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PREPARATION, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF NANOEMULSIONS CONTAINING ASCORBYL PALMITTE[1]

PREPARAÇÃO, CARACTERIZAÇÃO E ATIVIDADE ANTIOXIDANTE DE NANOEMULSÕES CONTENDO PALMITATO DE ASCORBILA

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ABSTRACT

Ascorbyl palmitate (or L-ascorbyl-6-palmitate), which is a more stable fat-soluble derivative of vitamin C, may be an agent for incorporation into topical formulations. Its main advantage is the vitamin C-type antioxidant capacity, which may be compromised due to the physicochemical instability of this active agent against photodegradation. Nanotechnology is an alternative found to improve stability and to prevent degradation of ascorbyl palmitate. Thus, the objective of this work was to develop, characterize and evaluate the antioxidant activity of ascorbyl palmitate-containing nanoemulsions. The nanoemulsions were prepared by the high and low energy method, and the results of the physicochemical characterization were 104 nm in diameter for the ascorbyl palmitate containing nanoemulsions prepared by the high energy method (T - NE - MP) and 168 nm for nanoemulsions prepared by the low energy method (R-NE-MP), a polydispersity index of 0.18 (T - NE - MP) and 0.312 (R-NE-MP), zeta potential of -18.88 mV (T - NE - MP) and -22.16 mV (R-NE-MP), pH 3.81 (T - NE - MP) and 3.93 (R-NE-MP) and morphology showed globular droplets. The content result was 100 % ± 1.42 for R-NE-MP. The antioxidant capacity of ascorbyl palmitate-containing nanoemulsions obtained by the low energy method was superior to the two associated free actives and was equivalent to palmitate activity free. The results demonstrate the viability of encapsulation of ascorbyl palmitate. The nanoemulsions prepared by the low energy method can be a strategy to obtain stable and effective cosmetic formulations.

Keywords: cosmetology, nanotechnology, ocimum basilicum, trolox, 2-diphenyl-1-picrylhydraza.

RESUMO

O palmitato de ascorbil (ou L-ascorbil-6-palmitato) é um derivado solúvel em gordura e mais estável da vitamina C, pode ser um agente para incorporação em formulações tópicas. Sua principal vantagem é a capacidade antioxidante tipo vitamina C, que pode ser comprometida devido à instabilidade físico-química desse agente ativo contra a fotodegradação. A nanotecnologia é uma alternativa encontrada para melhorar a estabilidade e impedir a degradação do palmitato de ascorbil. Assim, o objetivo deste trabalho foi desenvolver, caracterizar e avaliar a atividade antioxidante de nanoemulsões contendo palmitato de ascorbil. As nanoemulsões foram preparadas pelo método de alta energia e baixa energia, e os resultados da caracterização físico-química foram de 104 nm de diâmetro para as nanoemulsões contendo palmitato de ascorbila preparadas pelo método de alta energia (T - NE - MP) e 168 nm para as nanoemulsões preparadas pelo método de baixa energia (R-NE-MP), um índice de polidispersão e de 0,18 (R-NE-MP) e 0,312 (R-NE-MP), potencial zeta de -18,88 mV (T - NE - MP) e -22,16 mV (R-NE-MP), pH 3,81 (T - NE - MP) e 3,93 (R-NE-MP) e a morfologia mostraram gotículas globulares. O resultado de teor foi de 100 % ± 1.42 para R-NE-MP. A atividade antioxidante de

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nanoemulsões contendo palmitato de ascorbil, obtida pelo método de baixa energia, foi superior aos dois ativos livres associados e foi equivalente à atividade do palmitato livre. Esses resultados demonstram a viabilidade do encapsulamento do palmitato de ascorbil, sendo que as nanoemulsões preparadas pelo método de baixa energia podem ser uma estratégia para obter formulações cosméticas estáveis e eficazes.

Palavras-chave: *cosmetologia, nanotecnologia, ocimum basilicum, trolox, 2-difenil-1-picril-hidraza.*

INTRODUCTION

The concern for personal image has gained notoriety in recent years, which places Brazil in the fourth position in the world ranking of hygiene product, perfumery and cosmetic consumption. This sector has been highlighted by the fact that personal image can interfere in the daily life of people, and influence the coexistence in society, personal safety and even in the job market (ABIHPEC, 2017). Thus, the introduction of new assets in marketed products that reach consumer satisfaction has been promising in this still growing sector (MAGDASSI, 1997; SCHMALTZ; SANTOS;GUTERRES, 2005).

Ascorbyl palmitate (or L-Ascorbil-6-palmitate) is a liposoluble and more stable derivative of vitamin C. It appears as an agent for incorporation into topical formulations (Kristl *et al.,* 2003). Among the advantages of this derivative is that it presents an antioxidant activity very similar to vitamin C, it penetrates the skin more easily, and it allows greater protection of the cutaneous components against the action of free radicals. However, this compound has a low aqueous solubility and a low physicochemical stability (PANEVA *et al.,* 2011).

In this context, an alternative to circumvent the limitations of low solubility and stability of ascorbyl palmitate may be the use of nanotechnology. Nanotechnology has been employed in the cosmetics area for some years, including products available in the market, as it is considered an alternative to increase stability and allow a controlled and targeted release of active substances (FRONZA *et al.,* 2007).

In view of the studies carried out so far and the problems related to skin aging, the association of ascorbyl palmitate with nanoemulsion systems allows an improvement in its stabilization and may subsequently result in an effective beneficial formulation for topical application. Combined with the benefits of ascorbyl palmitate, it is estimated that the association of basil essential oil in these systems is a promising agent in promoting antibacterial action, due to the properties presented by the oil. The use of basil essential oil associated with ascorbyl palmitate in a nanoemulsion system is an innovation since no related studies are currently available in literature.

The aim of this study was to investigate the development, physicochemical characterization, and determination of the antioxidant activity of nanoemulsions containing ascorbyl palmitate to exploit its potential use as a cosmetic product. The development of nanoemulsions was carried out from two methods of preparation, one of high energy and another of low energy. The characteristics analyzed for

nanoemulsions containing ascorbyl palmitate were size, polydispersion index, zeta potential, pH and morphology. The antioxidant activity was determined by the DPPH method.

MATERIAL AND METHODS

Crodamol GTCC was obtained from Alpha Química LTDA, Sorbitan monooleate (Span 80®), basil essential oil and ascorbyl palmitate were acquired from Sigma-Aldrich, and polysorbate 80 (Twen 80®) and acetone P.A. from Synth.

PREPARATION OF NANOEMULSIONS

The nanoemulsions were prepared by two methods, being the first a high energy method (T-NE-MP) and the second low energy method (R-NE-MP). The quali-quantitative composition of the formulations is described in Table 1. As an oily component was used the basil essential oil due to already having antioxidant, antibacterial and antifungal activity described in the literature (GHOSH *et al.,* 2013), or in the case of blank nanoemulsions (R-NE-BL and T-NE-BL), the basil essential oil was replaced by medium chain triglycerides (Crodamol) and the presence of ascorbyl palmitate was omitted.

Table 1 - Qualitative and quantitative composition of nanoemulsions prepared by both methods. Final volume of formulations prepared by the high energy method = 40 mL; Final volume of formulations prepared by the low energy method = 25mL.

Components	T-NE-MP	T-NE-BL	R-NE-MP	R-NE-BL
Aqueous phase (AF)				
Polyssorbate 80	2%	2%	0.7%	0.7 %
Ultra-pure water	40 mL	40 mL	134 mL	134 mL
Oily Phase (OF)				
Essential oil	7.5 %	---	3.3 %	---
Crodamol GTCC	---	5 %	---	3.3 %
Ascorbyl Palmitate	0.03%	---	0.1%	---
Sorbitan Monooleate	2%	2%	0.7%	0.7%
Acetone	---	---	67 mL	67 mL

T-NE-MP - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by Ultraturrax. T-NE-BL - Blank nanoemulsions prepared by Ultraturrax. R-NE-MP - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by rotary evaporator. R-NE-BL - Blank nanoemulsions prepared by rotary evaporator.

In the preparation of the T-NE-MP the constituents of the aqueous phase (AF), polysorbate 80 and ultra-pure water were added in 10 mL beakers and homogenized with the aid of a magnetic stirrer at room temperature for 10 minutes. The oily phase (OF) containing sorbitan monooleate, basil essential oil and ascorbyl palmitate were placed in 10 mL beakers and homogenized for 10 minutes at room temperature. After the homogenization of both phases, AF was taken to the shaker (Ultraturrax) and kept under stirring at 10,000 rpm for 10 minutes. So, the OF was then injected into the AF using

a syringe, and the formulation was kept under stirring at 17,000 rpm for 30 minutes in an ice bath to prevent formulation heating (GODOI *et al.*, 2017).

The R-NE-MP, prepared by the low energy method, occurred as follows: initially the OF and the AF were weighed in beakers separately, homogenized for 10 minutes. Then the OF was poured into the AF and homogenized for 10 minutes, after which the formulation was evaporated from the organic solvent in a rotary evaporator, rotating at 80-90 rpm at 35 ° C for the time required for evaporation. total organic solvent of the formulation and final volume adjustment (Ourique *et al.*, 2008).

PHYSICOCHEMICAL CHARACTERIZATION OF NANOEMULSIONS

The formulations were characterized according to the following parameters: diameter, polydispersion index (PDI) and zeta potential using the equipment Zetasizer® nano-ZS model ZEN 3600 (Malvern Instruments). For the determination of zeta potential, the samples were diluted in solution of Na Cl 10 mM (500 times, V/V) and for the other analyzes in ultra-pure water (500 times, V/V). The pH of the dispersions was determined by potentiometer (Digimed®). The results were expressed as the average of three replications.

For the study of morphology and analysis of ascorbyl palmitate content, only the formulation that presented the best results in the antioxidant activity was chosen. The morphology was performed by Atomic Force Microscopy (AFM) using Agilent Technologies 5500 equipment. The samples were diluted with ultra pure water (1:10), then a drop was added under cleaved mica until dry. Some images were obtained at room temperature using the non-contact mode with high resolution SSS-NCL tips (nanosensors, constant force 48 N / m and resonant frequency 154 kHz). The results were obtained using PicoViwe 1.14.4 software (Molecular Imaging Corporation) and analyzed using PicoImage 5.

Ascorbyl Palmitate content was obtained by HPLC according to SILVA (2016) with modifications from the co-validation of the analytical method (BRAZIL, 2017). A Prominence High Performance Liquid Chromatograph with Lichrospher® 100 RP-18 Reverse Phase Stationary Phase Lichrospher® 100 RP-18 Pre-Column was used. The chromatographic conditions were as follows: mobile phase flow rate of 0.6 mL/min; wavelength 243 nm; injection volume of 20 µL and the mobile phase composed of acetonitrile: sodium phosphate buffer pH 2.5: methanol (85: 10: 5 v/v). For sample preparation the nanoemulsions were subjected to an extraction process, initially 150 µL of the formulation was added to a 10 mL volumetric flask and the volume was completed with the mobile phase, which corresponds to the midpoint of the analytical curve (15 µg mL^{-1}). Then, the volumetric flask containing the solution was taken to the ultrasound bath for 25 minutes. Following the procedure described, the sample was filtered using a 0.20 µm cellulose membrane prior to the HPLC analysis.

EVALUATION OF ANTIOXIDANT ACTIVITY BY DPPH METHOD

The determination of the antioxidant activity by the DPPH method was performed as described by CUVELIER; BERSET, (1995) with some modifications. For the evaluation of the antioxidant activity of the nanoemulsions, three controls were prepared for each method of preparation. For the high energy method, it was prepared: a) free associated assets = basil oil (7.5%) Ascorbyl palmitate (0.03%) polysorbate 80 (2%) 10 mL ultra-pure water, b) free basil oil = basil oil (7.5%) polysorbate 80 (2%) 10 mL of ultra-pure water and c) free ascorbyl palmitate = ascorbyl palmitate (0.03%) polysorbate 80 (2%) 10 mL ultra-pure water. For the low energy method: a) free associated assets = basil oil (3.3%) ascorbyl palmitate (0.1%) 10 mL acetone, b) free basil oil = basil oil (3.3%) 10 mL acetone and c) free ascorbyl palmitate = ascorbyl palmitate (0.1%) 10 mL acetone.

STATISTICAL ANALYSIS

Data were subjected to analysis of variance (ANOVA) and the difference between the samples was evaluated by the Tukey test at 95% confidence, $p < 0.05$. The program used was ASSISTAT, version 7.7.

RESULTS AND DISCUSSIONS

FORMULATION OPTIMIZATION STEP

Nanoemulsions containing essential oil of basil and ascorbyl palmitate prepared by the high agitation method in the Ultraturrax (T-NE-MP) were initially tested with different concentrations of ascorbyl palmitate, which were: 1%, 0.5%, 0.25%, 0.12%, 0.06%, 0.03%. The oily phases containing different concentrations of ascorbyl palmitate and surfactant were taken for agitation with the aid of a magnetic stirrer at room temperature for 10 minutes. After they were submitted to the ultrasound bath for another 10 minutes. Only the phase containing 0.03% of ascorbyl palmitate was totally solubilized. According to Spice, Gasperlin & Kmetec (2001) the recommended range for use of ascorbyl palmitate in cosmetic products is between 0.25% and 1%. However, despite the small amount solubilized, it was decided to follow up with this method, since it does not employ organic solvents and had temperature control throughout the process.

However, in order to increase the concentration of ascorbyl palmitate in the formulations, another method of nanoemulsions preparation was tested, a method of low energy, spontaneous emulsification, which is based on the evaporation of organic solvents. This method of preparation allowed the use of a higher concentration of ascorbyl palmitate, since acetone allows its solubilization. The ascorbyl palmitate concentration was 0.1%, still below that suggested by Spice, Gasperlin & Kmetec (2001), but it

increased considerably when compared to the amount used by the other method. Thus, from the choice of ascorbyl palmitate concentrations (0.03% for the high energy method and 0.1% for the low energy method), the nanoemulsions were prepared in triplicate by the two methods of preparation.

PHYSICOCHEMICAL CHARACTERIZATION OF FORMULATIONS

After preparation, all formulations were characterized according to diameter (nm), polydispersion index, zeta potential (mV) and pH, the results are described in Table 2.

Table 2 - Physicochemical characterization of the formulations.

	Average diameter (nm)	Polydispersion index	Zeta potential (mV)	pH
T-NE-MP	104 ± 5.06^{b}	0.18 ± 0.01^{b}	-18.88 ± 0.80^{ab}	3.81 ± 0.12^{a}
T-NE-BL	129 ± 0.59^{b}	0.24 ± 0.01^{ab}	- 08.06± 0.75^{b}	5.61 ± 0.84^{a}
R-NE-MP	168 ± 5.64^{b}	0.31 ± 0.01^{a}	-22.16 ± 0.41^{a}	3.93 ± 0.08^{a}
R-NE-BL	242 ± 5.66^{a}	0.34 ± 0.01^{ab}	-21.98 ± 0.67^{a}	5.51 ± 0.19^{a}

T-NE-MP - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by Ultraturrax. T-NE-BL- Blank nanoemulsions prepared by Ultraturrax. R-NE-MP - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by rotary evaporator. R-NE-BL - Blank nanoemulsions prepared by rotary evaporator. Values were expressed as average ± standard deviation. The numbers followed by equal letters in the same column did not show statistical difference between them, by Tukey test ($p < 0.05$). Means followed by the same letter do not differ significantly ($p = 0.05$).

The results of the physicochemical analyses of the nanoemulsions containing ascorbyl palmitate showed a diameter of 104 ± 5.06 nm for the T-NE-MP and 168 ± 5.64 nm for the R-NE-MP. Leong *et al.,* (2009) studied the optimization of the production of nanoemulsions by the high energy method in order to develop nanoemulsions with a diameter smaller than 100 nm, and the results showed that it was possible to obtain nanoemulsions with a diameter of 40 nm. The authors showed that during the preparation of the formulations the concentration of oil and surfactants used may be correlated with the diameter of the nanoemulsions produced by the high energy method. BOUCHEMAL *et al.,* (2004) carried out a study of the preparation of nanoemulsions by the method of spontaneous emulsification (low energy) in order to optimize a stable formulation. In this study, the authors obtained a diameter of 170 ± 2 nm corroborating with the result obtained for the nanoemulsions containing ascorbyl palmitate. KELMANN *et al.,* (2007) developed Carbamazepine nanoemulsions also by the method of spontaneous emulsification and the results of this study showed a size of the nanoemulsions with a range of 150-212 nm.

The polydispersion index values of the formulations showed the following results, 0.18 ± 0.01 for the T-NE-MP and 0.31 ± 0.01 for the R-NE-MP. The determination of the zeta potential of formulations (ʒ) is important to predict and control the stability of colloidal suspensions, this parameter measures the total load that a particle acquires in a specific medium (GOUVÊA; MURAD, 2001). In our study, the formulations presented a zeta potential of -18.88 ± 0.80 mV (T-NE-MP) and -22.16 ± 0.41 mV (R-NE-MP).

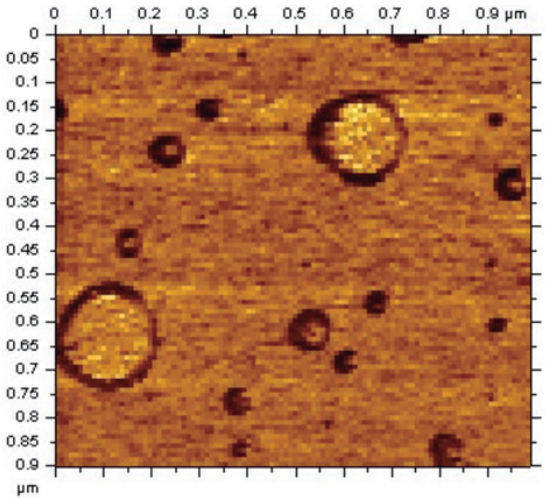
According to Kong and Park (2011), zeta potential values between 25 and 30 mV in module avoid the coalescence of dispersed droplets through the formation of a repulsion barrier. The authors show that the highest values of zeta potential in modulus result in a stable dispersion for a longer period. According to Tadros *et al.,* (2015), the integrity of the particles can be ensured through the steric hindrance, that is, by the presence of a component in the interface that ensures greater rigidity and stability of these particles. In the case of our formulation the steric hindrance is correlated with the polysorbate 80, which is an external surface coating material, and ensures the mechanism of stabilization of particles by steric hindrance (Jager, *et al.,* 2009).

The higher the zeta potential value, the greater the electrostatic repulsion between nanoparticles, thus decreasing the likelihood of aggregation (TAMJIDI *et al.,* 2014). One possibility for the low zeta potential observed in blank nanoemulsions produced by the high energy method is that the zeta potential is directly related to electrostatic repulsion between the nearby scattered globules, which is a parameter that indicates the behavior of the nanoemulsions interface composition, either in relation to surfactants or in relation to the presence or absence of drugs or other molecules associated with interface (SCHAFFAZICK, 2003). Regarding the fact that only the white formulation prepared by the high energy method presented a lower zeta potential value, it may be associated with the difference between the concentrations of surfactants and oil between the formulations.

The pH of the formulations T-NE-MP and R-NE-MP were in a range of 3.81 ± 0.12 and 3.93 ± 0.08, that is, they demonstrated an acidic pH due to the characteristics of ascorbyl palmitate which is a combination of an ascorbic acid attached to a fatty acid. According to Leonardi *et al.,* (2002) The pH values are determinant for the suitability of a formulation for topical route, and these values should be slightly acidic.

The results of the morphology of the nanoemulsions containing ascorbyl palmitate prepared by the low energy method performed by atomic force microscopy showed globular droplets and the diameter is in accordance with the results obtained by the technique of dynamic light scattering. The photomicrograph obtained by electron microscopy of atomic force can be visualized in Figure 1.

Figure 1 - Atomic force microscopy of nanoemulsions containing ascorbyl palmitate.

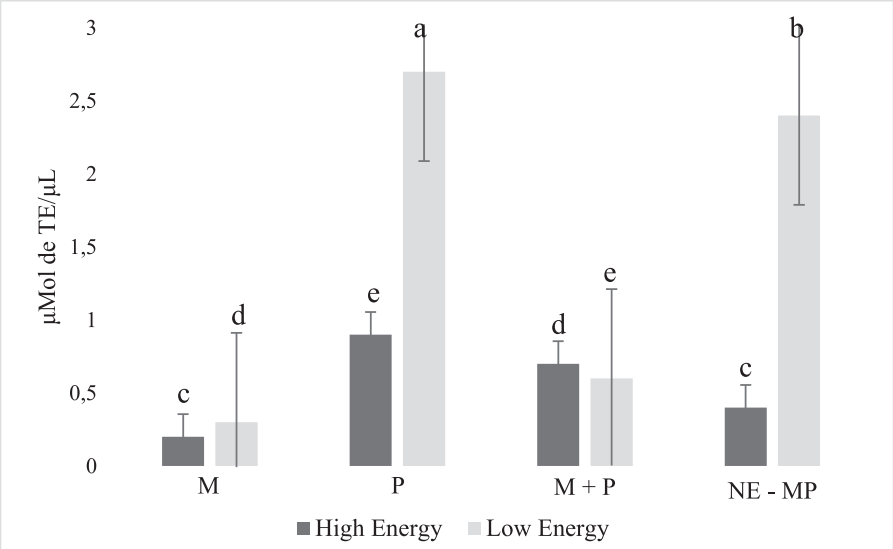


The Ascorbyl palmitate content was performed only with R-NE-MP and the result was 100 % ± 1.42. According to a study by Zatta (2011), nanoemulsions (NE), nanocapsules (NC) and nanodispersions (ND) containing ascorbyl palmitate were prepared and the quantification of the active showed the following results: 80.68% for NE, 97.51% for NC and 83.10% for ND. Our study showed a content equal to 100%, which demonstrates that it was possible to increase the values of ascorbyl palmitate content when compared to studies already performed.

DETERMINATION OF ANTIOXIDANT ACTIVITY - DPPH

After the preparation and physicochemical characterization of the formulations, the antioxidant activity was evaluated by the DPPH method as shown in Figure 2.

Figure 2 - Graphic representation of the antioxidant activity of the formulations.



Fonte: autor

Ultraturrax (dark gray): M- Free basil oil = basil oil (7.5%) + polysorbate 80 (2%) + 10 mL ultra-pure water; PA - Free ascorbyl palmitate = ascorbyl palmitate (0.03%) + polysorbate 80 (2%) + 10 mL ultra-pure water; M + PA - Free associated assets = basil oil (7.5%) + ascorbyl palmitate (0.03%) + polysorbate 80 (2%) + 10 mL ultra-pure water; NE-MP - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by Ultraturrax. Rotary evaporator (light gray): M - Free basil oil = basil oil (3.3%) + 10 mL acetone; PA - Free ascorbyl palmitate = ascorbyl palmitate (0.1%) + 10 mL acetone; M + PA - Free associated assets = basil oil (3.3%) + ascorbyl palmitate (0.1%) + 10 mL acetone; NE-MP-R - Nanoemulsions containing basil essential oil and ascorbyl palmitate prepared by rotary evaporator. The values were expressed as mean ± standard deviation, means followed by equal letters in the same bar showed no statistical difference between them, by the Tukey test ($p < 0.05$). *Means followed by the same letter do not differ significantly ($p = 0.05$).

According the results found in the determination of the antioxidant activity, it was observed that the nanoemulsions prepared by the low energy method presented a better anti-radical capacity when compared to the nanoemulsions prepared by high energy method. This difference in the anti-radical capacity presented by the nanoemulsions may be correlated with the concentration of ascorbyl

palmitate use, since the T-NE-MP contained 0.03% and R-NE-MP 0.1% of ascorbyl palmitate. It is noteworthy that this difference in the ascorbyl palmitate concentration of the formulations occurred due to the limitations presented by the high energy method for the dissolution of the asset, since this method does not use organic solvent. Another point observed in this study was that despite the anti-radical activity of the M+P (lower energy method) was less than nanoemulsions containing ascorbyl palmitate (NE-MP - lower energy method), furthermore, it was possible to show that the nanoemulsions containing ascorbyl palmitate maintained the anti-radical capacity after nanoemulsification.

CONCLUSION

This study demonstrated for the first time the feasibility of obtaining nanoemulsions containing ascorbyl palmitate, as active, and basil oil as an oily component by employing two methods of preparation. The formulations presented average droplet diameter lower than 200 nm, low polydispersion index, negative zeta potential and acidic pH, due to the characteristics of the do active. Moreover, it was possible to observe that the essential oil of basil presented a low antioxidant activity in its free form when compared to ascorbyl palmitate. Furthermore, it can be concluded that the association of these two compounds in nanoemulsions, prepared by spontaneous emulsification, potentiated the antioxidant activity when compared to the association of the compounds in the free form, which may be a potential formulation to be incorporated into a semi-solid vehicle for topical skin use.

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