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Development and Characterization of Nanoemulsion Containing Volatile Oil of *Matricaria recutita* L.

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HIGHLIGHTS

- The high energy method revealed the efficiency in obtaining the nanoemulsions.
- The nanoemulsions showed appropriate characteristics for this type of system.
- 4 °C was the best temperature to preserve the nanoemulsions characteristics.
- Lipophilicity of the volatile oil of *Matricaria recutita* L. was first announced.

Abstract: Nanoemulsions (NEs), emulsified systems with diameter between 20 and 500 nm, are promising in cosmetic science due to stability and efficient transportation of lipophilic substances, such as volatile oils, favoring the skin penetration to carry out the biological activity. Volatile oil of *Matricaria recutita* L. (VOMR) has several therapeutic effects, but the lability of the volatile oils requires appropriate vehiculation in NEs. Besides, the hydrophilic-lipophilic balance (HLB) parameter for VOMR is not described in the literature. Therefore, NEs composed of Span[®] 80, Tween[®] 80, PEG-7 glyceryl cocoate, glycerin and water were developed by the high-energy method. The NEs were characterized by organoleptic, physicochemical and morphological evaluations as well as the stability evaluation was conducted at different temperatures for 60 days. The NEs obtained with and without VOMR exhibited translucent aspect, pH of 4.82 and 5.62; average diameter of 104.97 ± 4.03 and 92.32 ± 6.17 nm; polydispersity index of 0.27 ± 0.06 and 0.31 ± 0.04 , and zeta potential of -28.37 ± 3.06 and -36.48 ± 6.56 mV, respectively. The encapsulation of VOMR was around 83% into the NE and confirmed by visualization through scanning electron microscopy - field emission gun. The stability study demonstrated that refrigeration is the optimal storage condition because the conservation of the NEs characteristics. As a result, the present study describes for the first time HLB value near 7 for VOMR. Thus, NE with this volatile oil was developed with suitable characteristics, contributing to technology development of phytopharmaceuticals for topical application to the skin.

Keywords: Nanoemulsion; German Chamomile; Volatile Oil; Nanotechnology; Skin.

INTRODUCTION

The volatile oils are secondary metabolites produced by plants. They are oily liquids at room temperature with aromatic characteristics that can be extracted by steam distillation from different parts of plants, such as stems, flowers, roots, and leaves [1]. Due to the diverse chemical composition and biological properties, volatile oils offer an economic potential that has attracted the interest of the food, pharmaceutical and cosmetic industries [2].

Matricaria recutita L., commonly known as German Chamomile, is one of the main species that can produce the volatile oils. This is an herbaceous, annual, and aromatic plant that belongs to the Asteraceae family. It is native to Western Asia and the Southern of Europe and is easily found in countries with subtropical climates such as Brazil [3]. The German Chamomile is known worldwide for the volatile oil extracted from its flowers, which consists of various terpene compounds, such as bisabolol oxides A and B, (-)- α -bisabolol, farnesene, and chamazulene. The chemical composition of the volatile oil also includes cis-ocymene, camphene, α -pinene and β -pinene, eucalyptol, camphor, thymol, and terpinen-4-ol [3-5]. The chamazulene ensures the bluish color and antioxidant activity, which, in combination with α -bisabolol provides to the volatile oil of *M. recutita* (VOMR) therapeutic properties such as anti-irritating, anxiolytic, analgesic, sedative, antiallergic, antimicrobial, anti-inflammatory, anticoagulant, and cicatrizing. Therefore, it is widely used on topical application through the dermatological and cosmetic products such as body lotions, ointments, moisturizers, hair care products, soothing lotions, and baby creams [3,4]. It can also be used a preservative system in food and cosmetics [3-6].

Considering the diverse biological activities of VOMR, some intrinsic aspects of the volatile oils may limit their use and application, such as high volatility, low water solubility, and instability in the presence of oxygen and light, leading to organoleptic and physicochemical alterations with loss of activity [2]. In this regard, nanotechnological-based systems (1×10^{-9} m), particularly nanoemulsions (NEs), represent a suitable strategy preserve the properties of volatile oils characteristics. NEs can provide higher physicochemical stability, promote controlled release and more effectiveness of the loaded substances. Besides, they protect from physiological reactions, increase the concentration at the application site and reduce toxicity compared to conventional formulations. As a result, they contribute to the development of innovative products containing natural compounds as the volatile oils [2, 7-10].

Nanoemulsions (NEs) (oil-in-water, O/W) are oil droplets, with diameters between 20 and 500 nm, stabilized by surfactants dispersed in an aqueous phase [11]. NEs are kinetically stable systems with transparent to translucent appearance and low viscosity. They are used in several applications, such as controlled delivery of substances to the skin layers due to larger surface area than emulsions, which makes them an effective transport system for volatile oils, facilitating distribution and penetration through the stratum corneum [12]. This last property is easily achieved by increasing skin hydration and elasticity, which has been widely explored in cosmetology [12,13]. Examples of NE for topical application have been developed with: isotretinoin and quercetin for the treatment of acne *vulgaris* [14]; pomegranate seed volatile oil to protect the skin from ultraviolet rays due to its antioxidant capacity [15]; and NE loaded with *Achyrocline satureioides* (Lam.) DC extract for external use in wound healing [16].

On the other hand, to obtain emulsified systems with physical and chemical stability, it is required enough concentration of surfactants to disperse the oil in the aqueous phase. The Hydrophilic-Lipophilic Balance (HLB) can guide this adjustment of surfactant amounts. HLB is a critical parameter that numerically classifies specific compounds according to their characteristics and by the hydrophilic and lipophilic portions on the molecules. It is useful to sort the surfactants and assist on the prediction of the emulsion type when oily substances are added, such as the volatile oils [17]. Nonetheless, VOMR does not have the HLB described in the literature to enable its vehiculation into emulsified systems through pharmaceutical and cosmetic formulations, limiting the stability and exploration of its therapeutic effects.

Therefore, regarding the benefits of NEs associated with the biological properties of the volatile oil of *M. recutita* (VOMR), this study aimed: to determine the HLB value of VOMR, develop and characterize the NEs containing VOMR in order to obtain formulations with appropriate physicochemical properties as an alternative cosmetic form for topical application.

MATERIAL AND METHODS

Determination of the Hydrophilic-Lipophilic Balance (HLB) from the volatile oil of *Matricaria recutita* L.

A series of emulsions (10 mL) containing VOMR were prepared using the reverse-phase method to obtain systems with different HLB values ranging from 7.0 to 15.0. The emulsions were composed of 5% (w/w) surfactants in different proportions from the mixture of sorbitan monooleate (Span[®] 80, Dinâmica[®]) and polysorbate 80 (Tween[®] 80, Labsynth) added to 2% (w/w) of VOMR (QUINARI[®], Brazil; SisGen: A4FBE21) and 93% (w/w) of ultrapure water (MilliQ, Merck Millipore[®], United States of America).

The oily phase consisted of the Span[®] 80 and the VOMR, while the aqueous phase was composed of the Tween[®] 80 and ultrapure water. Both phases were heated at 40 °C and homogenized, separately, with magnetic stirrer for 15 minutes. Subsequently, the aqueous phase was poured onto the oily phase and left under stirring for 15 minutes. The transmittance (%T) of the emulsions was measured by turbidimetry in a spectrophotometer (Thermo Scientific[®], Genesys 10S UV-Vis, United Kingdom) at 600 nm. Turbidity was calculated using the equation: $Turbidity = 100 - \%T$ [18, 19]. All samples were analyzed in triplicate.

Development of the nanoemulsion (NE) containing VOMR

The NEs were obtained by emulsifying method of high-energy through the high shear homogenizer (Ultra-turrax IKA[®] T 18 Basic, Brazil) based on previous method with modifications [20]. For the NE containing the VOMR (NEVOMR), the oily phase was composed of VOMR (2%, w/w) and sorbitan monooleate (3.8%, w/w), while the aqueous phase was composed of polysorbate 80 (1.26%, w/w), PEG-7 glyceryl cocoate (2%, w/w) (Dinâmica[®]), glycerin (2%, w/w) (Reatec[®]) and ultrapure water for. Both phases were previously homogenized, separately, by magnetic stirring at 40 °C to obtain a homogeneous solution. Thereafter, the oily phase was added dropwise to the aqueous phase under agitation of 6000 rpm. Afterwards, the homogenization was increased to 14000 rpm for 30 minutes. The NE without the VOMR (NEWVOMR) in the oily phase was considered the negative control and was prepared according to the methodology described above. All the NEs were produced in triplicate.

Evaluation of the organoleptic characteristics

To characterize the macroscopic features of the emulsions from the HLB study, they were classified according to visual aspect, color, and stability of the emulsified systems (formation of a phase).

After the preparation of the NEs, the visual and olfactory aspects were analyzed by comparison among the formulations during the storage period. The aspects were classified as altered and non-altered [21].

Physicochemical characterization

The analysis of average size and polydispersity index (PDI) were conducted using the dynamic light scattering (DLS) method. The zeta potential determination was made by electrophoretic mobility in equipment Zetasizer[®] Nano ZS90 (Malvern Instruments, United Kingdom) with a 90° detection angle at 25 °C. The emulsions obtained in the HLB study and developed NEs were diluted in water at 1:25 (v/v) to perform these assays.

The pH of the NEs was measured directly into the formulations with the potentiometer (Digimed[®], model DM-22, Brazil) previously calibrated with buffer solutions (pH 3.0 and 7.0).

Encapsulation efficiency of the VOMR (%EE)

The amount of encapsulated VOMR was determined according to the method of Manna and coauthors [22] and Paula and coauthors [23] with adaptations. Firstly, VOMR was scanned to define the maximum absorption wavelength in ethanol (99.5%, Synth) by UV-spectroscopy ranging from 190 to 500 nm. Different concentrations of VOMR (diluted in ethanol) were prepared to obtain a calibration curve at wavelength of 202 nm, which corresponds to the maximum absorption peak of VOMR ($n=3$, $y=8.62x - 2.6088$; $R^2= 0.9964$). To measure the loaded VOMR concentration, NEVOMR was diluted in ethanol (1:5000, v/v) and homogenized using a vortex mixer (Quimis[®], Model Q920-A2, Brazil) for 3 minutes at 3000 rpm to release the volatile oil from NE. Volatile oil content was measured the absorbance by UV spectrophotometry (Thermo Scientific[®], Genesys 10S UV-Vis, United Kingdom) at 202 nm based on the calibration curve above. Samples were performed in triplicate and represented as mean value \pm SD. The %EE value was calculated based on the

total volatile oil concentration used to prepare the NEVOMR and the volatile oil concentration quantified by the proposed method according to equation: %EE = (loaded VOMR concentration / total VOMR concentration) x 100.

Analysis by scanning electron microscopy coupled with field emission gun (SEM-FEG)

The morphological characterization of the NE was performed using SEM-FEG coupled on the *STEM-in-SEM* detector (Scanning Transmission Electron Microscopy in Scanning Electron Microscope), based on Ditzel and coauthors [24] with adaptations. Therefore, 1 mL of NE was diluted in 9 mL of ultrapure water (1:9, v/v). This solution was magnetic stirred for 30 minutes at room temperature (Fisatom®, model 752A, Brazil). Thereafter, the 10 mL solution was mixed, in equal parts, with a solution 1% (w/v) of phosphotungstic acid (Biotec®). The combination was kept under magnetic stirring for 30 minutes. One drop of the final solution was added on the copper grid (200 mesh), covered with carbon film and dried for 24 hours at room temperature. This was examined under the microscope (TESCAN®, model Mira 3, Brno, Czech Republic) operated at an accelerating voltage of 30 to 200 kV.

Stability study of the NEs

The NEs containing VOMR (NEVOMR) and without it (NEWVOMR) were evaluated under different storage conditions, protected from light, at: room temperature, oven (37 °C) and fridge (4 °C) for 60 days. The measurements were made at 0, 10, 20, 30, and 60 days with assessments of pH, particle size, PDI and zeta potential. All analysis were carried out in duplicate.

Statistical analysis

The data were presented as mean values ± standard deviation. For the determination of the hydrophilic-lipophilic balance (HLB) was used one-way analysis of variance (ANOVA) followed by multiple comparison Bonferroni post-test and for the stability study was used two-way analysis of variance (ANOVA) followed by multiple comparison Bonferroni post-test. Physicochemical characterization was analyzed by Student's t-test. The analyses were conducted with the software GraphPadPrism® 5.04 version (San Diego, CA, USA) and the significance level was $p < 0.05$.

RESULTS

Determination of the Hydrophilic-Lipophilic Balance (HLB) from the volatile oil of *Matricaria recutita* L.

Table 1 presents the HLB values (7 – 15) with the average sizes, polydispersity index (PDI), and zeta potential values, and in Figure 1 are outlined the measurements of turbidimetry obtained for the formulations containing the VOMR.

Table 1. Physicochemical characterization of the system to determine the HLB value for the VOMR.

HLB	% Tween® 80 (w/w)	% Span® 80 (w/w)	Diameter ± SD (nm)	Polydispersity index (PDI)	Zeta potential ± SD (mV)
7	25.23%	74.77%	560.8 ± 0.90	0.110*	-48.9 ± 6.20
8	34.58%	65.42%	388.7 ± 0.78	0.358	-45.6 ± 7.03
9	43.93%	56.0%	367.9 ± 0.82	0.415	-43.8 ± 6.07
10	53.27%	46.73%	547.7 ± 0.91	0.475	-41.1 ± 8.20
11	62.62%	37.38%	518.4 ± 0.87	0.485	-46.1 ± 7.13
12	71.96%	28.04%	501.3 ± 0.98	0.507	-35.7 ± 6.55
13	81.30%	18.70%	600.6 ± 0.93	0.551	-27.4 ± 5.72
14	90.65%	9.35%	678.8 ± 0.94	0.553	-22.4 ± 1.76
15	100%	0%	569.7 ± 0.99	0.618	-21.8 ± 3.59

* $p < 0.05$.

The samples that had similar visual characteristics of the emulsions were opaque and homogeneous, displaying a blue color due to the VOMR encapsulation. These emulsions presented HLB values between 7 to 13. The samples with HLB values of 14 and 15 did not exhibit turbidity and homogeneity. The average sizes ranged from 335.7 to 647.9 nm, with the smallest diameters occurring at HLB 8 (388.70 ± 0.78 nm) and HLB 9 (367.90 ± 0.82 nm). The emulsions with low PDI, high homogeneity, were those with HLB values 7 and 8. Zeta potential values were negative for all the emulsions.

After 24 hours of preparation, phase separation was macroscopically observed in the emulsions with HLB values 9 to 15, whereas the emulsions with HLB 7 and 8 presented signs of instability only after 7 days of preparation.

The turbidity values indicate the oily material dispersed in an emulsion. In Figure 1 is represented the emulsions containing VOMR with their different HLB values. Higher turbidity indicates more stable the emulsion is at the desired HLB value. It was observed that the highest turbidity values occurred at HLB values 7 to 10, being HLB 7 the emulsion with the lowest standard deviation and without phase separation.

The critical micelle concentration values for sorbitan monooleate and polysorbate 80 were 0.37 g (w/w) and 0.126 g (w/w), respectively, indicating that the emulsion with HLB 7 formed a suitable emulsified system according to the data. Despite the high average particle size, the emulsion with HLB 7 featured the lowest PDI value, indicating the homogeneity of the system and the efficiency of VOMR stabilization. In this way, it is indicated that the HLB value of VOMR is close to 7, which means that the emulsion with a composition of 74.77% (w/w) for sorbitan monooleate and 25.23% (w/w) for polysorbate 80 is the most suitable for transporting this volatile oil.

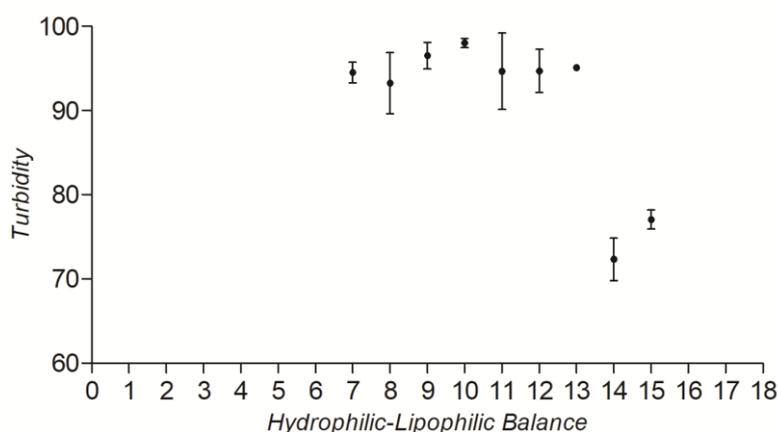


Figure 1. Comparative curve of turbidity values (mean \pm standard deviation) for emulsions with VOMR versus HLB.

Evaluation of the organoleptic characteristics of the nanoemulsions (NEs)

NEs containing volatile oil of *M. recutita* (NEVOMR) or without the volatile oil (NEWVOMR) were successfully obtained using the high-energy method. After the preparation, all formulations were translucent fluids and exhibited Tyndall effect, which is typical for NE system [25]. No phase separation or crystal formation was observed for either NEs.

Physicochemical characterization of the NEs

The table 2 presents the physicochemical characterization for the NEs.

Table 2. Average sizes, PDI, zeta potential and pH of the NEs containing the volatile oil of the *M. recutita* (NEVOMR) or not (NEWVOMR).

Formulation	Diameter \pm SD (nm)	Polydispersity index (PDI)	Zeta potential \pm SD (mV)	pH
NEVOMR	104.97 \pm 4.03	0.27 \pm 0.06	-28.37 \pm 3.06	4.82 \pm 0.07
NEWVOMR	92.32 \pm 6.17	0.31 \pm 0.04	-36.48 \pm 6.56	5.62 \pm 0.15*

*p < 0.05

The formulations presented nanometric sizes with no alteration after the VOMR encapsulation (p > 0.05). Potential zeta values were negative for both NEVOMR and NEWVOMR (p > 0.05). For pH, there was a significant statistical difference between NEVOMR and NEWVOMR (p < 0.05), indicating that encapsulation of the VOMR reduced pH value of the NE.

Encapsulation efficiency of the VOMR (%EE)

The encapsulation efficiency for the NEVOMR was found to be 83.05 \pm 0.12% after the measurements at 202 nm with the calibration curve through UV-spectrophotometry.

Analysis by scanning electron microscopy coupled with field emission gun (SEM-FEG)

It was demonstrated that the NEVOMR possessed well-defined spherical shapes with smooth and uniform surfaces (Figure 2A), which are common characteristics of NEs (o/w). The nanometric diameter of the particles ranged of 99.49 to 217.50 nm, being near to those determined by DLS, excluding the technique peculiarities. Furthermore, it was possible to evidence the VOMR encapsulation into the NE as demonstrated in Figure 2B by the presence of the surfactants film surrounding the VOMR droplet.

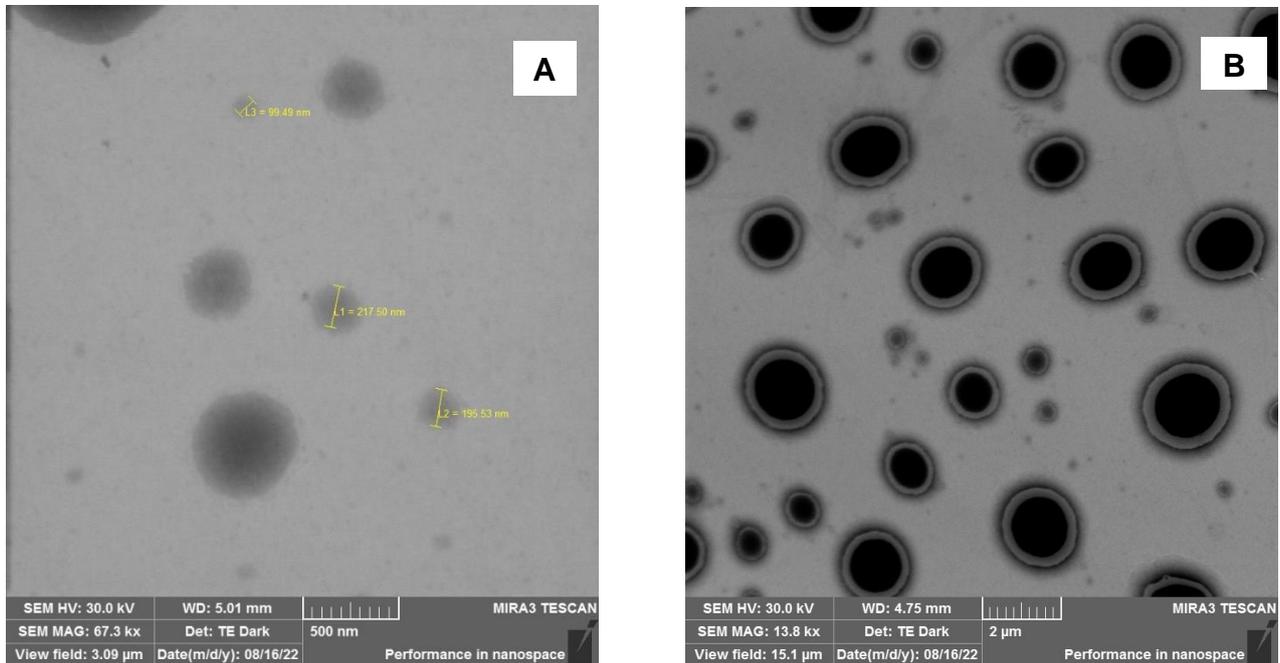


Figure 2. SEM-FEG of NEVOMR under acceleration tension of 30 kV. (A) Observation of the particles diameter at magnification of 67.300x; (B) Observation of VOMR encapsulation for NE at magnification of 13.800x.

Stability study of the NEs

In the stability study, the behavior of these NEs was examined at different temperatures over 60 days. Images of the NEs displaying the macroscopic aspects during the stability study can be found in the Figure 3 below. The results can be analyzed through the Figures 4, 5, 6 and 7.

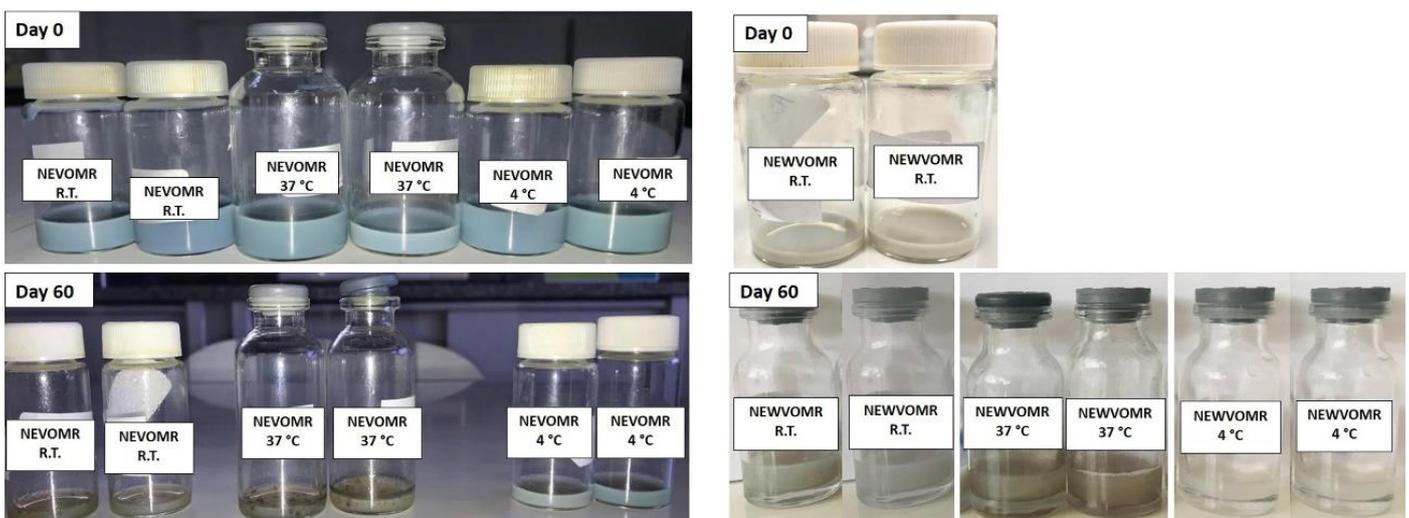


Figure 3. Macroscopic aspects of the nanoemulsions with the volatile oil of *Matricaria recutita* L. (NEVOMR) and without (NEWVOMR) stored at room temperature (R.T.), 37 °C (oven) and 4 °C (fridge) during 60 days for the stability study.

Based on this, it was observed that the NEVOMR and the NEWVOMR stored at room temperature (R.T.) and 37 °C from the 10th day ($p > 0.05$) and at 37 °C for the PDI ($p < 0.05$), which are shown in Figures 4 and 5.

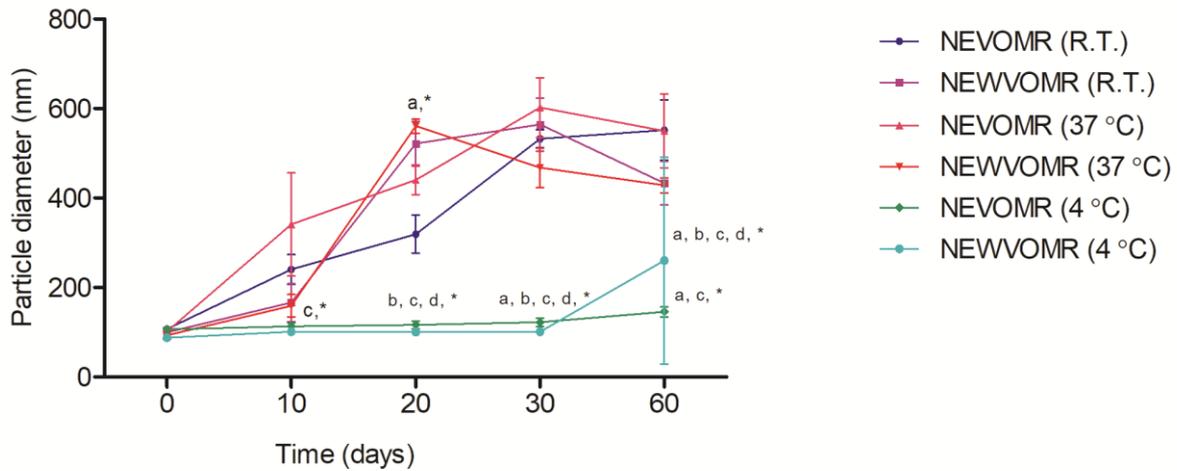


Figure 4. Stability study of the NEVOMR and NEWVOMR depending on the particle diameter in nm. Room temperature: R.T.* $p < 0.05$; ^a comparison with the NEVOMR stored at R.T.; ^b comparison with the NEWVOMR stored at R.T.; ^c comparison with the NEVOMR stored at 37 °C; ^d comparison with the NEWVOMR stored at 37 °C.

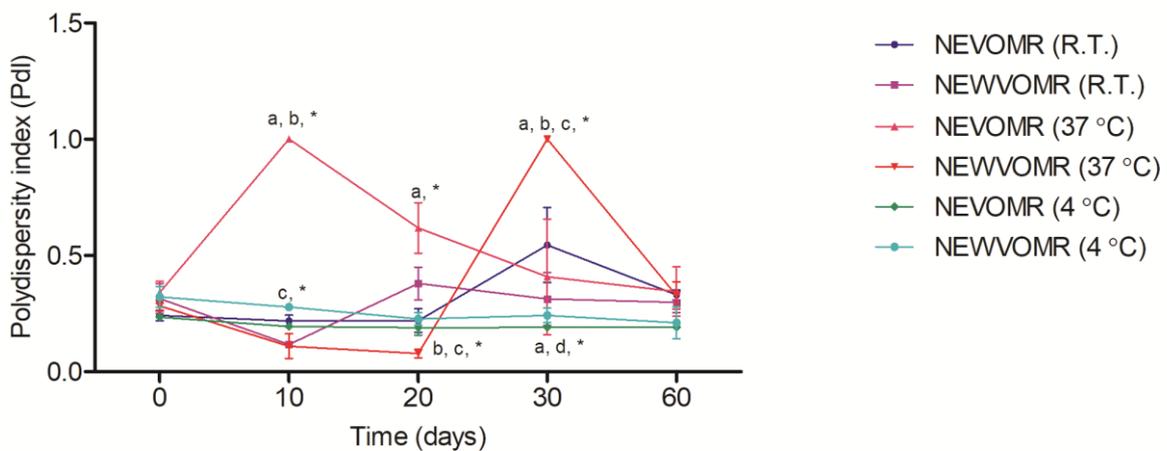


Figure 5. Stability study of the NEVOMR and NEWVOMR depending on the polydispersity index (PDI). Room temperature: R.T.* $p < 0.05$; ^a comparison with the NEVOMR stored at R.T.; ^b comparison with the NEWVOMR stored at R.T.; ^c comparison with the NEVOMR stored at 37 °C; ^d comparison with the NEWVOMR stored at 37 °C.

After 30 days of storage, NEVOMR showed a tendency of flocculation and color change to brownish when stored at R.T. and 37 °C, probably due to the oxidative process caused by the storage temperatures. The same profile of color change was observed in the NEWVOMR stored at R.T. and 37 °C, while the NEVOMR and NEWVOMR stored at 4 °C did not showed macroscopic change (Figure 3).

For the zeta potential, the samples stored at R.T., 37 and 4 °C without the VOMR demonstrated significant difference when compared to the NEVOMR stored at room temperature ($p < 0.05$) at time 0, indicating the influence of the presence of oil on the electrostatic stability of the system. However, along the time, it was not presented the same pattern (Figure 6). It was also observed that the zeta potential of NEVOMR changed after 60 days of storage, with values ranging from -26.65 ± 4.03 to -9.72 ± 1.39 (R.T.) and -31.55 ± 0.21 to -12.45 ± 0.35 (37 °C). However, this difference was not statistically significant ($p > 0.05$). NEWVOMR stored at R.T. and 37 °C indicated a slight variation in zeta potential, as did NEVOMR ($p > 0.05$). In contrast, NEs stored at 4 °C maintained constant zeta potential values, regardless of the storage time and the presence or absence of VOMR, indicating the stability of the system under this storage condition in comparison with R. T. and 37 °C ($p < 0.05$).

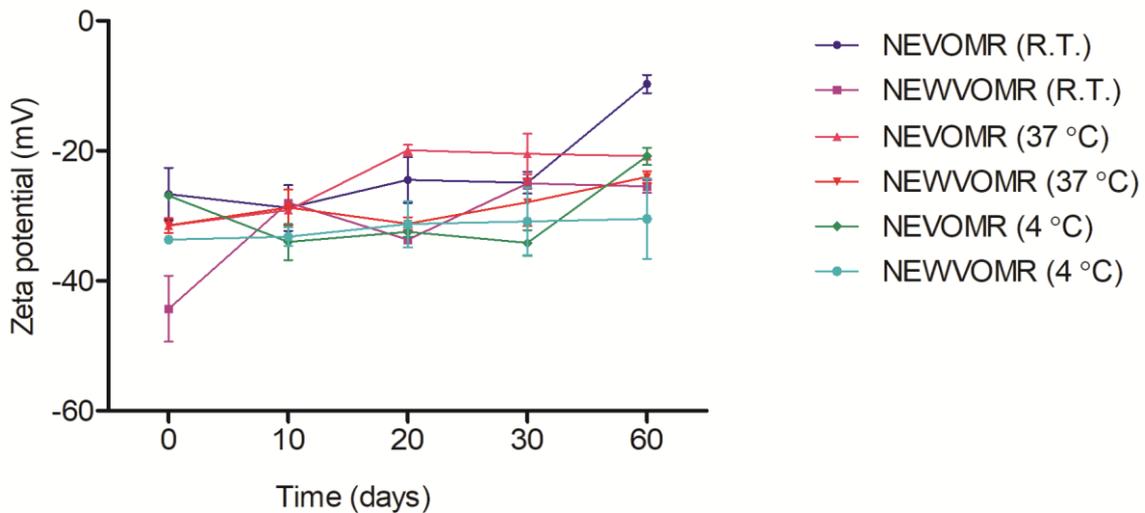


Figure 6. Stability study of the NEVOMR and NEWVOMR depending on the zeta potential (mV). Room temperature: R.T.* $p < 0.05$; ^a comparison with the NEVOMR stored at room temperature.

Figure 7 presents the pH values for the NEVOMR and NEWVOMR after 60 days of storage. The pH values decreased from the first month of storage for all NEs. For the NEVOMR, the pH values varied from 4.76 ± 0.06 to 2.98 ± 0.03 (R.T.); 4.83 ± 0.05 to 3.045 ± 0.02 (37 °C). For the NEWVOMR, the pH values varied from 5.52 ± 0.12 to 3.61 ± 0.11 (R.T.); 5.56 ± 0.04 to 3.37 ± 0.15 (37 °C). Except for the NEs stored at 4 °C, which did not change the pH.

The NEs stored at R.T. did not differ from each other, remaining stable for 30 days, whereas the NEs stored at 37 °C showed a progressive decrease in pH from the 20th day of storage. The NEVOMR exhibited pH from 4.83 ± 0.05 (day 0) to 3.05 ± 0.02 (day 20); the NEWVOMR from 5.56 ± 0.04 (day 0) to 3.37 ± 0.16 (day 20). There was a significant difference in pH values when comparing the R.T. and 37 °C storages with the presence of VOMR ($p < 0.05$).

There was correlation between the zeta potential and pH values of the system, since the NEs in R.T and 37 °C, after 60 days of storage, presented reduction in pH. Concurrently, the zeta potential values demonstrated more positive values, as showed in Figures 6 and 7.

Finally, it was evidenced that the storage at 4 °C proved to be the optimal condition for the NEVOMR and NEWVOMR because their macroscopic and physicochemical properties such as diameter, PDI, zeta potential, and pH were preserved.

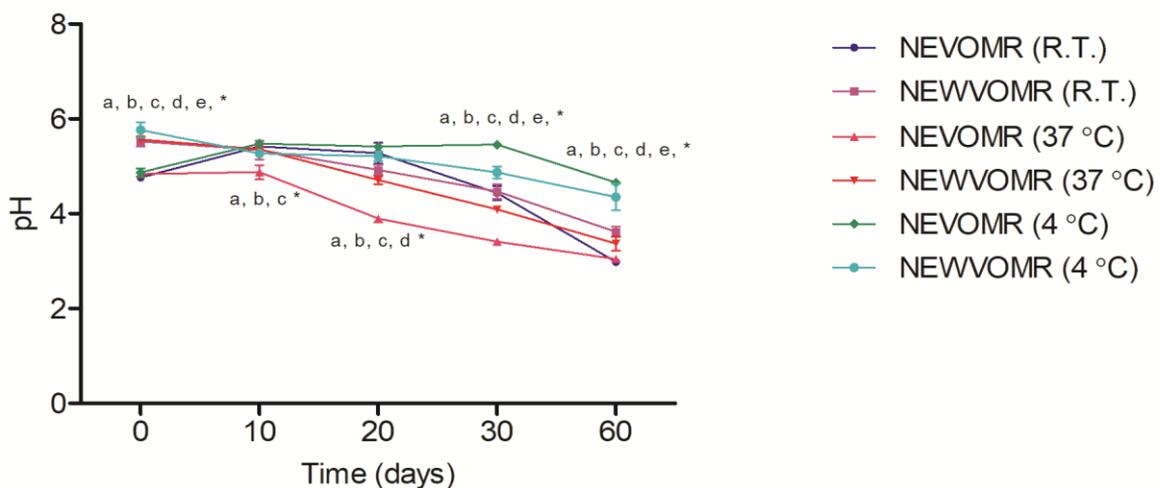


Figure 7. Stability study of the NEVOMR and NEWVOMR depending on the pH. Room temperature: R.T.* $p < 0.05$; ^a comparison with the NEVOMR stored at R.T.; ^b comparison with the NEWVOMR stored at R.T.; ^c comparison with the NEVOMR stored at 37 °C; ^d comparison with the NEWVOMR stored at 37 °C; ^e comparison with the NEVOMR stored at 4 °C; ^f comparison with the NEWVOMR stored at 4 °C.

DISCUSSION

The HLB is an important parameter that guides the vehiculation of oily substances in emulsified systems with physical stability through appropriate combination of surfactants [17]. The HLB study was performed with different HLB values from 7 to 15 of the emulsions, based on the variation of Tween® 80 and Span® 80 in the surfactant mixture (5%, w/w), in an attempt to produce a stable emulsion in which a layer of surfactants should be located at the oil/water interface. This physical intervention is possible by maintaining a critical HLB value corresponding to the required HLB value of the oily material (volatile oil of *Matricaria recutita* L.), such near 7. Furthermore, this value is consistent with those reported for oils from plant origin with HLB value between 6 and 12 [17,18].

The encapsulation of the VOMR into the NE resulted in a bluish coloration, due to chamazulene, one of the main components of the VOMR [3,5], as well as the characteristic odor. On the other hand, the NEWVOMR was a translucent, slightly bluish-white liquid, which was due to the formation of the NE [10, 25-27].

The nanosized obtained for NEVOMR and NEWVOMR is characteristic for the NEs, according to Codevilla and coauthors [11] and Bernardi and coauthors [28]. Particle size is influenced by the composition of the formulation, including the increased concentration of surfactants in association with cosurfactants, preparation method, and intrinsic properties of the oil phase, which is directly related to the formation of micelles and the reduction of surface tension between the aqueous and oil phases. And, consequently, it leads to the decrease in size of the dispersed droplets at nanometric scale [26,27,29]. It is noteworthy that the determination of the HLB value for the VOMR was crucial to adjust the surfactant proportion and further addition of cosurfactants for the NE.

The NEs displayed a homogeneous and unimodal distribution of size. The PDI indicated the system homogeneity, as monodisperse (≤ 0.3). This was achieved by selecting an appropriate process to produce the NEs using high-energy, which facilitates the mixing of the oily and aqueous phases [29, 30]. In addition, the NEs had pH values close to the skin (≈ 5.5), being suitable for topical formulation by showing biocompatibility [31].

The zeta potential provides information about the chemical composition of the formulation and the ionization of the groups that constitute the surfactant molecules when dispersed into the medium. According to Salvia-Trujillo and coauthors [32], the negative values may be related with the sesquiterpenes and oxygenated sesquiterpenes from the VOMR components. Furthermore, values higher or close to ± 30 mV create electric repulsion, contributing to the electrostatic stability, which reduce aggregation and flocculation [33].

Based on the images from SEM-FEG, it was possible to obtain morphological information about the NE particles [34], which were similar with those reported by Godoi and coauthors [20] for NE containing volatile oil of *Eucalyptus globulus* and by Gündell and coauthors [35] on the development of NE containing volatile oil of *Cymbopogon flexuosus*.

The lower values of the particle size and the surface properties perform important role on the bioactivity of the NEs, influencing the release of the molecules. Thus, the VOMR encapsulation into the NE near to 83% can potentialize its biological activities by promoting better interaction and the distribution over the skin, as well as its penetration through the stratum corneum [7,10, 13]. Plus, this high %EE of the VOMR allows the physicochemical protection and its controlled release from the nanotechnological-based system.

The NE is considered the most suitable system to vehiculate volatile oils due to its ability to form micelles in smaller sizes with lower concentration of surfactants resulting in a higher safety profile when compared to microemulsions and other nanoparticles. However, this achievement needs to be investigated in terms of physicochemical stability in storage for long periods [20, 30]. The stability study conducted at different temperatures verified the behavior of these NEs. Although NEs are temperature-sensitive due to the effect on the droplet movement, the tendency for droplets aggregation is lower compared to the larger emulsified systems, such as the macroemulsions and/or the vehiculation of volatile oil without appropriate formulation [29, 33,36].

The tendency of flocculation and color change observed in the stability evaluation was likely due to the oxidative process induced by the storage temperature. Thus, the addition of preservatives and/or antioxidants can help to delay this process, as well as the use of appropriate packaging [36].

Regarding zeta potential values, it is known that there is an interaction of nanoparticles with the dispersion medium, which is affected by pH, temperature, and light exposure [30]. This was observed for NEVOMR stored at room temperature and 37 °C, which showed significant differences between them [29,30].

The pH also provided information about stability of the components of the NEs, because variations in pH may indicate degradation reactions [10]. This was observed when comparing the storage conditions at

room temperature and 37 °C with the presence of VOMR ($p < 0.05$), confirming the influence of temperature on the stability of NEs. This behavior may indicate that the acidity of the medium was a result from the hydrolysis of the VOMR, causing changes in the surface charges of the NVOMR [37, 38]. According to Masmoudi and coauthors [37], emulsions prepared with vegetable oils may exhibit this behavior due to hydrolysis reactions on the volatile oil components, which acidify the system and can be accelerated at higher temperatures. Flores and coauthors [39] reported that pH of NE, containing *Melaleuca alternifolia*, decreased after 60 days of storage and attributed this effect to the greater contact of the oil with the aqueous phase, which may be due to the oily components' degradation. Godoi and coauthors [20] had also found a pH drop after 30 days in their stability study for the NEs containing *Eucalyptus globulus*.

CONCLUSION

A nanoemulsion containing volatile oil of *Matricaria recutita* L. was obtained, with appropriate physicochemical features of decreased nanometric size, low polydispersity index, negative zeta potential, as well as pH close to 5. High rate of volatile oil was encapsulated into the NE determined by UV-spectrophotometry and disclosure by SEM-FEG. Furthermore, the stability evaluation revealed that refrigerator storage is more recommended to preserve the physicochemical characteristics of NEVOMR. The present study also describes for the first time HLB value near 7 for *M. recutita* volatile oil. Considering these findings, NEVOMR has the potential to serve as a nanocarrier for the topical application of *M. recutita* volatile oil on the skin, enabling the delivery of its therapeutic benefits. In addition, this research provides valuable insights for the future development of dermocosmetic products containing nanotechnology and for the evaluation of safety and efficacy.

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REFERENCES

1. Baser KHC, Buchbauer G. Handbook of Essential Oils: Science, Technology, and Applications. Boca Raton, FL: CRC Press. 2010:235-80.
2. Turek C, Stintzing F. Stability of Essential Oils: A Review. Compr Rev Food Sci Food Saf. 2013;12: 40-53.
3. Brasil. Monografia da espécie *Matricaria chamomilla* L. (*Chamomilla recutita* L.) Rauschert, CAMOMILA. Brasília, DF: Ministério da Saúde. ANVISA; 2015.
4. Borsato AV, Doni-Filho L, Cocco LC, Paglia, EC. Essential oil yield and chemical composition of chamomile *Chamomilla recutita* (L.) Rauschert under drying air temperature of 70 degrees C. Semin Cienc Agrar. 2007; 28(4):635-43.
5. Presibella MM, Villas-Bôas LB, Belleti KMS, Santos CAM, Weffort-Santos AM. Comparison of chemical constituents of *Chamomilla recutita* L. Rauschert essential oil and its anti-chemotactic activity. Braz Arch Biol Technol. 2006;49(5):717-24.
6. Nowak K, Jabłońska E, Ratajczak-Wrona W. Controversy around parabens: Alternative strategies for preservative use in cosmetics and personal care products. Environ Res. 2021; 198:1-12.
7. Marzuki NHC, Wahab RA, Hamid MA. An overview of nanoemulsion: cosmeceutical development concepts and applications. Biotechnol Biotechnol Equip. 2019;33(1):779-97.
8. Cecchini ME, Paoloni C, Campra N, Picco N, Grosso MC, Soriano Perez ML, Alustiza F, Cariddi N, Bellingeri R. Nanoemulsion of *Minthostachys verticillata* essential oil. In-vitro evaluation of its antibacterial activity. Heliyon. 2021;7(1):e05896: 1-8.
9. Kamel RA, Teiama MS, El-Hagrassi AM, Elgayed SH, Khattab MA, El-Sayed EK, Ibrahim MT, Mady MS, Moharram FA. Appraisal on the wound healing potential of *Deverra tortuosa* DC. and *Deverra triradiata* Hochst essential oil nanoemulsion topical preparation. Front Pharmacol. 2022;13:940-88.
10. Aziz ZAA, Mohd-Nasir H, Ahmad A, Setapar HM, Peng WL, Chuo CC, Khatoon A, Umar K, Yaqoob AA, Ibrahim MNM. Role of nanotechnology for design and development of Cosmeceutical: Application in Makeup and Skin Care. Front Chem. 2019;7(739):1-15.
11. Codevilla CF, Bazana MT, Silva CB, Barin JS, Menezes CR. Nanostructures containing bioactive compounds extracted from plants. CeN. 2015; 37:142-51.
12. Yukuyama MN, Ghislenoi DDM, Pinto TJA, Bou-Chacra NA. Nanoemulsion: process selection and application in cosmetics -a review. Int J Cosmet Sci. 2015;38(1):13-24.

13. Daudt RM, Emanuelli J, Külkamp-Guerreiro IC, Pohlmann AR, Guterres SS. Nanotechnology as a strategy for the development of cosmetics. *Cienc Cult.* 2013;65(3):32-38.
14. Hosny KM, Al Nahyah KS, Alhakamy NA. Self-Nanoemulsion loaded with a combination of isotretinoin, an anti-acne drug, and quercetin: preparation, optimization, and in vivo assessment. *Pharmaceutics*, 2020;13(1),46: 1-16.
15. Baccarin T, Mitjans M, Ramos D, Lemos-Senna E, Vinardell MP. Photoprotection by Punica granatum seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction against UVB-induced DNA damage in human keratinocyte (HaCaT) cell line. *J Photochem Photobiol B.* 2015;153:127-36.
16. Balestrin LA, Kreutz T, Fachel FNS, Bidone J, Gelsleichter NE, Koester LS, Bassani VL, Braganhol E, Dora CL, Teixeira HF. *Achyrocline satureioides* (Lam.) DC (Asteraceae) Extract-loaded nanoemulsions as a promising topical wound healing delivery system: in vitro assessments in human keratinocytes (HaCaT) and HET-CAM irritant potential. *Pharmaceutics.* 2021;13(8):1241.
17. Zanin SMW, Miguel MD, Chimelli MC, Oliveira AB. Determinação do equilíbrio hidrófilo-lipófilo (EHL) de óleos de origem vegetal [Determination of Hydrophile- Lipophile Balance (HLB) of oils from vegetal origin]. *Visão Acad.* 2002;3(1):13-8.
18. Fernandes CP, Mascarenhas MP, Zibetti FM, Lima BG, Oliveira RPRF, Rocha L, Falcão DQ. HLB value, an important parameter for the development of essential oil phytopharmaceuticals. *Rev Bras Farmacogn.* 2013;23(1):108-14.
19. Orafiya LO, Oladimeji FA. Determining the required HLB values of some oils. *Int J Pharm.* 2002;237: 241-9.
20. Godoi SN, Quatrin PM, Sagrillo MR, Nascimento K, Wagner R, Klein B, Santos RCV, Ourique AF. Evaluation of stability and in vitro security of nanoemulsions Eucalyptus globulus oil. *Biomed Res Int.* 2017; 2017:1-10.
21. Brasil. Guia de Estabilidade de Produtos Cosméticos. 1. ed. Brasília, DF: Agência Nacional de Vigilância Sanitária (ANVISA); 2004.
22. Manaa AO, Baghdadi HH, El-Nikhely NA, Heikal LA, El-Hosseiny LS. Oregano oil-nanoemulsions: Formulation and evaluation of antibacterial and anticancer potentials. *J Drug Deliv Sci Technol.* 2022; 78: 103978.
23. Paula HCB, Sombra FM, Abreu FOMS, Paula RCM. Lippia siloides essential oil encapsulation by angico gum/chitosan nanoparticles. *J Braz Chem Soc.* 2010; 21 (12): 2359-66.
24. Ditzel FI, Prestes E, Carvalho BM, Demiate IM, Pinheiro LA. Nanocrystalline cellulose extracted from pine wood and cornob. *Carbohydr Polym.* 2017;157:1577-85.
25. Magalhães BQ, Machado FP, Sanches PS, Lima B, Falcão DQ, Von Ranke N, Bello ML, Rodrigues CR, Santos MG, Rocha L, Faria RX. *Eugenia sulcata* (Myrtaceae) nanoemulsion enhances the inhibitory activity of the essential oil on P2X7R and inflammatory response *in vivo*. *Pharmaceutics.* 2022; 14 (5): 1-25.
26. McClements DJ. Food Emulsions: Principles, Practices, and Techniques. 3. ed. Boca Raton, FL: CRC Press. 2015.
27. Fernandez P, André V, Rieger J, Kühnle A. Nanoemulsion formation by emulsion phase inversion. *Colloids Surf A: Physicochem Eng.*2002;251:53-8.
28. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, Rocha- Filho PA. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. *J Nanobiotechnology.* 2011;9(44);1-9.
29. Schaffazick SR, Guterres SS, Freitas LL, Pohlmann AR. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos [Characterization and physicochemical stability of nanoparticulate polymeric systems for drug administration]. *Quím Nova.* 2003;26(5):726-37.
30. Rai VK, Mishra N, Yadav KS, Yadav NP. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. *J Control Release.* 2018; 270:203-25.
31. Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. *Skin Pharmacol Physiol.* 2006; 19:296-302.
32. Salvia-Trujillo L, Rojas-Graü A, Soliva-Fortuny R, Martín-Belloso O. Physicochemical characterization, and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. *Food Hydrocol.* 2015; 43:547-56.
33. Campos PM, Praça FG, Mussi SV, Figueiredo SA, Fantini MCA, Fonseca MJV, Torchilin VP, Bentley MVLB. Liquid crystalline nanodispersion functionalized with cell-penetrating peptides improves skin penetration and anti-inflammatory effect of lipoic acid after in vivo skin exposure to UVB radiation. *Drug Deliv Transl Res.* 2020;10(6):1810–28.
34. Klang V, Matsko NB, Valenta C, Hofer F. Electron microscopy of nanoemulsions: an essential tool for characterisation and stability assessment. *Micron.* 2012;43(2-3):85-103.
35. Gündel SS, Souza ME, Quatrin PM, Klein B, Wagner R, Gündel A, Vaucher RA, Santos RCV, Ourique AF. Nanoemulsions containing Cymbopogon flexuosus essential oil: development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. *Microb Pathog.* 2018; 118:268-76.
36. Pavoni L, Perinelli, DR, Bonacucina G, Cespi M, Palmieri GF. An overview of micro- and nanoemulsions as vehicles for essential oils: formulation, preparation and stability. *J Nanomater.* 2020;10(1),135:1-24.
37. Masmoudi H, Dréau YL, Piccerelle P, Kister J. The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR. *Int J Pharm.* 2005;(289):117-31.

38. Duarte JL, Bezerra DC, Conceição EC, Mourão RHV, Fernandes CP. Self-nano-emulsification of chamomile essential oil: A novel approach for a high value phytochemical. *Colloids Interface Sci Commun.* 2020;34:100225:1-6.
39. Flores FC, Ribeiro RF, Ourique AF, Rolim CMB, Bona SC, Pohlmann AR, Bech RCR, Guterres SS. Nanostructured systems containing an essential oil: protection against volatilization. *Quím Nova.* 2011;34(6):968-72.



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