NANO VETORES® GROUP

NV CYSTEAMINE 15

FACIAL

Active Ingredient: Cysteamine HCL

Benefits

- Antioxidant;
- Illuminates the skin;
- Standardizes skin tone;
- Hyperpigmentation and Melasma treatment;

Application

Primers, emulsions (creams and lotions, masks, seruns, gels, facial gel-cream and products to standardize skin tone.

IN Harmony NANOVETORES

10.03.2021 - Rev.: 01

DESCRIPTION

Nanovetores innovative encapsulation system enables the intelligent and safe delivery of Cysteamine while also ensures greater cosmetics efficacy and stability for the treatment of hyperpigmentation and Melasma. Cysteamine is very challenging ingredient to work with, our encapsulated version grants the following benefits:

•Cysteamine characteristic odor reduction;
•Active ingredient stabilization;
•Improved active permeation and diffusion through the skin layers;
•Controlled release;
•Improved illuminating efficacy;
•Reduction of the active's irritating effects on the skin;
•Better treatment adherence;
•Acts in the barrier function maintenance preventing transpidermal water loss.

Cysteamine

Cysteamine (CSH) is a reducing aminothiol naturally formed in the body from the conversion of coenzyme A to panthetine. Considered one of the most potent intracellular antioxidants, Cysteamine cell redox homeostasis[1,2]. Cysteamine skin lightening effect is attributed to its antioxidant properties, with direct action in the stratum corneum[3,4]. With mechanism of action that involves its capacity to reduce melanin production, by inhibition of essential melanogenic enzymes , tyrosinase and peroxidase, also has a chelating effect on the copper ions needed in melanogenesis[5]. Besides the antioxidant properties, the free thiol group of Cysteamine can interact with the disulfide bonds of peptides and proteins, and interfere in their functions. This way, cysteamine can act in several signaling pathways involved in proliferation, survival and influence on the expression of several redox-sensitive cellular genes[6].

Despite its innumerous and noticeable biological applications, cysteamine commercial potential is limited due to its unpleasant odor (attributed to the sulfur present in the molecule). Besides this, hygroscopicity, poor pharmacokinetic profile and the fact that cysteamine is highly susceptible to degradation due to its rapid oxidation by air[7] difficult its application. With the goal to overcome these challenges, Nanovetores encapsulation technology is capable to reduce the active's odor, enhance its stability and make the incorporation in cosmetic products easier. This improvement in the organoleptic characteristics makes the use of Cysteamine more pleasant, reducing skin irritation and allowing its continuous use, with superior results in the treatment of hyperpigmentation and Melasma.

APPLICATION

NV Cysteamine 15 application in pigmentation disorder treatment

The skin has epidermal elements responsible for the production and distribution of melanin through the melanogenesis process. Hyper pigmentary skin disorders, such as Melasma, may result from epidermical diseases, melanocyte hyperactivity, which in turn can cause the increase of melanin production and accumulation. Although Melasma etiology is not well understood, some risk factors, such as sunlight exposure, cosmetics, hormonal therapies, birth control pills, photosensitizing agents, genetic susceptibility and anticonvulsant drugs represent the disorder triggers. Melasma can be categorized into four histological types based on the depth of pigment deposition, such as: epidermal, dermal, mixed and indeterminate.

Many strategies have been adopted in Melasma treatment, some based in mechanical removal of the pigment, like chemical peelings, pulsed light, different types of lasers, as well as dermabrasion and micro needling. Besides, photoprotective measures, such as avoiding direct sunlight exposure and the regular application of broad-spectrum sunscreens are constantly recommended. Currently, many topical lightening actives have been introduced and widely applied for hyperpigmentation treatment. With different mechanisms of action, the efficacy of these topical agents can be limited to its absorption in the stratum corneum, limiting its activity to more superficial skin regions. In addition, the use necessity of high active concentrations may lead to the increase of cellular toxicity and skin irritation[8].

As an alternative to invasive methods of treating hyperpigmentation, and increase in efficacy and comfort of using Cysteamine, Nanovetores Technology was applied in the development of nanoparticles with Cysteamine, the NV Cysteamine 15. This technology promotes an improvement in the permeation and diffusion of the active through the skin layers and its controlled release has a positive impact in its lightening efficiency. NV Cystemaine permeates the skin in a protected way till the dermis-epidermis junction when it is then released gradually by the enzymatic trigger action. Once released, cysteamine interacts with melanocyte and inhibits essential melanogenic enzymes, reducing melanin production, besides generating a powerful antioxidant action, producing the skin lightening effect (Figure 1).

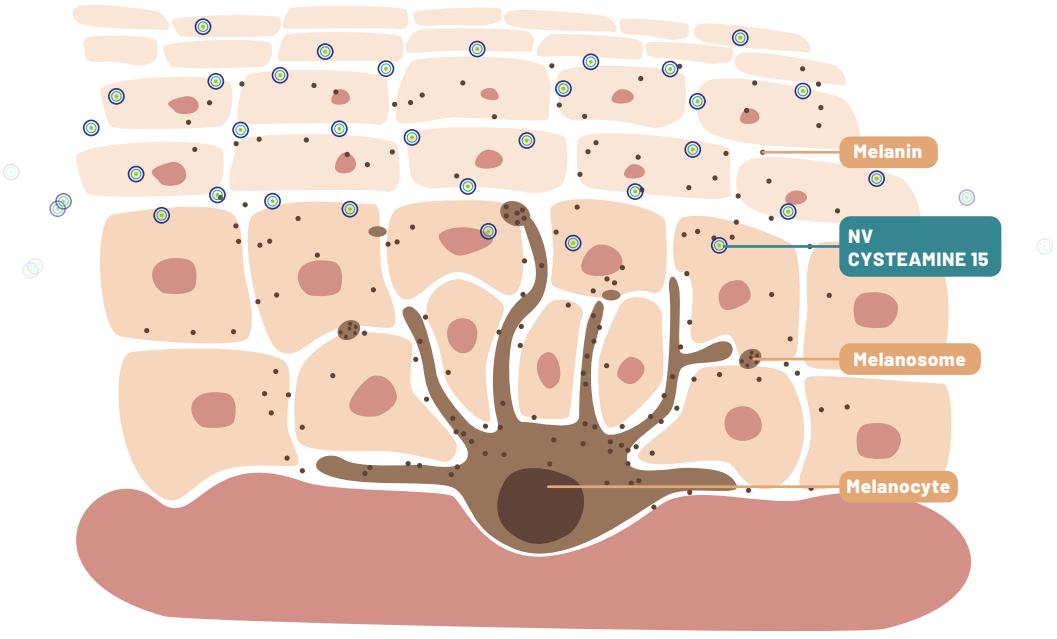


Figure 1: Schematic representation of NV Cysteamine action on the treatment of skin pigmentation disorders.

In vitro comparative evaluation between free cysteamine and NV Cysteamine 15 in melanin production

Samples: Free Cysteamine and NV Cysteamine 15.
Objective: Evaluate the product's illuminating potential through its capacity to control melanin granules production by melanocytes after exposure to the samples.
Metodologia: Evaluation and quantification of melanin granules production after the exposure of samples in melanocyte cell culture, kept in culture with DMEM (Dulbecco's Modified Eagle's Medium), stained with Fontana-Masson.

Actives with the power to control melanin production and accumulation are essential to the treatment of skin hyperpigmentation disorders, such as Melasma. NV Cysteamine 15 has a high capacity to reduce melanin granules, which is associated with the skin illuminating potential. As can be observed in Figure 2, where the melanocyte cell culture shown a lighter coloring, which indicates the lower melanin granulocyte concentration compared to Free Cysteamine and the control (Kojic Acid, 0,1%).

The studies demonstrated the superior depigmenting efficacy of NV Cysteamine 15, with the capacity to reduce 74,4% of melanin granules, compared to the reduction of 23,0% with the positive control group (kojic acid, 0,1%) and 57,2% Free Cysteamine. NV Cysteamine 15 contains in its formula 15% of Cysteamine HCL, therefore, the encapsulated active contains 6,6 times less active content in comparison with the non-encapsulated pure form, and even so presented 30% greater lightening effectiveness (Graph 1). These exceptional results can be attributed to the NV Cysteamine 15 nanoparticles permeation, which delivers the active stable in the action area, with less irritating potential, enabling the development of versatile cosmetics, secure, and with high lightening performance.

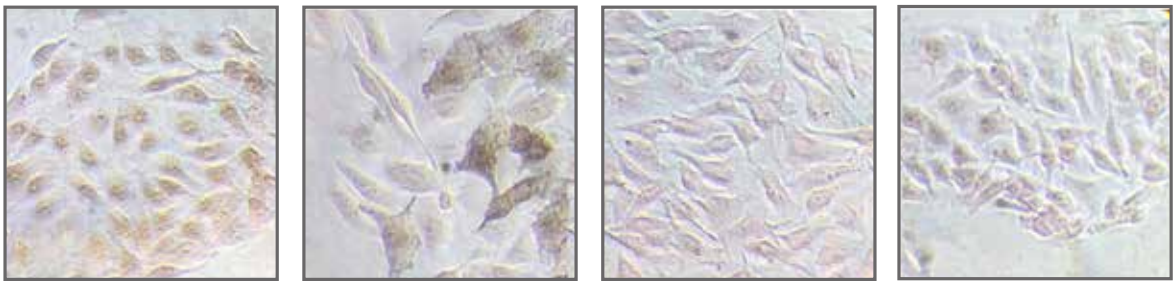
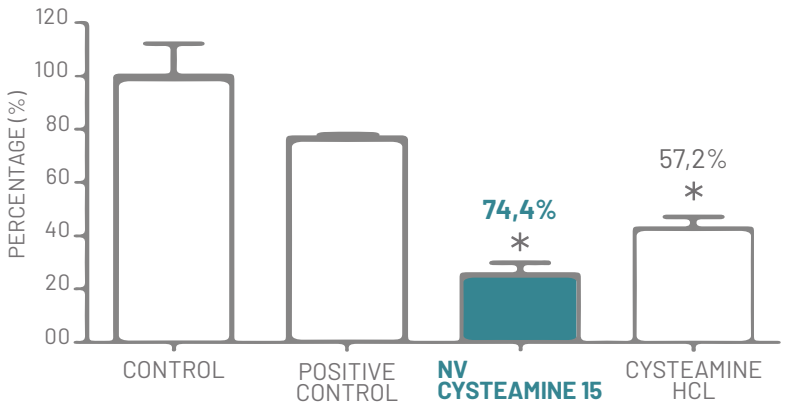


Figure 2: Representative images of melanin granules inside the cells after exposure to NV Cysteamine 15; Cysteamine HCL, and stained with Fontana – Masson.



Graph 1: Quantitative percentage of melanin pigment produced when exposed to NV Cysteamine 15; Cysteamine HCL, study observed results with the control group normalized at 100% (*p<0,01).

Melanin production comparison

The objective of this study is to evaluate the production of melanin granules by melanocytes after exposure to hydroquinone, **NV Cysteamine 15**, and positive control (kojic acid).

To evaluate the whitening potential, the samples were tested in order to obtain their cytotoxicity levels. Hydroquinone showed cytotoxicity at concentrations greater than 0.001 mg/mL, while **NV Cysteamine 15** and kojic acid at concentrations greater than 1 mg/mL and 0.1 mg/mL, respectively. Therefore, the analyzes were performed at these non-cytotoxic concentrations.

For analyzing the effectiveness of the samples, the control group (cell culture medium) was normalized to 100%. The positive control reduced 17.93% (±0.91) of melanin granules. The Hydroquinone sample showed a reduction of 46.8% (±0.69) while **NV Cysteamine 15** had a reduction of 71.12% (±0.69) when compared to the control group, that is, **NV Cysteamine 15** showed 1.5X more potent results when compared to Hydroquinone. These results can be seen in **Chart 2**.

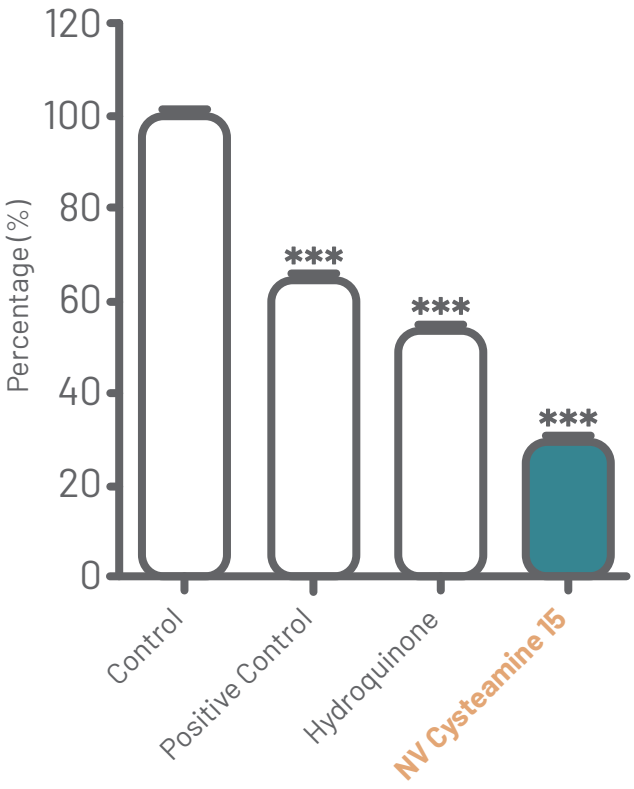


Chart 2: Quantitative chart of the results observed in the study, with the control group normalized to 100% for the respective non-cytotoxic concentrations of the evaluated samples (***p<0.001).

Additionally, the samples were subjected to staining of the treated cells with the respective non-cytotoxic concentrations. Fontana-Masson staining highlights melanin granules within the cell. As shown in **Figure 3**, it is possible to observe that there was a significant reduction of melanin granules when comparing the sample of **NV Cysteamine 15** to the control group, in turn, it is noted that **NV Cysteamine 15** has a potential whitening effect when compared to Hydroquinone.

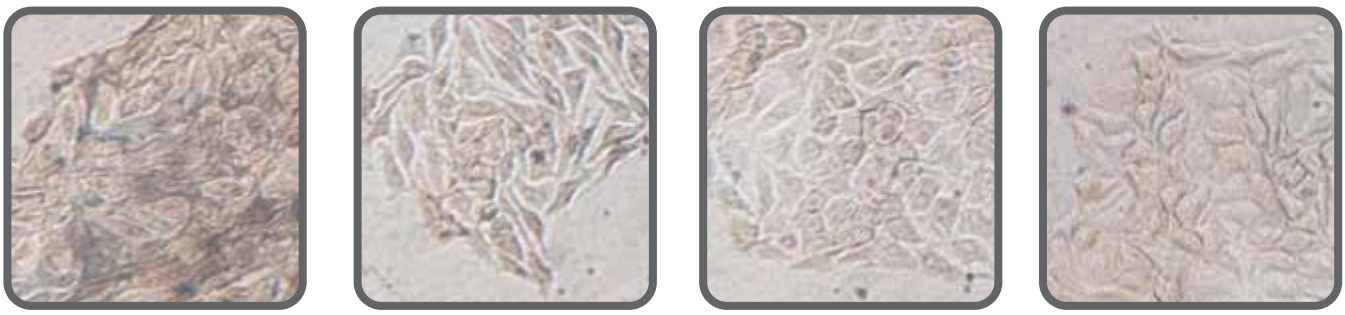


Figure 3: Representative images of the indicated groups with Fontana-Masson staining to highlight the melanin granules.

IN VITRO STUDY

With the results obtained, it can be stated that the **NV Cysteamine 15** sample presented better results compared to the hydroquinone sample, in the reduction of melanin granules, which is associated with the skin whitening potential. It is worth mentioning that the **NV Cysteamine 15** sample is presented in this study in its encapsulated form (15% of the active ingredient in the sample) while the hydroquinone sample is in its free form (100%), that is, **NV Cysteamine 15** is almost 7X more effective in reducing melanin granules compared to hydroquinone. In addition, hydroquinone was 1000X more cytotoxic than **NV Cysteamine 15**.

7X MORE EFFECTIVE IN REDUCING MELANIN GRANULES COMPARED TO HYDROQUINONE

1000X LESS CYTOTOXIC COMPARED TO HYDROQUINONE

Inflammatory markers

For the related study, the relative expression of markers related to the inflammatory process was compared among the control, positive control (sodium lauryl sulfate) and **NV Cysteamine 15** and Hydroquinone sample groups, at the same concentration assessed of 0.01 mg/ml. The objective of the study was to evaluate the interleukin-6 (**IL-6**), interleukin-8 (**IL-8**) markers and tumor necrosis factor alpha (**TNF-α**) using human keratinocyte cell culture.

IL-6 analysis

Chart 3 shows the analysis of IL-6 expression. It is possible to observe that the positive control increased 2.2X (±0.22) in relation to the control. The hydroquinone sample showed a 4.24X (±0.32) increase in IL-6 expression while the **NV Cysteamine 15** sample showed a 1.21X (±0.13) reduction in expression, that is, hydroquinone had an inflammatory potential 3.5X greater than **NV Cysteamine 15**.

3,5X LOWER INFLAMMATORY POTENTIAL THAN HYDROQUINONE

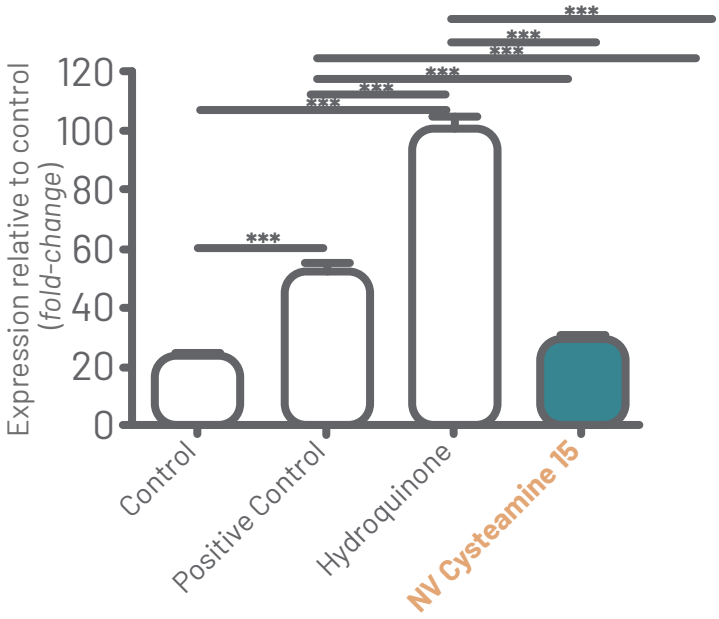


Chart 3: Result of the analysis of relative IL-6 expression among groups. Horizontal bars connect groups to demonstrate level of statistical difference (***p<0.001).

TESTE *IN VITRO*

IL-8 analysis

For IL-8 analysis, it was observed that the positive control showed an increase of 1.63X (±0.32) in relation to the control. The hydroquinone sample showed a 4.91X (±0.48) increase in IL-8 expression and the **NV Cysteamine 15** sample showed a 1.5X (±0.62) increase in expression. Therefore, hydroquinone was 3.3X more inflammatory than **NV Cysteamine 15**. The results are shown in **Chart 4**.

TNF-α analysis

For TNF-α analysis, it was possible to verify that the positive control showed an increase of 2.26X (±0.35) in relation to the control. The hydroquinone sample showed a level of 0.99X (±0.08) in the TNF-α expression related to the control group and the **NV Cysteamine 15** sample showed a 0.67X (±0.06) reduction in expression, that is, hydroquinone was 1.5X more inflammatory than **NV Cysteamine 15**. The results are shown in **Chart 5**.

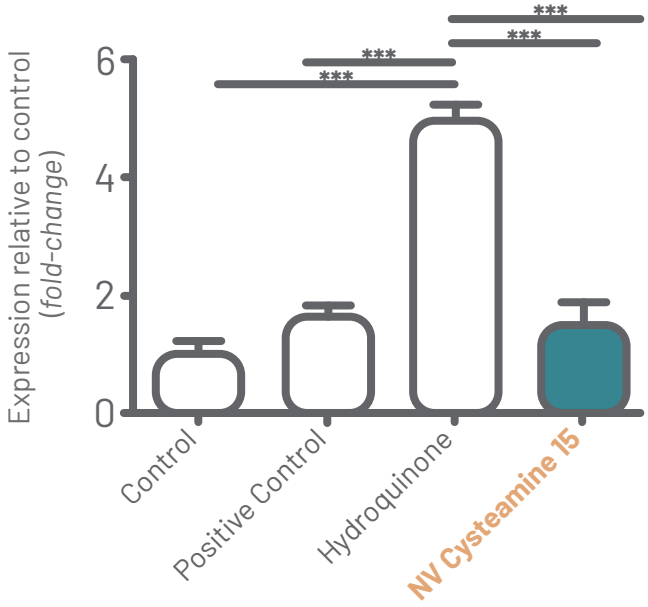


Chart 4: Result of the analysis of relative IL-8 expression among groups. Horizontal bars connect groups to demonstrate level of statistical difference (***p<0.001).

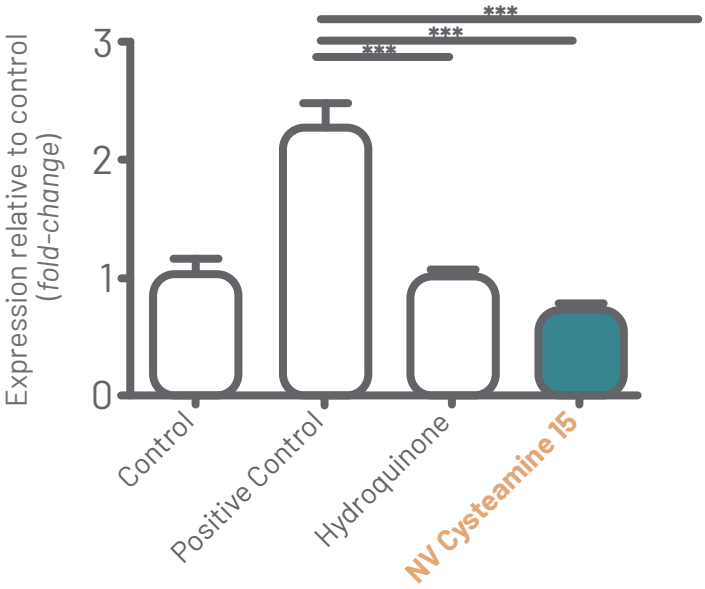


Chart 5: Result of the analysis of the relative TNF-α expression among groups. Horizontal bars connect groups to demonstrate level of statistical difference (***p<0.001).

With these results, it is possible to state that hydroquinone had a very high inflammatory potential compared to **NV Cysteamine 15**. Hydroquinone also has adverse effects in the regions where the product is applied, causing exogenous ochronosis (asymptomatic bluish--black or gray-brown hyperpigmentation), irritation, dermatitis, occupational vitiligo depigmentation, among others[9].

Thus, **NV Cysteamine 15** is the best option for the treatment of blemishes and melasma, since it has the benefit of whitening potential and simultaneously low inflammatory potential for the skin compared to other lighteners on the market.

NV CYSTEAMINE 15

Regullatory Information

INCI Name	Cas Number	EINCS Number
AQUA	7732-18-5	231-791-2
CYSTEAMINE HCL	156-57-0	205-858-1
CYCLODEXTRIN	7585-39-9	231-493-2
SODIUM BENZOATE	532-32-1	208-534-8
POTASSIUM SORBATE	24634-61-5	246-376-1
ALGIN	9005-38-3	-
CALCIUM CITRATE	813-94-5	212-391-7
DISODIUM EDTA	139-33-3	205-358-3

Physical-chemical data

Aspect	Transparent liquid
Color	Incolor to yellow
Odor	Characteristic
Dispersibility	Dispersion of encapsulated actives in water
Density	0,9 to 1,1 g/mL
pH	4,0 to 6,0

Usage Mode
Add to the formulation bellow 40°C under mild to moderate agitation.

Usage Concentration
2 to 10%.

Stability pH
4,0 to 6,0.

Storage
Keep in a well-ventilated place, away from light and heat. Shake before use.

Compatibilities
Anionic product, compatible with non-ionic and anionic bases.

Incompatibility
Incompatible with organic solvents.

Observations
The product is a suspension of nanoparticles, agitate before formula incorporation. For fluid formulations, the use of suspending agent is recommended.

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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2. Costa, F. V. et al. **Resposta terapêutica da cisteamina no tratamento do Melasma**. Research, Society and Development, 9, (6):759, 2020.

3. Grimes, P.E. et al. **New oral and topical approaches for the treatment of melasma.** International Journal of Women's Dermatology 5, 30–36, 2019.

4. Nguyen, J. et al. **Evaluation of the efficacy of cysteamine cream compared to hydroquinone in the treatment of melasma: A randomised, double-blinded trial**. Australas J Dermatol. 2020

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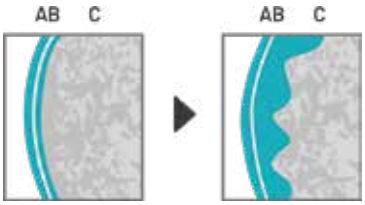
7. Hatem, S. et al. Background and different treatment modalities for melasma: Conventional and nanotechnology-based approaches. Journal of Drug Delivery Science and Technology 60, 101984, 2020.

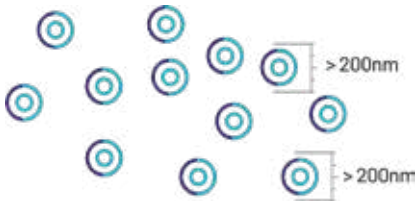
8. Atallah, C. et al. Challenges for cysteamine stabilization, quantification, and biological effects improvement. Journal of Pharmaceutical Analysis, 10, 6, 499-516, 2020.

Nanovetores Encapsulation Technology

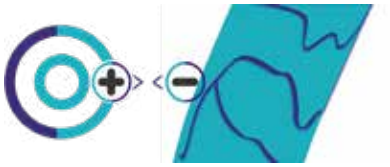
Active Ingredient Protection against oxidation resulted from interaction with external environment and other components of the cosmetic formulation.



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

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