Original Article

DEVELOPMENT OF NANOEMULSION CONTAINING *PELARGONIUM GRAVEOLENS* OIL: CHARACTERIZATION AND STABILITY STUDY

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ABSTRACT

Objective: To develop, characterize and evaluate the stability of nanoemulsions containing geranium oil (NEG) at different temperatures (4 °C, 25 °C and 45 °C) for 90 d.

Methods: The quantification of oil in the nanostructure was performed by gas chromatography–mass spectrometry (GC-MS). The NEG was prepared in Ultra-Turrex and characterized by determining the particle size, polydispersity indices and pH. The thermo gravimetric analysis (TGA), differential scanning calorimetry (DSC) and transmission electron microscopy (TEM) to evaluate the thermal stability of the compounds, the thermal events and morphological analyses of NEG, respectively.

Results: The results allow us to suggest that the use the ultra-turrax method is a strategy good to NEG preparation. The stability of the NEG was strongly influenced by storage temperature, with droplet size increasing rapidly at higher temperatures (45 °C), which was attributed to coalescence near the phase inversion temperature. The NEG submitted the low temperatures (4±2 °C) remained with the same particle size value (164 nm). However, the citronellol and geraniol showed a significant reduction throughout the test even in these conditions of temperature. Thermo gram of NEG shows the crystallization peak at the cooling cycle in-20.1 °C and a melting was observed at 1.5 °C. TEM images indicated that NEG was spherical and nanometric.

Conclusion: The proposed Ultra-Turrax method is simple which prevents volatilization of GO for the production of NEG. The formulations presented good physicochemical characteristics and stability for 90 d was only achieved in 4 °C.

Keywords: Geranium oil, Ultra-Turrax, Nanoemulsion, Citronellol, Geraniol

INTRODUCTION

Nanoemulsions are emulsified systems with droplets that are between 20 and 200 nm in size [1]. Due to their characteristic size appear transparent or translucent to the naked eye [2]. Depending on the preparation method, different droplet size distributions might be achieved, explaining why the route of preparation can have an influence on the emulsion stability [3]. The preparation of emulsions with droplet sizes in the submicrometer range may be performed mechanically, which involves high-energy input that is generally achieved by high-shear stirring, high-pressure homogenizers, or ultrasound generators [1].

In contrast, nanoemulsions produced with low energy methods depend on the spontaneous formation of emulsions based on the phase behavior of certain surfactant, oil, and water systems [4]. There is interest in using lower energy techniques in the emulsion formation process due to economic benefits and increasing amounts of research have been conducted to investigate the utility of different low-energy approaches [5, 6]. Self-emulsifying systems offer a strategy for dealing with the low bioavailability of compounds (drugs and oils) that are not easily dissolved in water [7, 8].

The oil from *Pelargonium graveolens*, also known as a geranium oil (GO) or mauve is extracted from the tree *Pelargonium odorantissimum* originating from South Africa. The *Pelargonium*

(Geraniaceae) *genus* is represented by many essential oil producing species inter alia: *P. graveolens, P. odoranissimum, P. zonale and P. roseum*. Geranium oil is obtained from leaves, flowers, and stalks by steam or hydrodistillation. The GO is composed of various chemical constituents such as linalool, citronellol, geraniol, and their esters [9, 10].

Further, the GO is non-toxic, non-irritant, generally non-sensitizing, and it is not known to cause any other side effects. Studies show that GO has therapeutic properties as antidepressant, antiseptic and healing. It is used to diverse dermatological problems such as oily or congested skin, eczema, and dermatitis [11].

However, few studies have explored the incorporation of GO in nanostructures [12, 13]. One of the main objectives of the current study was to investigate the formation of nanoemulsions by low energy isothermal methods using a well-defined model system: oil, non-ionic surfactant, and water. In addition, there is no study in the literature about the optimizing of nanoemulsion preparation in terms of droplet size, emulsion stability, and emulsification efficiency (EE). The produced formulations were analyzed and characterized in terms of physical properties such as particle size, zeta potential, morphology, entrapment efficiency and long-term stability.

MATERIALS AND METHODS

Acquisition of Geranium oil and reagents

The GO, geraniol, citronellol and sorbitan monooleate were purchased from Sigma-Aldrich Co (São Paulo, Brazil), polysorbate 80 was obtained from Henrifarma (São Paulo, Brazil) and all other chemicals and solvents presented pharmaceutical or GC grade and were used as received.

Geranium oil analysis

Oil composition and yield were analyzed by gas chromatography (GC) using a GC/MSD system (Agilent 6890N), equipped with DB-5 MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness) connected to a mass spectrometer detector. The injector and detector temperatures were set at 250 °C. Helium was used as the carrier gas, at a flow rate of 1.3 ml/min. The thermal programmer was 100-280 °C at a rate of 10 °C/min. Two replicates of samples were processed in the same way. Main components (citronellol and geraniol) were identified on the basis of retention times of the peaks of commercially available oils: geraniol, citronellol performed at under identical experimental conditions. Other components were identified by the comparison of mass spectra with the mass spectra library search (NIST) and with the mass spectra literature. Component relative concentrations were calculated based on GC peak areas without using correction factors. 1 µl of the GO in a diluted solution CH_3CN was injected. GC-Mass Spectroscopy (GC-MS) analysis was performed on an Agilent 5975B EI/CI–MSD system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). The transfer line temperature was 280 °C.

Preparation of nanoemulsions

Nanoemulsions (NEG) were obtained (n=3) after injection of oil phase (5% of geranium oil and 2% of sorbitan monooleate) in the aqueous phase (2% polysorbate 80 and ultrapure water) under high agitation employing a (Ultra-Turrax® T18, IKA®, Germany) at 10.000 rpm. After the stirring was increased to 17.000 rpm and maintained for 1 h. The nanoemulsion containing GO was called NEG. For comparison, blank formulations (NEB) were prepared (n=3) using capric/caprylic triglyceride mixture (MCT) instead of geranium oil. All formulations were prepared in triplicate and stored under protection from light and at room temperature. The nanoemulsions were prepared as reported by [13].

Physicochemical characterization of nanoemulsions

After preparation all formulations were characterized according to following parameters: particle size distribution by laser diffraction Microtrac S3500 (Microtrac Inc., North Largo, FL, USA), particle size and polydispersity indices (PDI) by photon correlation spectroscopy after dilution (500 times) of suspension in ultrapure water, zeta potential by electrophoretic mobility (Malvern Zetasizer Nanosizer®, Malvern Instruments Ltd, Worcestershire, UK) after dilution (500 times) of formulations in 10 mmol NaCl solution, and pH directly in formulations using a potentiometer DM 22 (Digimed, Campo Grande, Brazil) previously calibrated.

Emulsion stability tests

After optimizing the production of the nanoemulsions, their storage stability at three different temperatures (4, 25 and 45 °C) was tested. These experiments were carried out using pre-established optimized parameters. All formulations were analyzed in triplicate and were tested for both particle size, zeta potential, and pH after its production and after 0, 1, 7, 14, 30, 60 and 90 d.

Quantification of the constituents of NEG by GC/MS

Quantification analysis was performed into the CG-MS system by injection of 1 µl of the aliquot of 100 µl of NEG diluted in 2 ml of acetonitrile after 0, 1, 7, 14, 30, 60 and 90 d. Quantification of the constituents of NEG was performed at same chromatographic conditions than those describe in GO analysis section. The amounts of geraniol and citronellol of NEGs were determined based on the linear calibration curves obtained by chromatographic peak area measurement of pure reference compound sat various concentrations.

Thermogravimetric analysis (TGA)

The thermal stability of the compounds was determined by equipment Q5000 thermo-analyzer instrument (TA Instruments Inc., USA). The heating rate used was 10 °C/min and the inert atmosphere was N_2 (50 ml/min). The equipment was calibrated with $CaC_2O_4H_2O$ (99.9%). The mass has weighed a sample of approximately 10 mg. The data were processed using the Software TA Universal Analysis 2000, version 4.5 (TA Instruments Inc., USA).

Differential scanning calorimetry (DSC)

The thermal events were studied by Modulated Temperature Differential Scanning Calorimetry (MTSDC) in equipment DSC Q2000 instrument (TA Instruments Inc., USA) with option MTDSC, equipment with cooling accessory RCS and as a purge gas N_2 (50 ml/min). The heating rate used was 5 °C/min. The instrument was initially calibrated in the way DSC standard, with Indian (99.99%). The masses of pots and reference covers and samples weighed about of 50±0.02 mg. The samples were sealed in aluminum pans with lids. The masses of the samples were weighed on a balance Sartorius (M500P) with a precision of the (±0.001 mg). The data were processed using the Software TA Universal Analysis 2000, version 4.5 (TA Instruments Inc., USA).

Transmission electron microscopy (TEM)

Morphological analyses were carried out at the Microscopy Center Electronics of the Federal University of Rio Grande do Sul (Porto Alegre, Brazil) by transmission electron microscopy (TEM; Jeol, JEM 1200 Exll, Japan) operating at 200 kV. The combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. In order to perform the TEM observation, the nanoemulsion formulation was diluted with water (1/100).

Statistical analysis

A one-way analysis of variance (ANOVA) was used to analyze, followed by Tukey's test p statistically ≤ 0.05 indicated a statistically significant difference.
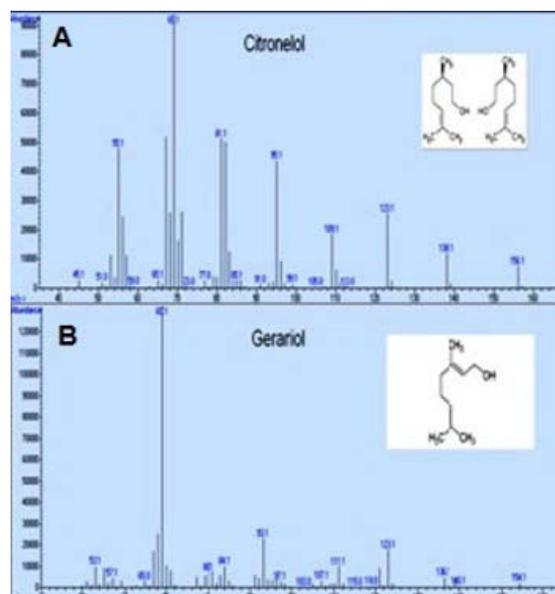
Fig. 1: Mass spectra of citronellol (A) and geraniol (B)

RESULTS AND DISCUSSION

GC analysis

Mass spectra in full scan mode are shown in fig. 1. In total, 20 compounds representing 83.5% of the GO were identified. The

main components were citronellol (17.74%) and geraniol (14.73%). The data presented here are consistent with previous reports, which demonstrated that geranium oils are characterized by citronellol (22-32.9%) as the most important component [10, 14]. However, our results diverge from those published by other studies in which the concentration of geraniol (23-38%) was higher than that of citronellol (21-28%) [15, 16]. The chemical composition of geranium oil will depend on a number of factors, including differences in climatic conditions and geographical locations, season at the time of collection, and fertilization [17].

Physicochemical properties of Nanoemulsions

The technique with Ultra-Turrax has enabled the formation of nanometric particles in formulations, independent of the presence or absence of GO. The mean+SD droplet size, PDI, zeta potential and pH of the NEG and NEB subjected to different temperatures on different days are given in table 1, table 2, table 3 and table 4, respectively.

Table 1: Nanoemulsions droplet size values (nm) subjected to different temperatures for 0, 1, 7, 14, 30, 60 and 90 d

↓ Time (d)	(4±2 °C)		(25±2 °C)		(45±2 °C)	
Formulations→	NEG	NEB	NEG	NEB	NEG	NEB
0	#	#	164+3.5	130+2.4	#	#
1	164+4.12	131+2.1	164+1.5	140+3.87	220+3.8[a]	130+4
7	161+4.47	127+2.3	183+1.2	153+4	712+0.07[a]	126+2.3
14	165+4.43	146+5.8[b]	185+0.92	169+2.6	*	182+2.5[b]
30	167+4.41	168+1.7[b]	276+14.9[a]	180+2.2	*	384+7.4[b]
60	160+0.92	178+0.97[b]	602+16.40[a]	291+3.6[b]	*	*
90	164+3.9	181+1.6[b]	*	1694+10.1[b]	*	*

NEG = nanoemulsions containing geranium oil, NEB = blank formulations. mean±SD values for triplicate samples. #:t = 0; *: phase separation; [a]: statistical comparisons of day 0 to NEG with 1, 7, 14, 30, 60 and 90 d. Indicate significance when p≤0,05; [b]: statistical comparisons of day 0 to NEB with 1, 7, 14, 30, 60 and 90 d. Indicate significance when p ≤0,05.

Table 2: Nanoemulsions PDI values subjected to different temperatures for 0, 1, 7, 14, 30, 60 and 90 d

↓ Time (d)	(4±2 °C)		(25±2 °C)		(45±2 °C)	
Formulations→	NEG	NEB	NEG	NEB	NEG	NEB
0	#	#	0.25+0.006	0.12+0.03	#	#
1	0.13+0.016[a]	0.22+0.015	0.25+0.013	0.15+0.009	0.22+0.011	0.21+0.02
7	0.13+0.011[a]	0.23+0.007[b]	0.38+0.006[a]	0.16+0.011	0.52+0.074[a]	0.21+0.011[b]
14	0.14+0.012[a]	0.26+0.05[b]	0.38+0.008[a]	0.27+0.008[b]	*	0.24+0.04[b]
30	0.14+0.010[a]	0.22+0.004[b]	0.30+0.039	0.29+0.02[b]	*	0.25+0.01[b]
60	0.14+0.01[a]	0.22+0.002[b]	0.51+0.02[a]	0.44+0.10[b]	*	*
90	0.15+0.01[a]	0.23+0.005[b]	*	0.93+0.14[b]	*	*

NEG = nanoemulsions containing geranium oil, NEB = blank formulations. mean±SD values for triplicate samples. #: t = 0; *: phase separation; [a]: statistical comparisons of day 0 to NEG with 1, 7, 14, 30, 60 and 90 d. Indicate significance when p≤0,05; [b]: statistical comparisons of day 0 to NEB with 1, 7, 14, 30,60 and 90 d. Indicate significance when p ≤0,05.

Table 3: Nanoemulsions zeta values (mV) subjected to different temperatures for 0, 1, 7, 14, 30, 60 and 90 d

↓ Time (d)	(4±2 °C)		(25±2 °C)		(45±2 °C)	
Formulations→	NEG	NEB	NEG	NEB	NEG	NEB
0	#	#	-10+1.7	-10+1	#	#
1	-10.8+1	-8.3+3.8	-11+1	-10+0.8	-10.6+1	-9.1+2.4
7	-10.2+1.6	-9.5+1	-14+1.2	-9.6+0.8	-10+1.2	-9.3+1.1
14	-9.6+3.2	-8.3+0.8	-13+1.6	-10+1.1	*	-9.3+0.2
30	-12.8+1.6a	-12+0.5	-12.5+0.5	-18+0.7[b]	*	-12.6+0.8
60	-11.6+2.6	-13+1.5	-11+0.1	-26+1.1[b]	*	*
90	-11.7+2.1	-9.3+0.3	*	-22+1.3[b]	*	*

 NEG = nanoemulsions containing geranium oil, NEB = blank formulations. mean±SD values for triplicate samples. #: t = 0; *: phase separation; [a]: statistical comparisons of day 0 to NEG with 1, 7, 14, 30,60 and 90 d. Indicate significance when p≤0,05; [b]: statistical comparisons of day 0 to NEB with 1, 7, 14, 30,60 and 90 d. Indicate significance when p ≤0,05.

Table 4: Nanoemulsions pH values subjected to different temperatures for 0, 1, 7, 14, 30, 60 and 90 d

↓ Time (d)	(4±2 °C)		(25±2 °C)		(45±2 °C)	
Formulations→	NEG	NEB	NEG	NEB	NEG	NEB
0	#	#	3.7+0.12	6.4+0.21	#	#
1	3.7+0.14	6.4+0.21	3.7+0.17	6.4+0.21	3.7+0.05	6.3+0.24
7	3.8+0.15	6.3+0.27	3.7+0.08	6.4+0.27	3.4+0.061	5.3+0.37[b]
14	3.7+0.16	6.4+0.19	3.6+0.07	6.2+0.18	*	3.8+0.18[b]
30	3.7+0.12	6.5+0.21	3.4+0.06	5.3+0.12[b]	*	3.4+0.21[b]
60	3.7+0.04	6.8+0.16[b]	3.3+0.04[a]	3.8+0.12[b]	*	*
90	3.4+0.06	6.8+0.11[b]	*	3.5+0.04[b]	*	*

mean±SD values for triplicate samples. #: t = 0; *: phase separation; [a]: statistical comparisons of day 0 to NEG with 1, 7, 14, 30, 60 and 90 d. Indicate significance when p≤0,05;[b]: statistical comparisons of day 0 to NEB with 1, 7, 14, 30, 60 and 90 d. Indicate significance when p ≤0,05.

NEG showed reduced particle size (164±3.5 nm) with a low polydispersity (0.25±0.006) characterizing, therefore, colloidal systems with narrow particle size distribution. Mean droplet size analysis indicated that NEB presented the smallest mean diameter (130±2.4 nm) and low polydispersity (0.12±0.021). Formulations where GO was added, on the macroscopic analysis, showed characteristics of nanoemulsions with tiny droplets size such as bluish reflex, translucency, and higher intrinsic stability. Nanoemulsion has been used as excellent vehicles to solubilize lipophilic drugs and significantly improve bioavailability [18–22].

The GO not decreased the droplet size and also no provided a better stability of the system. It is well accepted that, for dispersed systems, the smaller the droplet size, the higher the stability. The droplet size is an important data in the stability analysis, so faster the droplet size increases, faster will occur the instability process as creaming or phase separation [23].

The choice and amount of surfactant may influence the stability of the nanoemulsion. The proper balance between the surfactants and GO provides a smaller and uniform sized droplets because occurs uniform coverage of surfactant around the droplet which avoids aggregation. This clearly shows that the proper balance between surfactant and GO will not only yield smaller and stable particles in terms of size but also protect the emulsion from degradation. When there is excess surfactants system, there is increased the formation of micelles, which facilitate can the mass transport of oil molecules from smaller to larger globules, which may result in an increase in particle size as a function of time [24].

Some results suggest that every system should be investigated individually since factors such as the phase behavior of the surfactant–oil–water system and the physicochemical properties of the components greatly impact the effect of variables like stirring or mixing speed. It is frequently necessary to use blends, such as a pair of hydrophilic and lipophilic non-ionic surfactants, to achieve droplets with small diameter [3, 4, 25].

Our study showed that NEG has not significant variation in the particle size of the system in the temperature remained of 4±2 °C during the period of the study. At 25±2 °C and 45±2 °C, there was a change in the size of the droplets as well as in the polydispersity index, which is expected since the temperature increases the kinetic energy of the system, increasing the possibility to occur instability phenomena. Besides, the oil phase composed of volatile oil may have caused rupture of the interface by evaporation of its molecules.

When the formulations NEG were subjected to high temperature (45 °C) it was found that after the seventh days there was phase separation (table 2). This may be due to destabilization of the system by evaporation of its volatile constituents. The essential oils have a high vapor pressure, what means that it may volatilize in low temperatures. The phase separation can be due to the reorganization of the system, since the molecules of the essential oils, when submitted to heating might have caused the rupture of the interface followed by coalescence [26].

Zeta potential is a useful tool to predict the physical stability of colloidal systems. The zeta potential determines the electrostatic repulsion between the globules [27]. When the electrolyte concentration increases, the ionic double layer is compressed due to the ionic attraction forces, resulting in the reduction of its thickness and the extent of reducing electrostatic repulsion force.

The zeta potential values presented show that both the NEB as NEG (table 3) have negative charges. The zeta-potential was from NEB and the NEG was around-10 mV. However, there was no significant difference when comparing the zeta potential of the NEG when subjected to different temperatures, only in the analysis 30 d at 4±2 °C. The NEB showed significant variation in zeta values after the 30 d when subjected to room temperature. Some authors explain negative values due to the structural characteristics of the nanoemulsions interface components, especially polysorbate 80 used in high concentration in the formulation. In this case, the hydrocarbon chain of the surfactant interacts with the hydrophobic region of the oily phase and could induce negative charges on the surface of the system [28].

A reduction in the electrical potential of the double layer causes a reduction in total electric potential compromising the stability of the emulsion [29]. It is important to make a comparison between the zeta potential with the particle size results because an increase in particle sizes of nanoemulsions was accompanied by an increase in negative surface charge values. The droplets size and zeta potential are the most representative parameters in the control of emulsion stability. To evaluate the emulsion stability, these aspects were monitored for 3 mo (table 1 and 3). The zeta potential in NEG had a small variation during the stability test.

The pH value is a parameter for monitoring the stability since changes may indicate an occurrence of chemical reactions or microbial contamination. Formulations with vegetable oils may result in a decrease in pH from the hydrolysis of esters of fatty acid, which generate free fatty acids. The pH of the NEG had only a significant variation in temperature of 25±2 °C in an analysis of 60 d. However, it was observed that the NEB had their pH values decreased significantly after 30 d at 25±2 °C, 60 d at 4±2 °C and 7 d at 45±2 °C (table 4). The pH values were similar to those reported by Giongo *et al.* [13].

Evaluation of the constituents of NEG by GC/MS

The nanoemulsions containing GO were quantified by GC/MS. The fig. 2 shows the components found in GO and after extracted from nanoemulsions submitted to temperatures of 4±2 °C, 25±2 °C and 45±2 °C for 90 d.
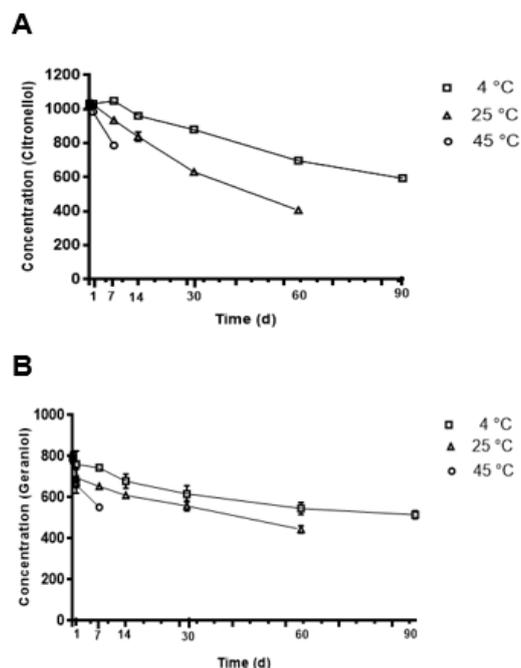
Fig. 2: Concentration of components (ppm) found in GO after extracted from nanoemulsion Citronellol (A) and Geraniol (B). The results represent the mean±SD values for triplicate samples

The method for quantification of the constituents of NEG demonstrated to be linear in the range of 430 mg/l–2000 mg/l and 420 mg/l–2000 mg/l for geraniol and citronellol. The calibration equations y = 1643x-5048 and y = 4179x-2933 showed excellent correlation coefficients, r = 0.9913 and 0.9911 for geraniol and citronellol. Therefore, the result confirms the absence of constant systematic error. The LOD and LOQ limits were 0.5 mg/l and 420 mg/l. The precision of the method was assessed considering repeatability. The precisions presented R. SD values lower or equal to 5.00% for geraniol and citronellol, which were considered satisfactory. The citronellol has a significant reduction of its concentration in the NEG from the day 7 in the temperatures of 25±2 °C and 45±2 °C. The geraniol showed a significant reduction of its concentration in the NEG from day 1 in these same temperatures.

The fig. 3 show a partial chromatogram performed after the production zero-day and after 90 d at 4±2 °C. The possible hydrolysis of this compound can relate to the reduction of pH

values observed in the formulations submitted to stability tests. Our results are in accordance to those reported by other studies [30-32].
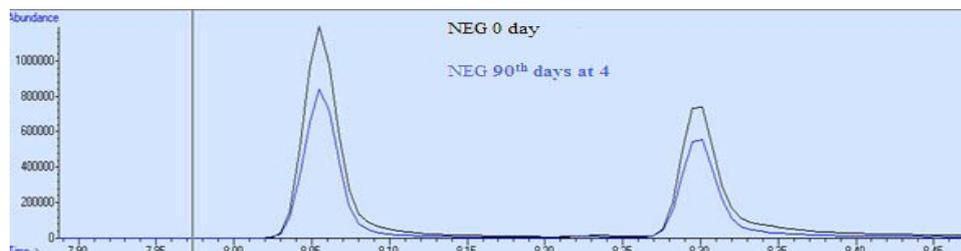
Fig. 3: Partial chromatogram performed to NEG (0 and after 90 d at 4±2 °C)

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC)

Thermograms of DSC were obtained for citronellol, geraniol, GO, NEG and NEB at same experimental conditions (table 5).

Table 5: Thermic properties of NEGs determined by DSC and TGA

Sample	T_g (°C)[a]	T_d (°C)[b]	T_i (°C)[c]	T_f (°C)[d]
Geraniol	-57	126	33	180
Citronellol	-59	145	32	197
Geranium oil	-58	155	32	504
NEB	-[e]	54	29.3	177
NEG	-[e]	104	32	153

NEG = nanoemulsions containing geranium oil, NEB = blank formulations. [a]Glass Transition (Tg). [b]Decomposition temperature (Td). [c]Inicial decomposition temperature (Ti). [d]Final decomposition temperature (Tf). [e]Thermogram show only thermal transitions (Tg and fusion) of water present in the formulation.

The citronellol, geraniol thermogram display a glass transition temperature (Tg) at-59 and-57 °C respectively. Similarly, GO thermogram shows a Tg at-58 °C corresponding probably to main components citronellol and geraniol. The absence of endothermic and exothermic peaks in thermograms of citronellol and geraniol and GO indicated that they not crystallize and/or melting in the temperature range evaluated. Thus, it is possible to affirm that they have an amorphous structure but are able to organize themselves at low temperatures (approximately-60 °C). On the other hand, DSC thermogram (not demonstrate) of NEB and NEG show a single thermal transition of water.

Thermogram of NEB shows the crystallization peak at the cooling cycle in-14.1 °C and a melting was observed at 0.3 °C. Thermogram of NEG shows the crystallization peak at the cooling cycle in-20.1 °C and a melting was observed at 1.5 °C. Results are indicating that the presence of GO in the nanoemulsion cause the greater shift of crystallization and melting of water. At the same time, the enthalpy of crystallization and melting was similar for NEB and NEG indicating that all water content that crystallized in cooling cycle had to melt in the heating cycle. However, it is important to note that the enthalpy of crystallization and melting of NEG was about 8 times greater than the enthalpy of crystallization and melting of NEB. The increase in the enthalpy of crystallization and melting in the presence of GO can be attributed to the rising of a complex lattice of intermolecular interactions between water, GO and other components of nanoemulsion that led to droplets formation. It was proved by thermogravimetric analysis that the initial decomposition temperature (T_d) is low for citronellol, geraniol, GO, NEB and NEG (around 30 °C), indicating that at this temperature about 5% of mass was lost. However, when we look at decomposition temperature, we can see that citronellol and GO were thermally more stable than geraniol. An interesting observation was the thermal stability of NEG when compared with NEB. The T_d of NEG (104 °C) was two times greater than T_d of NEB. In addition, GO and NEG show the profile of decomposition in two steps. These two funds show that GO is a nanoemulsion. However, when we compared T_d of GO with T_d of NEG, we observe a decrease in the T_d of GO when compared the NEG.

Finally, it is worth note that GO was totally carbonized at 504 °C while NEG was totally decomposed at 153 °C.

This methodology was used recently by Hosseini *et al.* [33] to determine the encapsulation efficiency and loading capacity of OEO-loaded chitosan nanoparticles. There are no studies that use this methodology to assess the GO or NEG to compare the results. This methodology is being reported for the first time in this study.

Transmission electron microscopy (TEM)

In addition, the morphology of NEG and NEB were characterized by TEM (fig. 4) and the particles size were approximately the same as the diameters measured by the dynamic light scattering instrument. TEM images indicated that all the nanoemulsions particles were spherical and nanometric. These results corroborate those found for the particle size and polydispersity index obtained for suspensions of these nanoemulsions by dynamic light scattering technique.
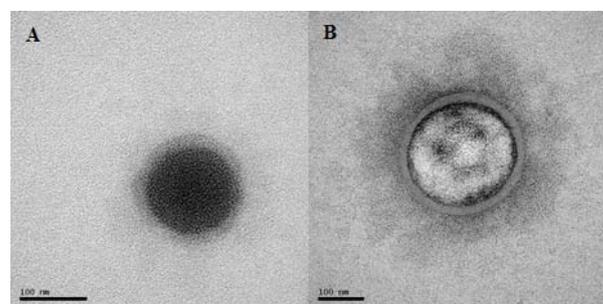
Fig. 4: TEM images of droplets in the NEG (A) and NEB (B). Scale bars 100 nm

Recently, Senthil Kumar *et al.* [34] reported the use of TEM in a study of enrofloxacin solid lipid nanoparticles (SLNs) and observed a

spherical in shape. Our study is the first to report the use of this technique for the characterization of NEG.

CONCLUSION

In this work, we proposed a strategy to obtain nanoemulsions containing GO due to its important pharmacological properties already reported in the literature. The formulations were produced under high agitation employing a Ultra-Turrax, in order to incorporate the GO and prevent its volatilization. The formulations presented good physicochemical characteristics. However, the physicochemical stability for 90 d was only achieved in 4 °C. These results allow us to suggest that the use the Ultra-Turrax is a strategy good to prepare nanoemulsions containing essential oil. Other studies can be realized with these formulations to check their potential biological activities.

CONFLICT OF INTERESTS

There is no conflict of interest

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