Development of Nanoemulsion with Vegetal Oils Enhanced by Melaleuca and Lavender Essential Oils

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Abstract

Vegetable oils have been widely used as a basic component or active ingredient in cosmetic formulations, based on the concept that they are safe and biocompatible with the skin. Oil in water (O/W) Nanoemulsions were produced using Rice bran and Babassu oils and low energy emulsification methods, to evaluate their physical stability. The addition of lavender (Lavandula officinalis) (LEO) essential oil in those NANOEMULSIONS promoted a reduction in droplet size, which can be explained by the possibility of the essential oil molecules to penetrate the interface of the droplet and change its properties, promoting stability to the nanoemulsion. This effect is more pronounced when the essential oil is used in association with other vegetable oils, indicating a synergy action on the stability mechanism. The nanoemulsion was stable over the course of this study. In vitro assays showed that this formulation has a low irritation potential, and when applied to human skin during in vivo studies, improved the skin's moisture content and maintained normal pH value. In this research stable nanoemulsions obtained using essential oil and vegetable oils were evaluated after a period of 60 days. All nanoemulsions physicochemical properties were maintained and particle size were around 77nm.

Keywords: *Nanoemulsion, Essential oils, Babassu, Rice, Lavender, Melaleuca, Stability, Droplet size.*

1. INRODUCTION

Emulsions are vehicles of great importance in cosmetic and pharmaceutical industries, it allows the encapsulation of active hydrophilic and lipophilic drugs in the same formulation, also allowing the control of the formulation's sensory characteristics.

Nowadays, pharmaceutical and cosmetic industries have shown great interest in nanoemulsions due to its characteristics such as high kinetic stability; efficiency in delivering active substance or drugs; excellent sensorial and aesthetic aspects, besides the need for less surfactant (5-10%) when compared to microemulsions, reducing the occurrence of skin irritation as well as production costs [8]. Nanoemulsions (NE) are special systems with uniform and extremely small droplet size, in the range of 20-500 nm. Due to its characteristic size, NEs can be optically transparent or translucent and have low viscosity, excellent spread ability and humectation, resulting in a more homogeneous film after application on the skin. These benefits make NE very interesting in many applications on cosmetic and pharmaceutical fields, as personal care or skin care formulations and as drug delivery systems [9].

Vegetable oils has been widely used as a basic component or active ingredient in cosmetic formulations, based on the concept that they are safe and biocompatible with the skin. The cosmetic industry has used rice (*Oryza sativa*) bran oil in sunscreen formulations, in topical aging prevention products and in treatments for skin diseases, based on the presence of high levels of antioxidant-rich components, such as tocopherols/ tocotrienols and gamma-oryzanol, which could be useful for topical formulations [1].

Rice bran oil NE was produced, and its physical stability, irritation potential and moisturizing activity on volunteers with normal and diseased skin types was evaluated. The NE was stable over the course of this study. *In vitro* assays showed low potential for irritation, and when applied to human skin during *in vivo* studies, improved the skin's moisture and maintained normal skin pH values [1].

Babassu is considered one of the greatest native resources in the world and its oil is used in body and hair formulations. An O/W NE containing babassu oil was prepared and its short-term stability was evaluated. The formulation showed no modification in terms of droplet size, polydispersity index, pH, and electrical conductivity values after thermal stress and heating/cooling cycle tests. Based on these results, the NE obtained can be considered as promising disperse systems for pharmaceutical and cosmetic applications [2].

Essential oils are natural compounds that shows a high range of properties, from aromatics to antioxidant and antifungal activities. As they have volatile compounds, encapsulation in NEs may enhance their efficacy ads stability [6].

According to the literature, besides its low skin toxicity, the lavender essential oil (*Lavandula officinalis*) shows anti-inflammatory and regenerating activity for the skin tissue. Lavender essential oil (*Lavandula officinalis*) is employed as antimicrobial, anti-inflammatory and healing in case of burns, insect bites and other skin inflammation. Studies show that the anti-inflammatory effect is directly related to linalool and linalyl acetate, the major compounds. However, this effect is more pronounced when the essential oil is employed associated to other oils than when used alone, showing a synergy mechanism on inflammatory skin process [8].

Tea tree oil (TTO), an essential oil extracted from the leaves of *Melaleuca alternifolia* (a native Australian tree species), has a wide range of pharmacological actions including antibacterial, anti-fungal, anti-inflammatory, anti-oxidant, anti-tumoral, and immune response regulation effects. The strong antibacterial efficacy of TTO has long been known and widely used in skin and hair care [5].

The aim of this research was to study the influence of lavender and tea tree essential oils addition on the particle size of vegetable oil-based NE, using babassu or rice bran oil.

2. MATERIAL AND METHODS

2.1. Material

Oils: Babassu oil (*Orbignya oleifera* seed oil) was supplied by Croda (Campinas, Brazil) and Rice bran oil was kindly provided by Lipo do Brasil (Brazil). Tea Tree oil (TTO) (*Melaleuca alternifolia* leaf oil) was provided by Fagron. Lavender (*Lavandula officinalis)* Essential Oil (LEO) was purchased from Bioessencia (Brazil).

Surfactants: Sorbitan oleate (HLB= 4.3) was donated by Lipo do Brasil (Brazil); PEG 30 castor oil (HLB= 11.7) and PEG 54 castor oil (HLB=14.4) was donated by Oxiteno (Brazil).

2.2. Nanoemulsion Preparation

The O/W NE were prepared using either babassu oil or rice bran oil (5.0%), PEG-54 castor oil or PEG-30 castor oil and sorbitan monooleate surfactants (10.0%). The hydrophilic-lipophilic balance (HLB) value was 10.0 for babassu oil and 8.0 for rice bran oil NE. The method of preparation was a low energy method, known as Emulsion Phase Inversion (EPI), in which the aqueous phase is heated until 75±5ºC and poured over the oily phase containing surfactants, also heated up at the same temperature. Mechanical stirring

(Ika RW20 digital, São Paulo, Brazil) was maintained at 600 rpm for about 15 minutes, until the emulsion reaches room temperature (25±5°C).

2.3. Macroscopic Analyses

Macroscopic analyses were conducted in all formulations, 24 hours after preparation to observe any sign of macroscopic instability, such as creaming, sedimentation, flocculation or coalescence before/after centrifugation, thermal stress and heating/cooling cycles.

2.4. Centrifugation, Thermal Stress, and Heating/Cooling Cycles

Stability tests were conducted after 24 hours preparation as follows: (i) centrifugation test: samples (5.0 g) were taken and submitted to three increasingly rotation speeds (1000, 2000, and 3500 rpm) (Fanem 206-R, São Paulo, Brazil), for 15 minutes at each speed [10]. Only the formulations that remained stable after the centrifuge test were submitted to thermal stress and heating/cooling cycles; (ii) thermal stress test: vials containing a 5.0 g sample were submitted to a range of temperature, from 40 up to 85±2°C, increasing by 5°C intervals. The samples were kept in each temperature for 30 minutes. The temperature was adjusted using a thermostatic bath (Nova Tecnica NT281, Piracicaba, Brazil) [10] and (iii) heating/cooling cycles: heating/cooling cycles were performed to assess the samples stability to extreme ranges of temperature. This test consisted in submitting the samples at 45±5°C temperature in air stove (Fanem 002-CB, São Paulo, Brazil) for 24 hours and then at 4±2°C temperature in refrigerator (Electrolux RDE-37, São Carlos, Brazil) for another period of 24 hours, thus completing one cycle (2 days). Six cycles (12 days) were performed in total [11] Physicochemical characteristics (pH, electrical conductivity, density refractive index and viscosity values, droplet size, zeta potential and polydispersity index) were evaluated before and after thermal stress and heating/cooling cycles. All testes were made in triplicate.

2.5. Droplet Size and Polydispersity Index Determination

Droplet size and polydispersity index of NE were determined by dynamic light scattering (DLS) (Nanosizer Malvern ZS, Worcestershire, UK) at a scattering angle of 173°. The samples were diluted in purified water by 1:100 ratio at 25°C. Samples were taken in two different conditions: (1) slightly stirred with a glass rod and (2) not stirred, to determine particle size.

2.6. pH and Electrical Conductivity

pH was determined by pH meter (Analion PM-608, Ribeirão Preto, Brazil), previously calibrated, by direct insertion of the electrode on the sample [4]. The conductivity was measured in the emulsions at room temperature with the help of conductivity meter (Tecnopon MCA150), previously calibrated [7]. Measurements were made in triplicate.

2.7. Viscosity Determination

The viscosity of the formulations (1.5 mL) was determined without dilution using an Ostwald viscometer in thermostatic bath at 25 ^{0}C. Measurements were made in triplicate.

2.8. Refractive Index (RI)

The RI of the system was measured by an Abbe refractometer (Quimis, Equipalabor, Ribeirão Preto, SP, BR) by placing one drop of the formulation on the slide, at 25 ± 2 ^{0}C (compared to purified water). Measurements were made in triplicate.

2.9. Statistical Analysis

All the measurements were performed in triplicate and the results were presented as mean and standard deviation (SD). Analysis of variance values obtained in tests with a significance level of 95%, nonparametric Mann-Whitney were performed.

3. RESULTS AND DISCUSSION

3.1. Nanoemulsion Preparation

Table 1 presents the NE compositions at HLB values of 10.0 and 8.0, respectively.
NE were prepared by low energy emulsification technique involving transitional inversion induced by increasing the dispersed phase volume fraction. The same technique was also studied by [3] that compared phase inversion composition methods and high-pressure homogenization methods. The results showed more stable NE with low energy methods [3].

3.2. Centrifugation, Thermal Stress, and Heating/Cooling Cycles

After centrifugation and heating/cooling cycles, no signs of instability were observed. Some samples showed a slight ring formation on the surface and it may be caused by junction of smaller globules with bigger globules originated by different values of Laplace tension. As the surfactant curvature is not continuous, like showed in figure 1, the junction of NE globules may occur. This phenomenon is known as Ostwald ripening and may be responsible for the existence of more than one population, of different sizes, in some NEs.

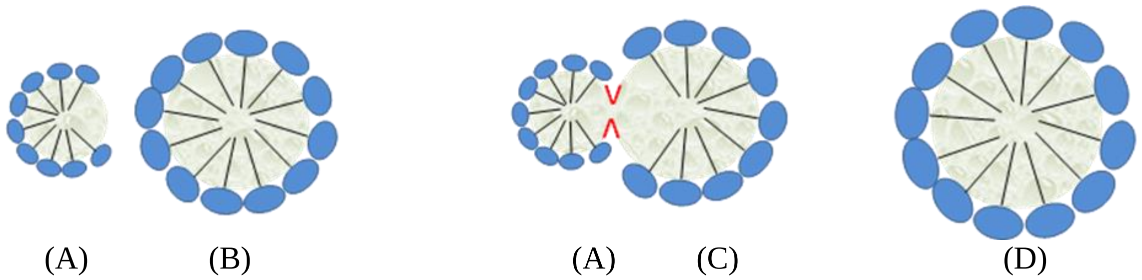

Figure 1. *Ostwald ripening junction where: (A) smaller globules with deficiency in the surfactant curvature; (B) globule with perfect surfactant curvature; (C) larger globules with deficiency in the surfactant curvature; (D) greater NE globules. (A)+ (B)= repellency; (A) + (C)= junction forming (D).*

Table 1. *NE composition (% w/w).*

Components	*HLB value*	
	10.0	*8.0*
Rice bran oil (R nano)	-	5.0
Babassu oil (B nano)	5.0	-
Sorbitan oleate	4.36	5.0
PEG-30 castor oil	-	5.0
PEG 54 castor oil	5.64	-
Purified water	85.0	85.0

3.3. pH Value

pH value for both babassu and rice bran oil NE (Tables 3 and 4) were compatible with skin's pH value, allowing topic applications of both NE. Surfactants employed were also known as safe for topic application.

3.4. Electrical Conductivity

This test was used to determine the character of NE external phase. As showed on tables 3 and 4, the high values in electrical conductivity show the hydrophilic character of NE external phase. Babassu oil NE has hydrophilic character higher than rice bran oil NE, almost the double. TTO addition causes slight increase for both NE and LEO have contrary effect - it diminishes conductivity values. For both NE the addition of TTO+LEO causes an increase in conductivity values, therefore in the hydrophilic character of NE.

3.5. Density

The density values for all NE were approximately 1.0 g/cm^3, the density value of water, showing the lightness of the nano dispersions, a desirable characteristic for cosmetic products.

3.6. Viscosity Determination

The viscosity values of the NE are given in Table 3 and 4. The viscosity of NE formulation was found to be as low as water viscosity. It can be observed that, in general, viscosity of all formulations was very low.

3.7. Refractive Index

No significant difference was observed in the refractive indexes of formulations. RI is an optical property that can be used to describe the isotropic nature of the NE. From our results, we may suppose that NE besides its isotropic character were very stable, after the stress thermic test evaluation

3.8. Globule Size and Size Distribution Analysis

For understanding the stability of NE, the information on droplet size and size distribution is very important and can reflect the release and absorption of the drug dispersed in these systems.

Polydispersity is the ratio of standard deviation to the mean droplet size and denotes the uniformity of droplet size within the formulation. The lower the polydispersity value, the higher is the uniformity of the droplet size in the formulation.

***Table 3**. Physicochemical properties of rice bran oil NE (R nano).*

	Formulation			
Properties	Rice bran oil NE	Rice bran oil NE + TTO	Rice bran oil NE + LEO	Rice bran oil NE+ TTO+ LEO
pH	5.58 ± 0.098	4.89 ± 0.173	5.41 ± 0.155	5.14 ± 0.219
Electrical conductivity (µS/cm)	246.93 ± 0.896	261.36 ± 1.106	250.4 ± 0.435	262.33 ± 0.493
Density (g/cm^3)	0.99 ± 0.009	0.99 ± 5.77E-05	0.994 ± 0.001	0.995 ± 5.77E-05
Relative density	0.994 ± 0.009	0.998 ± 5.77E-05	0.996 ± 0.000	0.997 ± 5.77E-05
Refractive index	1.5835 ± 0.0	1.592 ± 0.0	1.5933 ± 0.0	1.5980 ± 0.0
Viscosity (N.s/m^2)	3.835	2.720	3.972	3.373

Table 4. *Physicochemical properties of Babassu oil nanoemulsion (B nano).*

Properties	Formulation Babassu NE	 Babassu NE + TTO	 Babassu NE + LEO	 Babassu NE+ TTO+ LEO
pH	6.24 ± 0.094	5.94 ± 0.028	5.84 ± 0.011	5.67 ± 0.030
Electrical conductivity (µS/cm)	528.63 ± 0.808	530.36 ± 2.247	512.10 ± 3.292	513.76 ± 1.517
Density (g/cm^3)	1.00 ± 1E-04	1.00 ± 5.77E-05	0.979 ± 0.033	0.978 ± 0.032
Relative density	1.00 ± 1E-04	1.00 ± 5.77E-05	0.9808 ± 0.033	0.979 ± 0.032
Refractive index	1.6283± 0.0	1.6145± 0.0	1.5916 ± 0.0	1.6050 ± 0.0
Viscosity ($N.s/m^2$)	2.075	1.889	1.695	1.750

Table 5*. PdI and particle size of NE.*

Stirred (*)					Not stirred					
Nanoemulsion	**PdI**	**Peak**	**Size (d. nm)**	**Intensity**	**St. Dev. (d. nm)**	**PdI**	**Peak**	**Size (d. nm)**	**Intensity**	**St. Dev. (d. nm)**
R nano	0.454	1 2	143 4592	96.5 3.5	96.63 832	0.287	1 2 3	106.6 15.13 4236	94.6 3.9 1.5	62.82 3.427 991.6
28 days	0.398	1 2	124.7 4767	96.9 3.1	106.0 745.0	0.267	1 2	98.95 3900	97 3	65.41 1118
60 days	0.489	1 2	205.4 37.34	76.8 23.2	49.55 5.713	0.418	1 2	189.8 40.0	76.0 24.0	44.62 7.293
R+ LEO	0.245	1 2	69.75 4288	96.7 3.3	36.27 971.4	0.243	1 2	77.49 4478	98.9 1.1	55.27 889.2
28 days	0.283	1 2	218.9 17.46	99.5 0.5	120.5 2.659	0.287	1 2	244.2 52.96	85.2 14.8	87.32 12.81
60 days	0.318	1 2	302.4 48.76	87.1 12.9	57.83 5.67	0.318	1 2	307.0 54.01	88.2 11.4	6749 7.889
R+ TTO	0.445	1 2	194.2 36.52	82.0 18.0	94.54 12.28	0.442	1 2	184.6 31.51	84.6 15.4	93.76 9.944
28 days	0.448	1 2	262.8 49.51	72.4 27.6	93.87 11.77	0.440	1 2	251.5 47.13	77.0 23.0	105.6 12.39
60 days	0.542	1 2	326.8 53.58	67.6 32.4	88.33 13.75	0.591	1 2	277.3 40.63	72.6 27.4	68.14 7.809

(*) slightly stirred with a glass rod.

Stirred (*)						Not stirred				
Nanoemulsion	**PdI**	**Peak**	**Size (d. nm)**	**Intensity**	**St. Dev. (d. nm)**	**PdI**	**Peak**	**Size (d. nm)**	**Intensity**	**St. Dev. (d. nm)**
B nano 28 days 60 days	0.138 0.132 0.212	1 1 1 2	236.7 234.8 201.7 4904	100.0 100.0 96.5 3.5	79.15 87.11 51.90 662.4	0.156 0.143 0.153	1 1 1	234.3 248.0 234.3	100.0 100.0 100.0	96.6 101.2 58.24
B + LEO 28 days 60 days	0.154 0.134 0.168	1 1 1	158.9 154.0 161.8	100.0 100.0 100.0	63.97 63.74 93.98	0.142 0.134 0.159	1 1 1	153.5 154.0 135.6	100.0 100.0 100.0	56.75 63.74 30.66
B + TTO 28 days 60 days	0.211 0.191 0.239	1 1 2 1 2	215.3 193.5 4857 225.0 64.48	100.0 98.8 1.2 88.5 11.5	100.7 82.74 695.0 57.07 10.89	0.195 0.171 0.213	1 2 1 2 1	198.9 4812 193.5 4857 196.9	98.9 1.1 98.8 1.2 100.0	86.48 718.7 82.74 695 50.87
B + TTO + LEO 28 days 60 days	0.213 0.192 0.241	1 2 1 2 1 2	204.0 44.55 177.4 4928 183.3 54.58	95.4 4.6 98.6 1.4 89.3 10.7	89.18 8.99 73.22 650.9 46.42 7.432	0.176 0.192 0.220	1 1 2 1 2	189.2 177.4 4928 195.0 51.84	100.0 98.6 1.4 88.7 11.3	69.68 73.22 650.9 52.50 7.486

(*) slightly stirred with a glass rod.

As we can see in Table 5, not stirred NE show smaller particle size when compared to those that were slightly stirred with a glass rod. Stirred babassu NE has the smaller PdI value (0.138) and LEO addition causes a slight increase in PdI values compared to TTO addition, changing from 0.154 with the first one to 0.211 for the last one.

NE with smaller particle size were obtained for stirred R+LEO (69.75 nm) and not stirred R+LEO (77.49) and both NE have similar PdI values (0.245 and 0.243, respectively).

It can also be observed that TTO addition increases particle size of both, babassu and rice bran oil NE, in contrast to that observed with LEO addition. Both nanoemulsions added with TTO and LEO simultaneously show only a population (100%) in a specific particle size, ie, 130.4 nm for rice bran oil NE and 189.2 nm for babassu NE. We may suppose components of LEO act synergically to TTO components which makes possible to obtain NE with smaller particles size and therefore more stable systems.

4. CONCLUSIONS

Stable NE were obtained by low energy methods and the stirring rate most effective was 600 rpm. B nano and R nano shows hydrophilic external phase and B nano was the most hydrophilic and most stable. Both NE have pH value near to pH skin, allowing topic use. LEO cause reduction in size particle and we can observe a sinergical effect when associated to TTO and in this case forming a NE with an only size particle population and very stable NE.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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