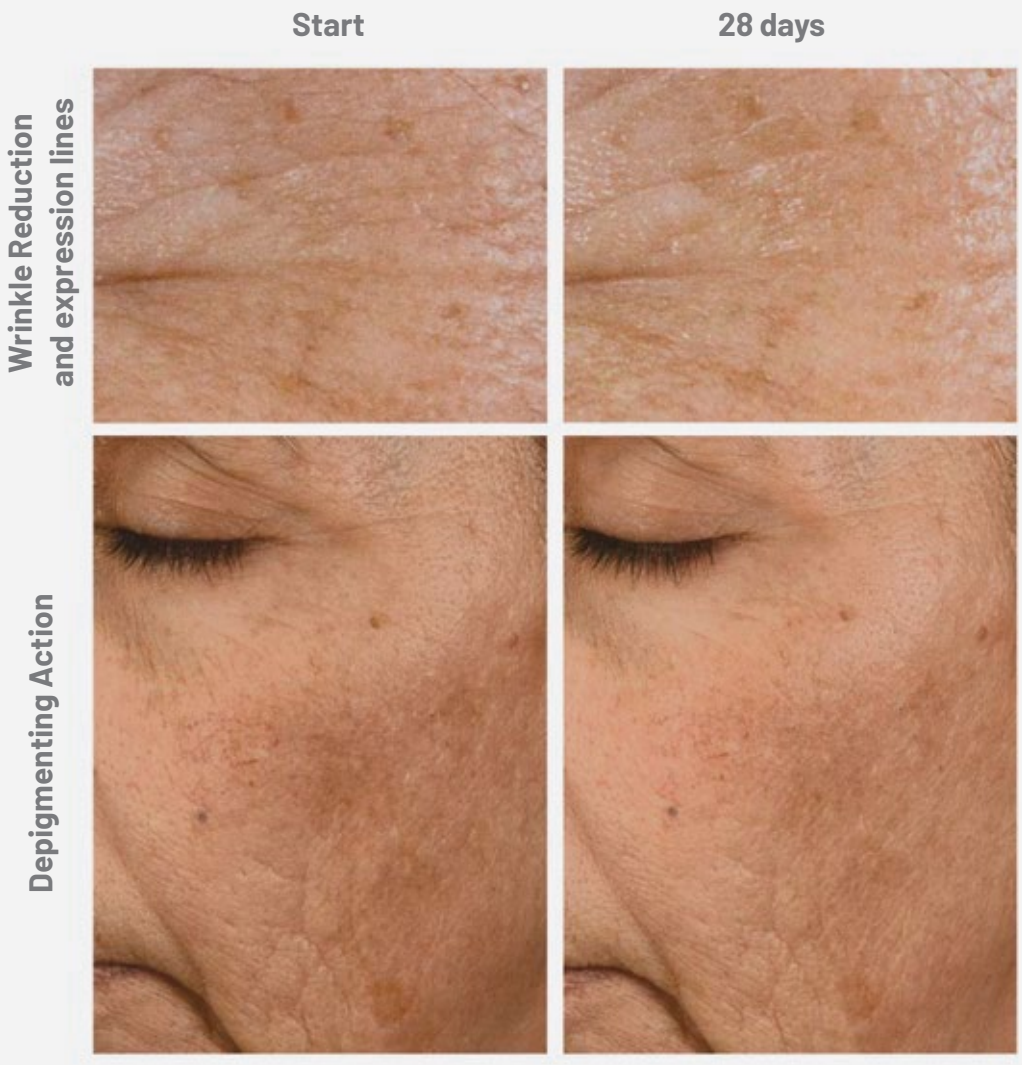
Evaluated product: Cream with NV Ascorbic Acid 10%

Objectives: Assess product effectiveness in providing reduction of wrinkles and expression lines as well as even skin tone through subjective analysis by perceived effectiveness.

Number of volunteers: 18 participants

Metodology: For the performance of the instrumental tests, the methodology consisted of the evaluation of wrinkles and facial expression lines reduction and uniformization of skin tone through objective image analysis. Digital photographic images of the face were obtained at the beginning of the study, after 7 and 28 days of home use of the product.

Directions for use: Apply the product evenly on clean and dry skin twice a day by spreading a small amount of the product lightly on the face until completely absorbed.



Graph 10: Results of the evaluation of wrinkles and expression lines reduction in the facial skin and skin tone standardization.

Results:

After 7 days of home use:

- **67%** of the study participants noticed a reduction in wrinkles and expression lines.
- **78%** of the study participants noticed improvement in skin firmness.
- **78%** of the study participants noticed skin rejuvenation, giving it a younger look.
- **78%** of the study participants noticed improvement in skin hydration, leaving it smoother and more hydrated.
- **72%** of the study participants noticed improvement in skin tone, leaving it more even.
- **78%** of the study participants noticed improvement in the skin freshness, leaving it brighter and revitalized.

After 28 days of home use:

- **94%** of the study participants noticed a reduction in wrinkles and expression lines.
- **100%** of the study participants noticed improvement in skin firmness.
- **100%** of the study participants noticed skin rejuvenation, giving it a younger look.
- **100%** of the study participants noticed improvement in skin hydration, leaving it smoother and more hydrated.
- **100%** of the study participants noticed improvement in skin tone, leaving it more even.
- **100%** of the study participants noticed improvement in the skin freshness, leaving it brighter and revitalized.

Overall Conclusion

Thus, it is concluded that the encapsulation process performed by Nanovetores is able to protect the active ingredient against oxidation resulting from interaction with external environment and other components of the cosmetic formula, providing NV Ascorbic Acid greater permeation power and delivery of its clinical benefits.

Marketing Appeal

- Reduces wrikles and expression lines;
- Stimulates collagen synthesis:
- Prevents skin aging;
- Assists in skin firmness;
- Improves skin elasticity;
- Promote hydratation;
- Dermatologically tested.

Our production process is based on Green Chemistry, being water-based and free of organic solvents, totally sustainable. We do not generate waste that could be harmful to users or the environment

We do not test on animals. All tests are conducted in trustworthy laboratories with human volunteers.

Essential oils, Vitamins, Acids and Natural Extracts are highly oxidative substances that degrade quickly and react constantly with the medium and other cosmetic compounds (light, oxygen, packaging, preservatives, fragrances, surfactants, etc.). By encapsulating it, we guarantee the stability of the active ingredients and protect them from potential reactions with the formulation or the environment.

References

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Regulatory Information

INCI Name	Cas Number	EINECS Number
Aqua	7732-18-5	231-791-2
Peg-12 Dimethicone	68937-54-2	-
Dimethicone	63148-62-9	-
Propylene Glycol	57-55-6	200-338-0
Ascorbic Acid	50-81-7	200-066-2
Sodium Metabisulfite	7681-57-4	231-673-0
Sodium Chloride	7647-14-5	231-598-3

Physical-chemical Information

Aspect	Milk
Color	White to cream
Odor	Characteristic
pH	1,5 - 3,5
Dispersibility	Dispersion of encapsulated actives in water
Relative Density	0,9 a 1,1 g/mL
Characterization	Blend

Usage Method:

Add to the formulation under 40 Celcius with moderate mixing.

Storage:

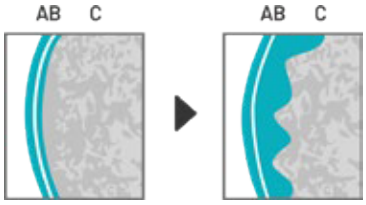
Keep in dry place, protected from light, with temperature between 20-25 C. Agitate before using as there can be separation characteristic of suspensions.

Incompatibility

Ethanol. The activity of the NV Ascorbic Acid is related with its pH, that we recommend to be lower than 4,0.

Nanovetores Encapsulation Technology

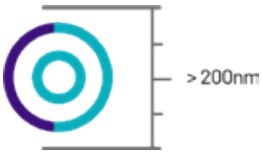
Active Ingredient Protection against oxidation resulted from interaction with external environment and other components of the cosmetic formulation.



Greater Permeation on the contact surface due to the small size of the capsule.

Monodispersity, that ensures control of the particle size, providing adequate permeation to its proposed action.

Surface Charge Control of the particle, promoting greater affinity with the contact surface.



Secure particles larger than 200nm, biocompatible and biodegradable.

Water Base. Active ingredients are manufactured without the use of organic solvents, ensuring safety for users and the environment.

Use Encapsulated Active Ingredients and Ensure:

- Stability Improvement
- Increased compability in the formulation
- Occlusion of odors
- Increased skin permeation
- Reduced dose
- Use of sensitive active ingredients (without refrigeration)
- Increased Solubility
- Prolonged release
- Increased effectiveness

NANO VETORES® GROUP

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