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Characterization and Stability Studies on Vegetable Nanoemulsions Obtained by Low Energy Process

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Abstract

Background: The use of vegetable oils in the pharmaceutical and cosmetic industry is becoming increasingly attractive worldwide. Native to Cerrado biome of Brazil, we can mention baru seeds, which oil has a high degree of unsaturation. Its major constituents are the fatty acids oleic and linoleic, and, besides that it contains significant amounts of tocopherols and phytosterols. Emulsified systems are the most common form presentation of industrial products. Its excellent ability to solubilize hydrophilic and lipophilic compounds explains its scientific and commercial importance. The design of these systems requires knowledge of the main factors that influence their formation, consequently, stability and performance. The objective of this research was the development and characterization of a nanoemulsion with baru oil using an economic process. The steps involved in the planning process of development of the systems were: 1) choice of emulsification methodology 2) development and characterization of formulations; 3) stability studies.

Results: The use of low energy emulsification methodology so, a low-cost process, allow obtention of stable nanoemulsion having particle size distribution between 100.67 ± 8.39 and 111.45 ± 5.44 nm. The study of the physicochemical properties of the formulations showed no signs of instability of the formulations during the storage period under specific temperature conditions and the nanoemulsion has fine appearance, translucent aspect and bluish reflect, which are in accordance with the concept of nanoemulsions.

Conclusions: The optimization of composition variables to minimize droplet size and polydispersity index allowed the obtention of a stable nanoemulsion by using a classic and low cost methodology. The developed system may be a good alternative delivery system, particularly because the use of vegetable oil follows a global trend of using renewable resources for sustainable development in cosmetic and pharmaceutical industry.

Keywords: Baru oil; Nanoemulsion; Stability; Low cost; Cutting-edge system

Background

Studies carried out by Almeida; Silva and Ribeiro [1] on the productivity of Baru native of the Savana do Brazil biome, revealed great economic and sustainable potential being used regionally in human food for presenting significant amounts of lipids, proteins, fibers and minerals.

Their concentration in phenolic compounds is high when compared to macadamia nuts, Brazil nut, cashew nuts and walnuts. Baru oil is rich in polyunsaturated fatty acids, terpenes (mono and sesquiterpenes) and phytosterols [2,3]. Thus, the chemical composition associated with economic potential stimulates the use of baru oil (*D. alata*) as raw material for different industries such as food, pharmaceutical and cosmetic [4-7]. Nanodispersed systems are prepared by methods divided into two categories: high or low energy emulsification, depending on the physical parameters involved in the formation of globules [8].

Phase inversion emulsification methods induce the inversion of water-in-oil (O/W) systems to oil-in-water (O/W) or vice versa, and the curvature of the O/W interface changes gradually. These methods are divided into Phase Inversion Temperature Method (TIF) and Phase Inversion Emulsification (EIF) depending on the phase inversion point and the volumetric ratio between the amounts of the aqueous and oily phases. TIF and EIF are also called Transitional and Catastrophic Phase Inversion, respectively [9].

The principle of Transitional Phase Inversion was introduced by Shinoda and Saito [10] and is based on changes in the properties of surfactants induced by temperature-. Conversely the Catastrophic Phase Inversion (CPI) occurs due to changes in the composition of the formulation (increase in the volumetric ratio of the dispersed phase) during the emulsification process at constant temperature [11-13].

The nanoemulsions present globules in the 50-200 nm size range and for this reason have a larger contact surface allowing greater wettability, spreadability and penetration of the active substances in the skin, besides providing differentiated sensorial. Macroscopically present as liquid systems of low viscosity and with intense blue reflection [14-16].

Nanoemulsion systems are susceptible to instability process, with being Ostwald ripening more common. The phenomenon of instability of higher occurrence in nanoemulsions is known as Ostwald ripening: a process in which the greater solubility of the dispersed phase in the dispersant, conferred by the greater interfacial curvature of the dispersed globule, allows the larger globules to increase in size to the detriment of the smaller ones causing an increase in the average radius of the nanoemulsion globules [9,17,18].

The objective of this research was to propose an economical method to obtain nanoemulsions using baru oil.

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Methods

Chemicals

Croda do Brasil-Sorbitan oleate (HLB value=4.3, Polysorbate 80 (HLB value=15.0);

Oxiteno do Brasil-Ceteareth 5 (HLB value=9.2; Ceteareth 20 (HLB value=15.7); Steareth-2 (HLB value=4.9), PEG-15 Castor oil (HLB value=8.3) PEG-30 castor oil (HLB value=10.8) and PEG-40 Castor oil (HLB value=13.0).

Lipo do Brasil-Polysorbate 80 HLB value=15.0); Chemyunion-*DMDM Hydantoin (and) Iodopropynyl Butylcarbamate* was employed as preservative.

Purified water obtained by reverse osmosis as aqueous phase.

Emulsification method

The formulations were developed according to the phase inversion technique where the aqueous phase heated to 75 ± 2°C was added to the oil and surfactant system at the same temperature while maintaining the mechanical stirring (IKA® RW Digital) at controlled speed (600 rpm) until complete cooling (25 ± 2°C).

Study of the HLB required value for baru oil

The pair of surfactants selected for the study of the HLB value was Sorbitan oleate and Polysorbate-80. The study was divided into two stages (step I and step II) (Figure 1).

Choice of surfactant

Different chemical classes of hydrophilic surfactants and/or lipophilic surfactants in predetermined HLB value were used: Sorbitan oleate and Ceteareth-5; Sorbitan oleate and Ceteareth-20; Steareth-2 and Ceteareth-5; Steareth-2 and Polysorbate-80; Sorbitan oleate and PEG-15 castor oil; Sorbitan oleate and PEG-30 castor oil; Sorbitan oleate and PEG-40 castor oil.

Ternary diagram

Three different conditions were worked based on ternary diagrams:

- The concentrations of each component being varied by 5.0 at 5.0% only in the region where the aqueous phase concentration was greater than 75.0% and the maximum concentration of surfactants 10.0%. Thus ten formulations were obtained using only PEG-15 Castor oil;

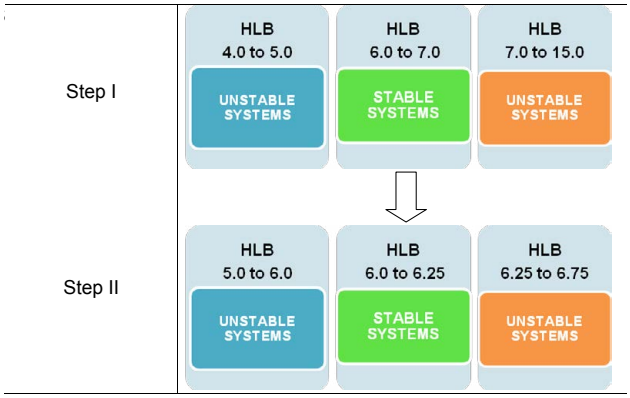


Figure 1: Formulations obtained in the HLB determination test of baru oil.

- The concentrations of each component being varied by 5.0 at 5.0% only in the region where the aqueous phase concentration was greater than 75.0% and the maximum concentration of surfactants was 10.0%. Thus, five formulations were obtained using the surfactant pair PEG-15 Castor oil+Sorbitan oleate.
- The concentrations of each component being varied by 2.5 at 2.5% only in the region where the aqueous phase concentration was greater than 75.0% and the maximum concentration of surfactants was 10.0%. Thus, five formulations were obtained using the surfactant pair PEG-15 Castor oil+Sorbitan oleate.

Preliminary stability test

Centrifugation test: Five g of each nanoemulsion were submitted to centrifuge cycles (70 g; 440 g and 863 g) during 15 min each, at room temperature (25 ± 5°C). Only formulations that did not show creaming or phase separation signs (N=normal or SL=slightly modified) were considerate stable. The tests were performed in triplicate.

Thermal stress

The emulsions were subjected to thermal stress in a thermostated bath at a temperature range of 40 ± 2°C to 80 ± 2°C, increasing the temperature in 5°C steps. The samples were held at each temperature for 30 min and then evaluated macroscopically. For this test, the following nomenclature was used to classify them: N=Normal; without change; LM=Slightly Modified; M=Modified; IM=Heavily Modified.

Physical-chemical characterization of formulations

Macroscopic evaluation: The homogeneity of the formulations were observed after 24 hours of preparation, identifying possible instability processes such as cream, flocculation and/or coalescence. It was also take pictures of nanoemulsion using an inversion light illumination and in different agitation time.

Microscopic evaluation: The distribution and morphology of the globules of macroscopically more stable formulations were subjected to optical microscopy with and without polarized light (Olympus® BX 50 Microscope).

pH value determination: With the aid of a digital pH meter (PG 1800-Gehaka®), determination was made by inserting the electrode directly into the sample at a temperature of 25 ± 2°C.

Electrical conductivity determination

The electrical conductivity was determined at 25 ± 2°C by insertion of the conductivity electrode (DM 32-Digimed®) directly into the sample [17].

Particle size and polydispersity index determination

It was determined by laser diffraction in Beckman Coulter® LS 13 320 equipment and by dynamic light scattering in Zetasizer NanoS Malvern equipment. The readings were performed at 25 ± 2°C after dilution of the samples in purified water in the proportion of 1:10. Particle size results are expressed in terms of the hydrodynamic diameter (Z-average).

Multiple light scattering analysis

The samples were followed for 24 hours with consecutive readings every 1 hour in Turbiscan® Lab Expert-Formulation, France. The system temperature was maintained at 25 ± 2°C.

Accelerated stability test

The formulations were submitted to different values of storage temperature (5 ± 2°C, 25 ± 2°C, 40 ± 2°C) for a period of 90 days (Brazil, 2004). Subsequently, the formulations were evaluated for macroscopic and microscopic appearance, pH value, electrical conductivity and size of globules on the 1st, 7th, 15th, 30th, 60th and 90th days after the preparation.

Results and Discussion

Study of the HLB required value for baru oil

Two classical surfactants Polysorbate 80 and Sorbitan oleate were chosen to find the HLB value for baru oil and a wide range of HLB values was tested in two steps (Figure 1). The results for baru oil required HLB value show a stability range between values 6.0 to 7.0 in step I and between 6.0 and 6.25 in step II.

The results showed that baru oil exhibits an HLB value near 6.25. After HLB determination, others surfactant pair were investigated.

Choice of surfactants: For the development of the nanoemulsified system, the surfactant Sorbitan oleate was associated with other surfactants derived from castor oil having different degrees of ethoxylation. It was concluded that only the use of PEG15 castor oil allows the obtaining of a macroscopically stable nanoemulsified system after the centrifugation test. Similar results were found by Maruno [19] in which the use of this surfactant allowed to obtain a stable nanoemulsified system containing different vegetable oils.

Ternary diagram: As shown in Figure 2, the region around point 36 of the ternary diagram was explored at 5.0% w/w and 2.5% w/w concentration intervals in order to obtain a nanoemulsified system. From 10 formulations point 36 included we have 5 unstable systems tree unstable systems after centrifugation test and only two formulations were selected after the centrifugation test, they were:

1-85.0% purified water: 5.0% baru oil: 10.0% PEG 15 castor oil

5-90.0% purified water: 5.0% baru oil: 5.0% PEG 15 castor oil.

From these results and to obtain formulations with higher oil content, four new formulations were developed so that the oil: surfactant ratio was the highest possible, as shown in Table 1.

Formulations 1.a and 1.b remained stable after the centrifugation test. However, formulation 1.b presented with globules of variable sizes, observed under the optical microscope, which discarded the possibility of being a nanoemulsion system.

Preliminary stability assessment

Formulation 1a was subjected to the macroscopic, microscopic and centrifugation evaluation tests and then to the thermal stress test [20] and after 24 hours of preparation were evaluated for pH, conductivity electric, particle size and evaluated by multiple light scattering technique. The results are shown in Table 2 and formulation 1a (Figures 3 and 4) was chosen for subsequent studies.

The technique of evaluation of the stability by multiple light scattering is considered an important tool in the evaluation of emulsified systems, since it allows predicting phenomena of instability such as sedimentation, cremation, flocculation and coalescence in a short period of time. Modifications along the backscattering chart are related to the phenomena of flocculation or coalescence. Thus, as shown in Figure 5, it is suggested that at untill this step the nanoemulsion system under study, will show changes in particle size during the storage period, which suggests that it is unstable and unsuitable for the proposed objectives [21].

From this result, new formulations containing the pair of surfactants Sorbitan oleate and PEG-15 castor oil were developed (Figure 6) with the addition of a cosurfactant (PEG-40 castor oil HLB value=13.0).

As shown in Figure 6, point 36 and the region around it (4 points A, B, E, F) was studied in order to improve the developed nanoemulsion system. All formulations, except for Formulation A, remained stable after 24 hours of preparation, but showed signs of instability after the centrifugation test.

Formulas	Oil (%w/w)	Surfactants (%w/w)
1.a	6	9
1.b	7	8
1.c	8	7
1.d	9	6

Table 1: Formulations around the point 36 of the ternary diagram.

Nanoemulsions (24 hours)	
pH value	5.66 ± 0.01
Electrical conductivity (µs/cm)	47.23 ± 2.94
Particle size (nm)	85.90 ± 1.61
Polidispersity índex (PdI)	0.188 ± 0.01

Note: results expressed as mean ± SD (n=3).

Table 2: Preliminary stability test - physicochemical characterization.

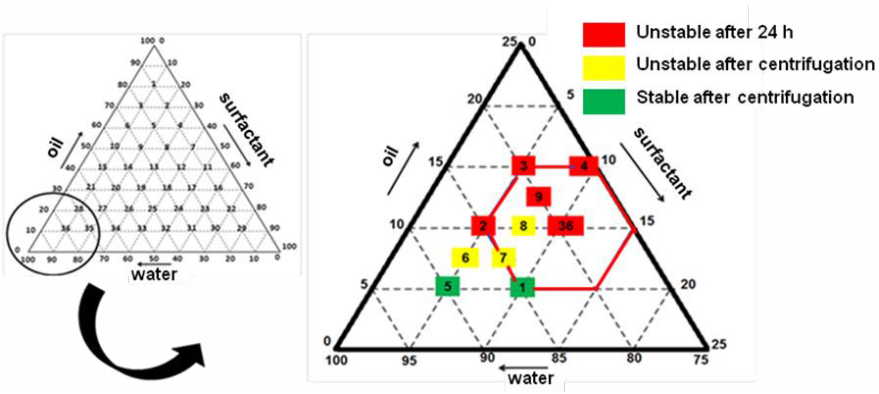
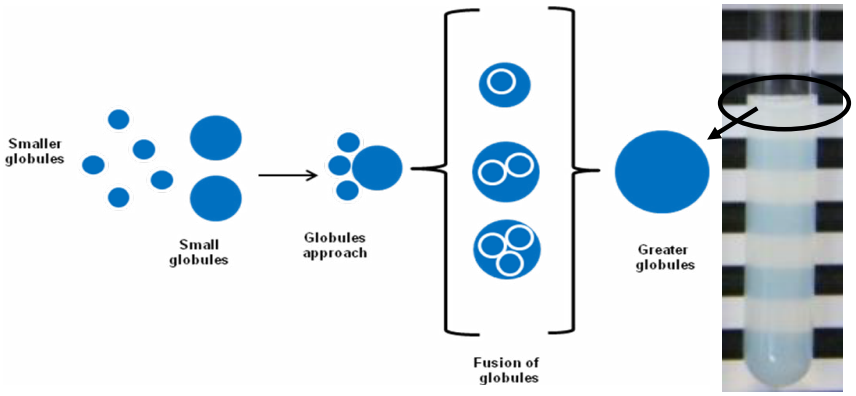


Figure 2: Ternary diagram - nanoemulsified system.



Ostwald ripenig scheme

Formulation 1a

Figure 3: Formulation 1a.

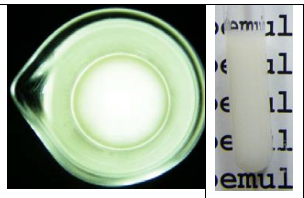
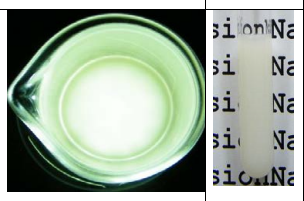
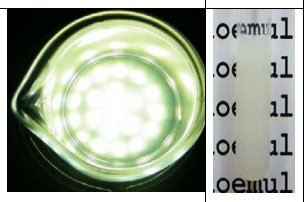
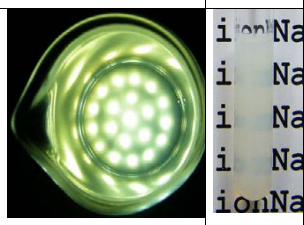
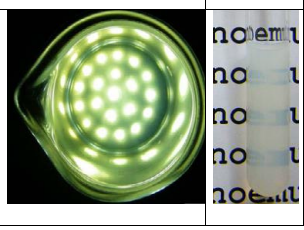
Time 1: 1 minute		**OPAQUE**
Time 2: 2 minutes and 30 seconds		**OPAQUE**
Time3: 5 minutes		**NEARLY TRANSLUCENT**
Time 4: 7 minutes and 30 seconds		**TRANSLUCENT**
Time 5: 10 minutes		**NEARLY TRANSPARENT**

Figure 4: Macroscopic analysis of formulation 1a at different agitation time.

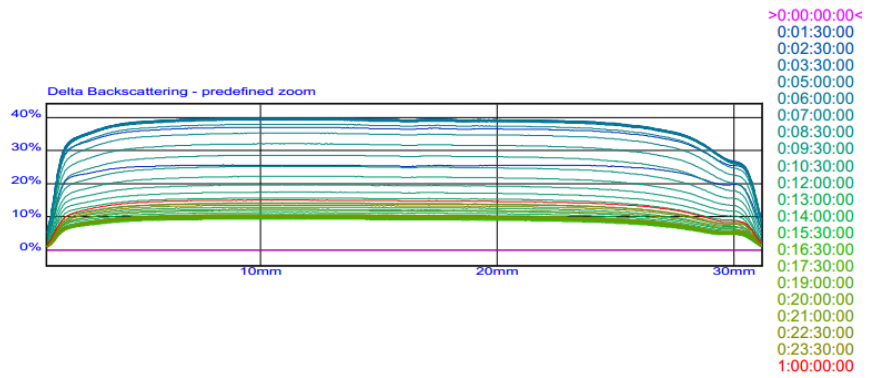


Figure 5: Multiple light scattering analysis of the nanoemulsified system.

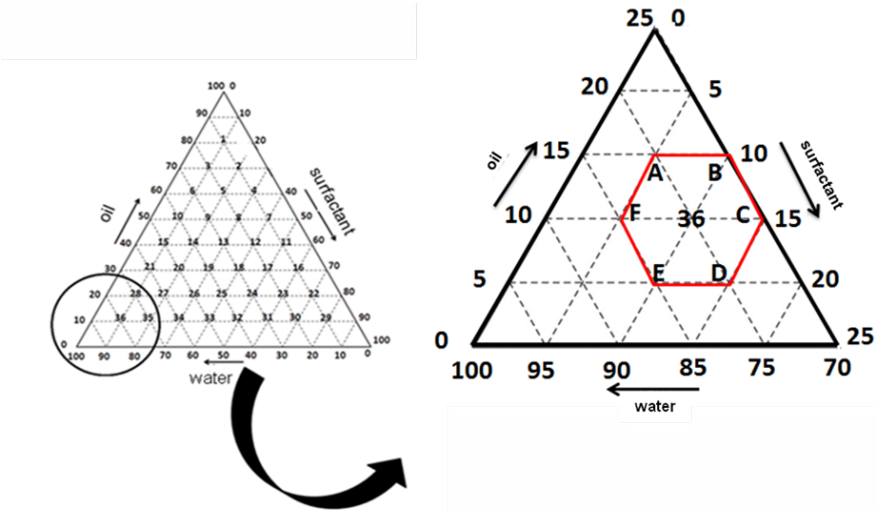


Figure 6: Ternary diagram - nanoemulsified system (PEG-15 castor oil+Sorbitan oleate).

According to the oil: surfactant ratio (10O: 5S) the formulation named as F was chosen and it was added with 2.0% co-surfactant. Thus, the final formulation HLB value would be 8.2 value very close to the HLB value of the surfactant PEG-15 castor oil (HLB=8.3).

As shown in Figure 7, the added cosurfactant formulation presented better profile in the evaluation by the light scattering technique, being, therefore, the one chosen for the tests of accelerated stability.

Stability tests

As shown in Table 3, formulations stored at 5 ± 2°C did not show significant variation in any of the evaluated parameters suggesting that this should be the ideal storage condition for nanoemulsion. The formulations stored at temperatures of 25 and 40 ± 2°C varied from 30 and 7 days, respectively. In the storage conditions at 25 ± 2°C, the formulations showed significant variation in pH and electrical conductivity values while the particle size remained unchanged after the entire storage period-90 days. The detected alterations can be justified by a possible chemical instability due to degradation of formulation compounds caused by hydrolysis of the oil, or even greater solubility of specific components of the oil in the continuous phase of the emulsified system.

Under high temperature storage conditions (40 ± 2°C), the nanoemulsion system may be considered unstable because on the 7th day of the study, significant variations were detected in the following physicochemical characteristics: pH and electrical conductivity. The macroscopic aspect of the formulations remained normal, nanoemulsion has fine appearance, translucent aspect and bluish reflect, which are in accordance with the concept of nanoemulsions, without alterations or signs of phase separation, and only from the 90th day of analysis there was a significant difference in particle size, which results can be justified by the occurrence of the Ostwald ripening phenomenon (Figure 3). This phenomenon, common in nanoemulsions, is a process in which the larger globules grow to the detriment of the smaller ones, due to the greater solubility of the smaller globules in the continuous phase.

The smaller globules diffuse through the continuous phase and are deposited on the larger globules, which causes an increase in the average radius of the globules of the system. According to the Lifshitz-Slyozov-Wagner (LSW) [22] theory the Ostwald ripening rate is dependent on the solubility of the oil, therefore, the addition of a second component with less solubility in the aqueous phase would prevent the occurrence of such phenomenon. Additionally, recent studies suggest that the use of nonionic surfactants associated with amphiphilic polymers could contribute to the formation of a viscoelastic (and therefore more

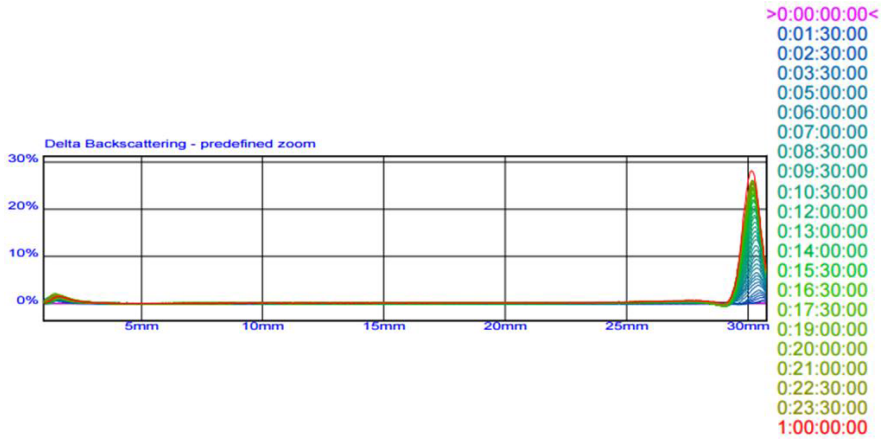


Figure 7: Multiple light scattering analysis of the added nanoemulsified system with cosurfactant.

	Time (days)					
Temperature (± 2ºC)↓	***1***	**7**	**15**	**30**	**60**	**90**
5						
pH value	4.97 ± 0.07	4.99 ± 0.05	4.97 ± 0.02	4.99 ± 0.03	4.97 ± 0.01	4.98 ± 0.02
Electrical conductivity (µs/cm)	70.91 ± 0.47	71.43 ± 0.89	69.06 ± 0.50	71.05 ± 0.36	70.24 ± 0.68	71.10 ± 0.65
Particle size (nm)	106.95 ± 0.92	103.30 ± 3.68	106.20 ± 3.25	108.85 ± 0.78	93.71 ± 2.57[a]	107.35 ± 0.92
Polidispersion índex	0.233 ± 0.03	0.243 ± 0.01	0.225 ± .06	0.251 ± 0.05	0.240 ± 0.00	0.188 ± 0.00
25						
pH value	4.95 ± 0.05	4.90 ± 0.13	4.92 ± 0.06	4.71 ± 0.10	4.48 ± 0.01[a]	4.59 ± 0.08[a]
Electrical conductivity (µs/cm)	70.33 ± 2.30	73.56 ± 2.29	72.45 ± 1.91	78.29 ± 1.92[a]	86.89 ± 1.53[a]	88.55 ± 1.28[a]
Particle size (nm)	111.45 ± 5.44	109.17 ± 4.76	111.85 ± 3.75	106.20 ± 0.71	116.25 ± 0.64	104.29 ± 7.66
Polidispersion índex	0.188 ± 0.01	0.193 ± 0.01	0.197 ± 0.01	0.232 ± 0.03	0.233 ± 0.04	0.231 ± 0.01
40						
pH value	4.90 ± 0.02	4.61 ± 0.17[a]	4.41 ± 0.08[a]	3.98 ± 0.02[a]	3.46 ± 0.06[a]	3.38 ± 0.04[a]
Electrical conductivity (µs/cm)	68.66 ± 1.35	77.37 ± 2.12[a]	84.64 ± 1.99[a]	100.11 ± 4.69[a]	160.00 ± 4.24[a]	198.34 ± 2.56[a]
Particle size (nm)	100.67 ± 8.39	91.25 ± 5.39	95.56 ± 5.49	105.80 ± 1.84	115.80 ± 4.81	140.20 ± 7.07[a]
Polidispersion índex	0.237 ± 0.01	0.170 ± 0.00	0.149 ± 0.2	0.200 ± 0.02	0.089 ± 0.01[a]	0.127 ± 0.05

Note: Results expressed as mean ± SD (n=3). There was a significant difference between the superscript values found ($p<0.05$) when compared to the results of 24 hour analyzes.

Table 3: Accelerated stability test - added nanoemulsified system of cosurfactant.

Figure 8: Photo of added nanoemulsified co-surfactant (PEG-40 castor oil) system.

resistant) interfacial film at the oil/water interface and thus prevent the occurrence of this phenomenon. Such strategy can be used in later studies to improve this nanoemulsified system (Figures 8 and 9) [17,23-27].

Conclusions

Slowly increasing the water volume fraction (without surfactant) allows obtaining O/W nanoemulsions from water in oil emulsions by phase inversion emulsification. Quantitative composition variables were optimized to minimize droplet size and polydispersity index. It was found that addition of the continuous phase to the dispersed phase resulted in the formation of oil droplets with diameters of 100-150 nm. The developed system may be a good alternative skin-care delivery

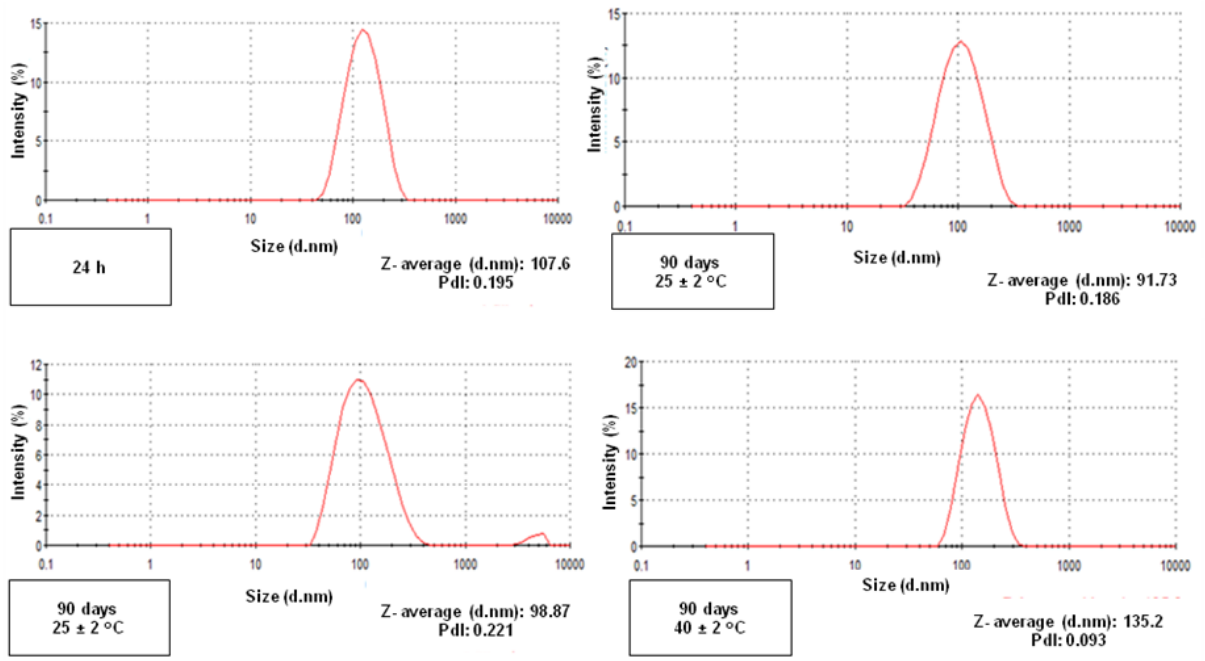


Figure 9: Particle size distribution of the nanoemulsified system added of co-surfactant after 90 days at the different storage temperatures studied.

system, particularly because the use of vegetable oil follows a global trend of using renewable resources for sustainable development in cosmetics.

Acknowledgements

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