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Rheological behavior, zeta potential, and accelerated stability tests of Buriti oil (*Mauritia flexuosa*) emulsions containing lyotropic liquid crystals

Cinthia Fernanda Zanatta, Anne Miwa Callejón de Faria Sato, Flavio Bueno de Camargo Junior, Patrícia Maria Berardo Gonçalves Maia Campos and Pedro Alves Rocha-Filho

Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, São Paulo, Brazil

Abstract

Background: It is well known that the Amazon region presents a huge biodiversity; therefore, countless natural resources are being employed in the production of phytocosmetics and phytomedicines. *Objective*: The purpose of this work was to obtain emulsions produced with Buriti oil and nonionic surfactants. *Methods*: Two surfactant systems were employed (*Steareth-2* associated to *Ceteareth-5 and to Ceteareth-20*) to produce the emulsions using phase diagram method. Emulsions were obtained by echo-planar imaging method at 75°C. Rheological behavior and zeta potential were evaluated, and accelerated stability tests were performed. *Results*: All emulsions analyzed presented pseudoplastic behavior. Zeta potential values were obtained between −14.2 and −53.3 mV. The formulations did not show changes in either physical stability, pH, or rheological behavior after accelerated stability tests. Significant differences were observed only after temperature cycling test. *Conclusion*: Based on these results, the emulsions obtained could be considered as promising delivery systems.

Key words: *Buriti oil; emulsion; liquid crystal; nonionic surfactant; rheology*

Introduction

Usage of natural ingredients in the cosmetics is steadily increasing. As a result, formulators are being offered a host of newly derived lipids, mostly from renewable plants sources[1]. It is well known that the Amazon region presents a huge biodiversity; therefore, countless natural resources are being employed, by the industry, in the production of phytocosmetics and phytomedicines. Nowadays, products obtained from the Brazilian flora are being tested in cosmetics formulations, such as the oils extracted from the Amazonian plants, Copaiba (*Copaifera L.*), Annato (*Bixa orellana*), Andiroba (*Carapa guyanensis*), and Buriti (*Mauritia flexuosa*), whose fruits and seeds are very rich in phytochemicals with antioxidants properties[2,3]. Unfortunately the knowledge of the benefits of a large variety of natural products is still the heritage of a native population. Exploiting and understanding the physical and chemical properties of these natural products has been a challenge to researchers for the past few years[4].

The Buriti is a palm tree that grows up in the north and central regions of Brazil and is called by the natives as the 'tree of life'. The oval fruit has a yellow to reddish pulp and is considered as one of the most notorious sources of carotenoids in nature. The Buriti oil contains about 1706 ± 54 μg of total carotenoids/g, and β-carotene is considered the major carotenoid performing 90% of total content[5]. The oil also presents high levels of oleic acid (60.3%) and considerable amounts of alfa-tocopherol (643.2 mg/g)[6]. Several authors studied the antioxidant activity of carotenoids and vitamin E in the skin and the synergism of their association[7,8]. According to Mortensen[9], carotenoids showed to present a peroxide radical scavenging activity in human skin and also a capacity of inhibiting lipid peroxidation. Stahl and Sies[10] also reported an ability of avoiding the formation of epidermal erythema during sun exposition.

Address for correspondence: Dr. Cinthia Fernanda Zanatta, PhD, Department of Pharmaceutical Sciences, University of São Paulo, Av do Café s/ n/, Ribeirão Preto 14040903, Brazil. Tel: + 55 11 4524 0766, Fax: + 55 11 4524 0618. E-mail: cinthiafz@yahoo.com.br

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Fluid and semi-solid emulsions structured by surfactant mixtures are widely used in pharmacy and cosmetics for their therapeutic properties and as vehicles to deliver drugs and cosmetics agents to the skin. Through the use of nonionic surfactants, long-term stability is conferred to the product by their ability to prevent the close approach of the oil droplets by structuring the continuous phase. They are also used to control rheological properties of the formulation between wide limits[11]. In addition, a reversible increase in the permeability of the stratum corneum can be induced by nonionic surfactants, without irreversible skin irritation[12].

However, cosmetics emulsions, such as lotions and creams, are rarely simple two-phase oil-in-water systems, and their study and development is one of the most complex subjects in dermatopharmacy. Such preparations often contain several interacting excipients and may be composed of additional phases to oil and water. In aqueous systems containing surfactant/fatty alcohol combinations, the additional phases generally form when the emulsifier, above the CMC, interacts with the continuous phase (water) to form a gel network of vastly swollen bilayer structure[13,14].

These structures are called liquid crystals and can be formed by two main mechanisms and are referred to as thermotropic or lyotropic. The thermotropic liquid crystals are formed by the action of heating and controlled cooling and the lyotropic liquid crystals (LLC) are formed by the solvent interaction of three components—usually water, oil, and surfactant, therefore being the most commonly found form in cosmetics[15,16]. Further, these LLC take on a structural arrangement depending on the type of surfactant and its concentration. These structures, when surrounding droplets in emulsion, can enhance stability by preventing droplet coalescence because of viscosity increase[16].

The LLC systems exhibit good penetration, because of the very low interfacial tension arising at the oil/water interface[17,18], and they may facilitate the progressive diffusion of biologically active substances into the skin or systemic circulation[19,20]. They can bring about a considerable increase in the solubility of drugs by means of solubilization, which are either insoluble or slightly soluble in water[21,22].

The study of phase diagrams of surfactant–oil–water not only allows a better understanding of complex interactions between the systems' components but also permits the attainment of different cosmetic forms using a unique water–Buriti oil–surfactant system. Furthermore, we should take into account that there are important reasons to encourage the use of plant-derived fats and oils. The belief that not only these lipids are similar (or even identical) to those normally found in the human skin, but also these edible substances are safer for use and the presence of active substances might bring benefits to the skin and human health. Owing to these properties, emulsions containing Buriti oil should not be considered simply as a vehicle to carry drugs or cosmetics agents but also as a product that possesses a therapeutic or cosmetic activity.

Because of this, the aim of this work was to obtain stable sustained release emulsions with liquid crystals using Buriti oil and nonionic surfactants. Subsequently we evaluated the rheological behavior, zeta potential, and performed an accelerated stability test of the selected formulations.

Material and methods

Material

The Buriti (*M. flexuosa*) oil was supplied by Croda Ltd. (Campinas, Brazil). Three nonionic surfactants were used: *Steareth-2* (Brij®72) supplied by Beraca Sabará (Santa Barbara, Brazil), *Ceteareth-5* (Unitol® CE 50) and *Ceteareth-20* (Unitol® CE 200F) acquired from Oxiteno S.A. (São Paulo, Brazil). As watery phase, freshly distilled water was used.

Methods

Emulsion preparation using phase diagram method

Two diagrams were prepared varying the concentrations of the three components by 10% intervals to cover the whole area of the triangle and by 5% to define the limits[23,24]. Emulsions were prepared by emulsion phase inversion method. Two surfactants systems were used, *Steareth-2* (HBL = 4.9) associated with *Ceteareth-5* (HBL = 9.2) (phase diagram A or combined with the *Ceteareth-20* (HBL = 15.7) (phase diagram B) both at critical HBL = 7.25, which one was previously determined. The oily phase was added to the surfactant systems and both were heated up to 75 ± 2°C. The heated watery phase was shed latter under constant stirring (Mechanic Mixer Fisatom Mod. 713 D) at 600 rpm until it reached room temperature (25 ± 2°C).

Macroscopic analysis

Macroscopic analyses were conducted in all formulations, 24 hours after preparation to observe any sign of macroscopic instability, such as creaming or coalescence (phase separation).

Optical microscopy

Macroscopically stable formulations were submitted to microscopic analysis performed on a polarized light view microscope (Olympus BX 50). The occurrence of anisotropic areas was observed by optical microscopy under polarized light indicating the presence of the LLC phases.

Preliminary stability test

Emulsions that were macroscopically stable were submitted to preliminary centrifugation tests, performed in a Excelsa Baby II centrifuge (Fanem Ltd., São Paulo, Brazil) at 1000, 2500, and 3500 rpm (70, 440, and 863 × *g*, respectively) for 15 minutes each[25].

Study of viscosity and rheological behavior

The shear stress as a function of shear rates and viscosity measurements of the emulsions were obtained with a cone and plate rheometer (Brookfield RVDV III) operated by Rheocalc software. The measurements were carried out at 25°C, using a Spindle CP-52. For the characterization process, analyses were performed in duplicate after 24 hours and 7 days of emulsions' preparation. For the accelerated stability test, measurements were conducted after 24 hours of preparation and 15 days under stress conditions, in triplicate.

Determination of zeta potential

Electrophoretic mobility was determined using a Delsa 440SX equipment (Coulter Electronics, Boston, MA, USA) and thereby the zeta potential. The system analyzes particle and colloid mobility sized from 0.02 to 30 µm in liquid dispersions by independent and synchronized measures with a laser Doppler in four different angles (8.9, 17.6, 26.3, 35.2°). Samples were diluted with distilled water in a ratio of 1:200 and added to the equipment sampler[26].

Accelerated stability test

Samples were weighted (30 g) and packaged in transparent polyethylene flasks with 50 g of content capacity, respecting the head-space[27]. Samples were analyzed after 24 hours of preparation (t_0), and all assays were conducted at room temperature (24.0 ± 2.0°C) in triplicate.

Emulsions were stored at room temperature (24.0 ± 2.0°C), low temperature (4.0 ± 1.0°C), and high temperature (40.0 ± 1.0°C) during 15 days. The temperature cycling test was also performed during 12 days; six cycles were conducted in a range of 4.0–40°C, changing the temperature every 24 hours[28].

At the pre-determined times, samples were removed from the storage conditions and allowed to achieve room temperature (24.0 ± 2.0°C) prior to the evaluation of pH, viscosity, stability test in centrifuge, and macroscopic analysis.

Results and discussion

Fifty-seven emulsions were produced in the phase diagram A, using Buriti oil–water–*Steareth-2*/*Ceteareth-5*. The macroscopic analysis revealed that 22 of these remained stable after 24 hours and were submitted to preliminary centrifugation tests. The results of the centrifugation tests showed that only 13 emulsions did not present instability signs, such as creamy or coalescence, after the stage of 3500 rpm. The emulsions that remained stable were obtained at surfactant concentrations from 10% to 30% and from 5% to 30% of oil (Figure 1A).

As for the phase diagram B, produced with Buriti oil–water–*Steareth-2*/*Ceteareth-20*, similar results were found. From 55 emulsions produced, 22 were macroscopically stable after 24 hours and 12 remained stable after centrifugation tests. Similar to the phase diagram A, the stability region was characterized by emulsions prepared with surfactant concentrations between 10% and 30% and from 5% to 20% of oil (Figure 1B). Although both hydrophilic surfactants have the same carbon

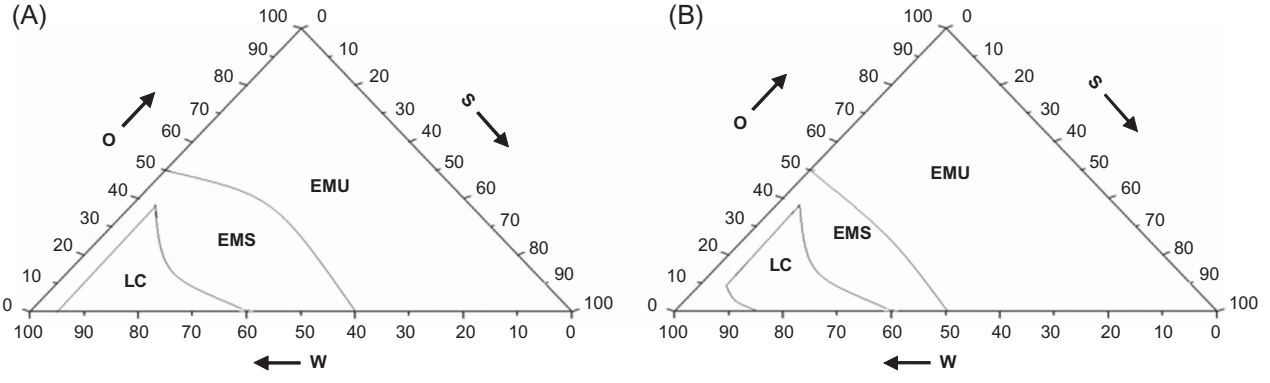

Figure 1. Scheme of results obtained in phase diagram A (Buriti oil : water : *Steareth-2*/*Ceteareth-5*—HLB = 7.25); phase diagram B (Buriti oil:water:*Steareth-2*/*Ceteareth-20*—HLB = 7.25). LC: emulsions containing liquid crystals and stable after centrifugation test; EMS: emulsions macroscopically stable before preliminary stability test, EMU: emulsions macroscopically unstable after 24 hours of preparation. Emulsions were produced at 75°C.

chain, the difference on the ethoxylation rate allowed the emulsification of 10% more oil by the *Ceteareth-5*, compared to the *Ceteareth-20*, because of its higher hydrophilicity.

The microscopic evaluation revealed the presence of homogeneous anisotropic regions in almost every emulsion stable to centrifugation tests (9 emulsions produced with *Ceteareth-5* and 10 with *Ceteareth-20*). These anisotropic regions are characteristic to liquid crystalline phases and were identified as lyotropic lamellar liquid crystals as it can be seen in the photomicrographs presented in Figure 2. Several authors have

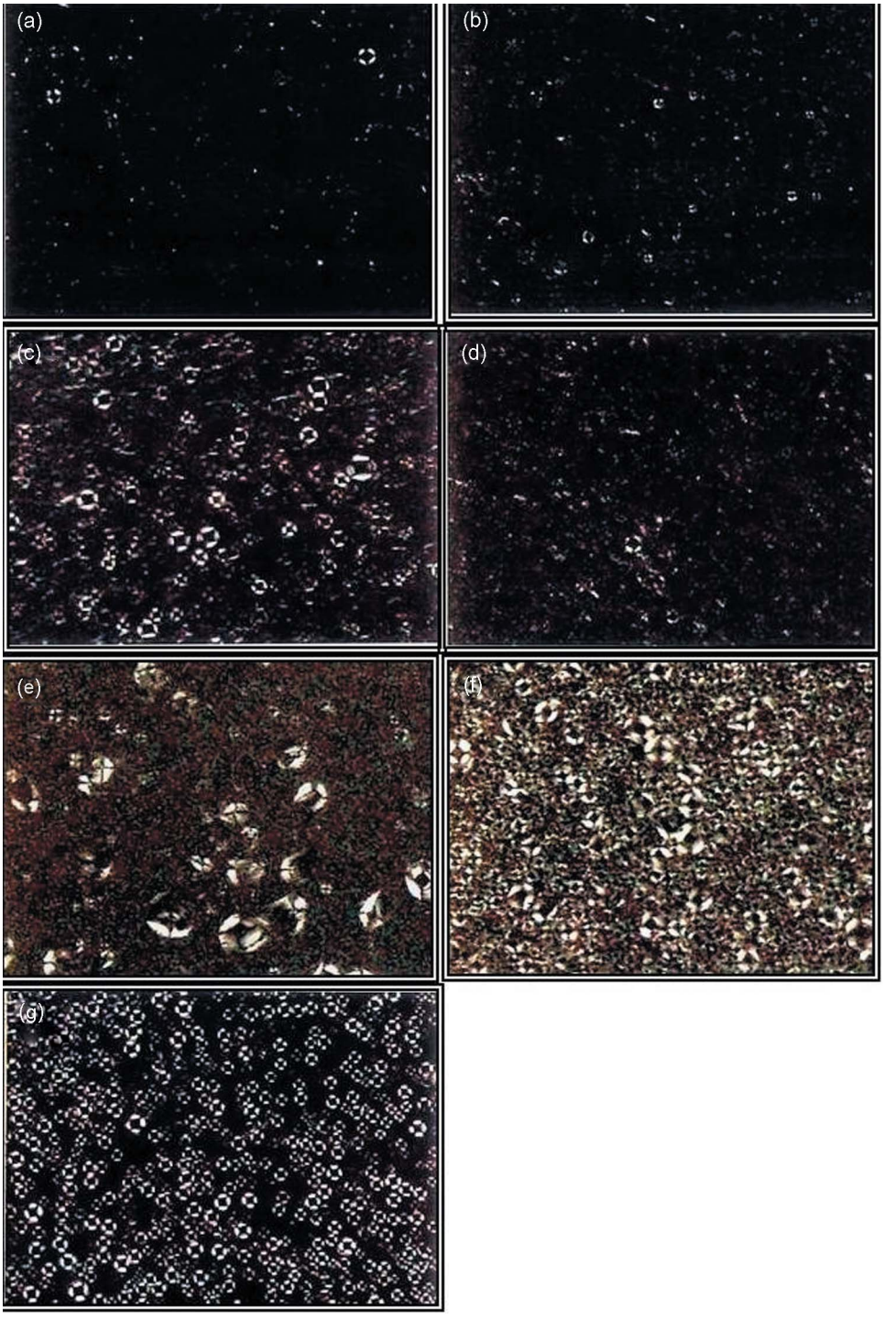

Figure 2. Photomicrographs, under polarized light, of the formulations F1A (a), F2A (b), F3A (c), and F4A (d) obtained from phase diagram (A) of Figure 1; F1B (e), F2B (f), and F3B (g) obtained from phase diagram (B) of Figure 1.

already reported the formation of lamellar liquid crystal phases in emulsions produced with ethoxylated fatty alcohols and vegetal oils[2,26,29] and its changes according to chain size and ethoxylation number.

In accordance to literature data[13,15,16], the majority of the Buriti oil emulsions, stable to centrifugation, contained liquid crystalline phases, suggesting that lamellar phases contributed to emulsion's stabilization by increasing viscosity and acting as a barrier to coalescence. Among the stable emulsions with liquid crystals, those containing higher water ratios presented an increased liquid crystalline phase formation.

It was also noticed, for both surfactant systems, that the region of the diagram where 5% of surfactant was used produced barely stable emulsions and no liquid crystalline phases were observed. A restricted number of formulations remained stable after centrifugation test when liquid crystals were absent, confirming its important role in these emulsions' stability. After the centrifugation test, the stability region in diagram A comprised 10–15% of surfactants (45.4% of *Steareth-2* and 54.6% of *Ceteareth-5*) and 10–20% of oil. As for the diagram B, the stable emulsions were composed of 15–20% of surfactants (80.7% of *Steareth-2* and 19.3% of *Ceteareth-20*) and from 5% to 15% of Buriti oil.

Many factors are known to change the stability of emulsions, for example, components, composition, and preparation method are important factors. According to Chung et al.[30] oil components greatly affect the stability of emulsions, as most of the vegetable, animal, and mineral oils, usually, are not pure compounds. This factor could explain the few stable emulsions obtained in the ternary diagrams, considering the characteristics of the Buriti oil[5]. The authors also observed that more hydrophobic the oils (higher O/W interfacial tension), more stable are the emulsions formed and smaller is the average particle size when compared to less hydrophobic ones.

A characterization process was performed to select an emulsion of each diagram (A and B) that would be submitted to an accelerated stability test. Macro- and microscopic analyses associated with the results of stability centrifugation tests reduced the number of samples from 24 to 7. Four emulsions prepared with *Steareth-2*/*Ceteareth-5* and three produced with *Steareth-2*/*Ceteareth-20* (component's concentrations described in Table 1) had their rheological behavior, viscosity, and zeta potential evaluated.

Measurements after 24 hours showed that all the formulations analyzed presented pseudoplastic behavior, but only one did not show thixotropy. No changes on the rheological behavior were observed after 7 days (Figure 3). We could not establish a correlation between the shear stress values and the surfactant system used to prepare the formulations. The variation of the shear stress seems to be more related to the formulations' composition (ratio of water/oil/surfactant system) and the interactions between the components than to the type of surfactant used or its concentration isolated, as emulsions with the same surfactant concentration presented different shear stress values. Lee et al.[31] reported that the rheological behavior of an emulsion is highly dependent on their composition and therefore the volume fraction of the dispersed phase is one of the most influencing factors on emulsion's viscosity. Considering that the liquid crystalline phase, which governs the viscosity of the continuous phase, is formed by the interaction of the three components of the emulsion, we assumed that their structuration are highly dependent on the components' concentration, which could affect the viscosity and would explain the difference in the obtained results.

Table 1. Percentages of the components used to produce the emulsions obtained in both phase diagrams that were characterized.

Formulation[a]	Oil (w/w) %	Water (w/w) %	Surfactant (w/w) %[b]		
			Total	Surfactant A	Surfactant B
F1A	30	60	10	4.9	5.1
F2A	20	70	10	4.9	5.1
F3A	10	80	10	4.9	5.1
F4A	10	70	20	9.78	10.22
F1B	20	60	20	15.64	4.36
F2B	10	70	20	15.64	4.36
F3B	5	80	15	11.74	3.26

[a]Phase diagram A composed of Buriti oil : water : *Steareth-2*/*Ceteareth-5*; phase diagram B composed of Buriti oil : water: *Steareth-2*/*Ceteareth-20*.
[b]Surfactant A, *Steareth-2*; Surfactant B, *Ceteareth-5* or *Ceteareth-20*.

Emulsions containing liquid crystals normally present an increase of viscosity with age because of the reorganization of the microstructure of the emulsion and the liquid crystalline phases[26,32]. After 7days of preparation, four emulsions (F2A, F3A, F2B, and F3B) showed this increase of viscosity, the results of which are presented in Table 2. On the other hand, two of them showed lower viscosity values after 7 days, as the formulation F4A and F1B. The formulation F1A did not present any change in the same period.

This behavior could be explained by the quantity of liquid crystals that were formed in these emulsions. The photomicrographs presented in Figure 2 show that the formulations F2A, F3A, F2B, and F3B presented higher amounts of liquid crystals when compared to the F1A, F4A, and F1B. The analyses also showed that the emulsions prepared with *Ceteareth-20* (F1B, F2B, and F3B) presented higher apparent viscosity in comparison to those produced with *Ceteareth-5*. This increase could be explained by the differences in the formation and structuration of liquid crystalline phases. These differences are influenced by the curvature and the undulations of the liquid crystals, which depend on the kind of surfactant used and its concentrations[33].

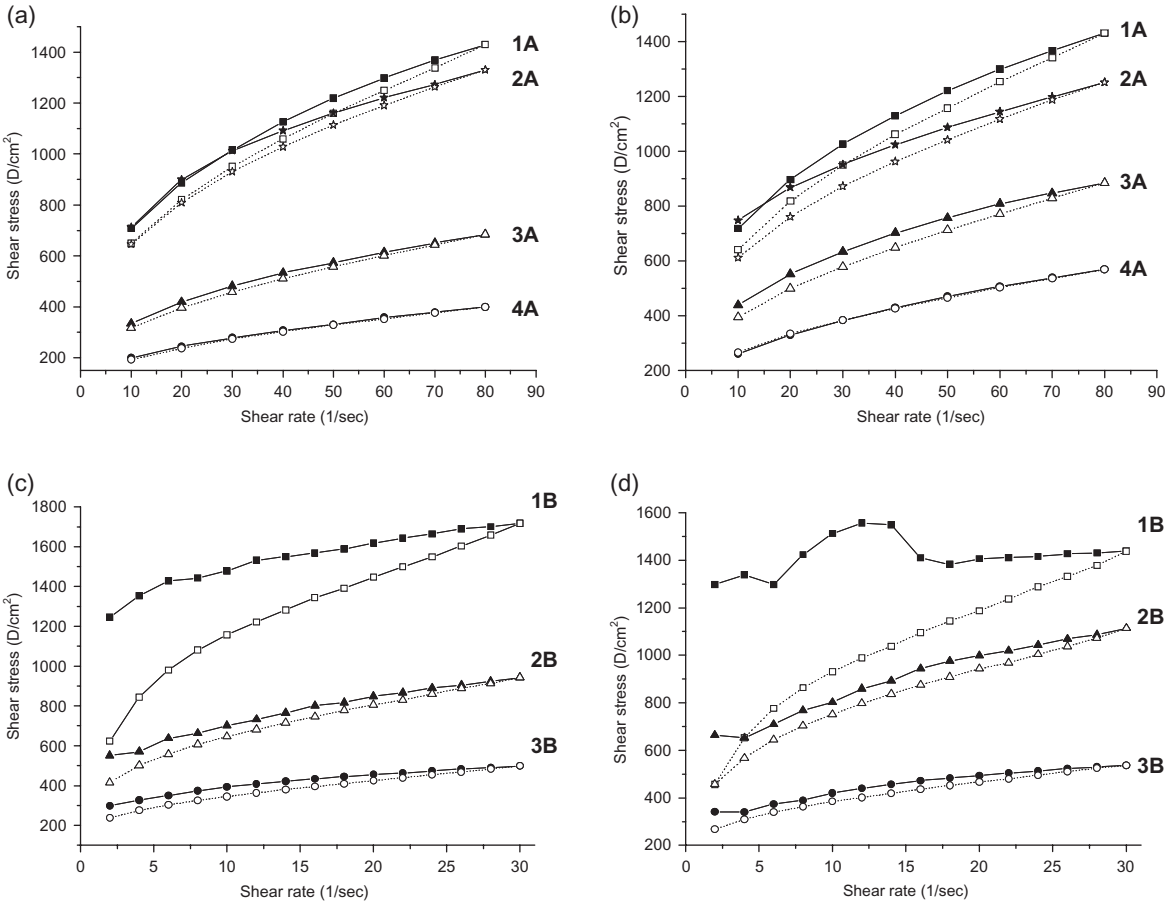

Figure 3. Graphics of the rheological behavior of the formulations 1A, 2A, 3A, and 4A after 24 hours (a) and 7 days of preparation (b), and of the formulations 1B, 2B, and 3B after 24 hours (c) and 7 days of preparation (d).

Table 2. Consistency and flow index values of the selected formulations from phase diagram (A) and (B), after 24 hours and 7 days of preparation.

	Consistency index (K)[b]		Flow index (ε)[b]		
Formulation[a]	24 hours	7 days	24 hours	7 days	Zeta potential[b]
F1A	27,407 ± 7181.4	29,516.5 ± 4309.8	0.36 ± 0.01	0.355 ± 0.01	−42.8 ± 9.2
F2A	14,156 ± 3181.9	17,843.5 ± 655.5	0.36	0.365 ± 0.01	−43.1 ± 8.9
F3A	8795 ± 726.9	11,058 ± 63.3	0.34 ± 0.01	0.37 ± 0.01	−32 ± 10.8
F4A	32,624 ± 4599	33,886.5 ± 663.3	0.32 ± 0.4	0.29 ± 0	−32.5 ± 8.4
F1B	76,118.5 ± 3103.5	75,233.5 ± 8917.3	0.235 ± 0.01	0.24 ± 0.03	−14.2 ± .2
F2B	37,967 ± 7611.3	42,677 ± 5351.4	0.26 ± 0.01	0.275 ± 0.02	−53.2 ± 7.1
F3B	21,750 ± 4187.5	24,284.5 ± 2049.9	0.235 ± 0.01	0.225 ± 0.02	−53.3 ± 7.1

[a]Phase diagram A composed of Buriti oil:water:*Steareth-2*/*Ceteareth-5*; phase diagram B composed of Buriti oil:water:*Steareth-2*/*Ceteareth-20*.
[b]Mean and SD of duplicates.

The values of zeta potential obtained for all formulations analyzed were in a range of –14.2 and –53.3. Zeta potential is one of the main forces that mediate interparticle interactions. An increase in the absolute value of zeta potential is correlated with a lesser tendency to flocculate. Generally high negative or positive zeta potential values (<–30 mV and >+30 mV) may stabilize emulsions by preventing droplets coalescence and increasing electrostatic repulsion between the emulsion droplets surfaces. The stabilization of emulsions is achieved by the addition of emulsifying agents capable of lowering the interfacial tension of the system and by forming an electric interfacial film around the oil globules[34]. The results presented in Table 2 showed high zeta potential values for the majority of the emulsions analyzed, especially when nonionic surfactants were used.

Some researchers have suggested that ether oxygen atoms in polyoxyethylene groups are slightly positively

charged and therefore they would attract negatively charged ions to the surface of the globule, forming a negative Stern layer[35,36]. Another approach suggests that the complex composition of Buriti oil could interfere in the zeta potential value[37]. According to the literature, the density of an oil droplet depends on the type and concentration of nonpolar molecules contained within it, and therefore the creaming stability of emulsions depends on droplet composition[38,39]. The major differences between the molecules normally found in oil droplets are their polarity and chain length. The interfacial tension at an oil–water interface decreases as the polarity of oil molecules increases, which would be expected to influence droplet coalescence and Ostwald ripening[37,40], hence we believe that some compounds present in the Buriti oil, such as the carotenoids, could be attracted to the droplets surface, modifying the barrier potential by ionizing in contact with the external watery phase.

After the characterization phase, we have selected two emulsions to perform the accelerated stability tests, owing to their rheological behavior, zeta potential values, macro-, and microscopic analyses. The formulations F3A and F3B did not show relevant alterations after 15 days at room temperature (24.0 ± 2.0°C), low temperature (4.0 ± 1.0°C), and high temperature (40.0 ± 1.0°C), or after the temperature cycling test.

The values of apparent viscosity, flow, and consistency index measured after 24 hours, 6 temperature cycles (12 days), or 15 days on stress conditions are presented in Table 3. According to the results, F3A showed significant differences in the values of flow and consistency index only after the temperature cycling test, whereas the F3B presented significant differences in the consistency index and apparent viscosity when exposed to the same condition.

The significant differences observed only after cycles of heating and freezing (40°C and 4°C) could be explained by the stress caused in this condition, as they are considered more aggressive when compared to the other conditions analyzed. The constant change of temperature could be responsible for modifying the liquid crystalline structure promoting an increase in the consistency index and apparent viscosity.

The consistency index reflects the property of the material by which it resists permanent change to its shape, whereas the fluidity is related to its resistance to flow[41]. It is known that the consistency index k can be enhanced with an increase in liquid crystals concentration and also with changes on their structures. The micellar interaction forces present on these systems make the structure more resistant to deformations in its form caused by flow. The lower temperatures promote a disorientation of the liquid crystalline structures and when the temperature is raised they suffer a reorientation, leading to an increase in the consistency index, as was observed for the F3B. At the same time, a decrease in the flow index ε can be observed, which enhances the plastic behavior of the system[41,42].

Table 3. Values of apparent viscosity, flow, and consistency index of the formulations F3A and F3B after 24 hours of preparation and 15 days of accelerated stability test.

Stress condition[a]	Apparent viscosity (cP)[b]	Flow index (ε)[b]	Consistency index (K)[b]
24 hours			
F3A-RT	1736.25 ± 130.3	0.345 ± 0.02	30,193 ± 4624.5
F3A-LT	1454.85 ± 180.7	0.35 ± 0.01	25,200.5 ± 4555.9
F3A-HT	1479.65 ± 121.3	0.34	29,316.5 ± 1907.1
F3A-CT	1792.75 ± 33.0	0.34	31,955 ± 309.7
F3B-RT	888.4 ± 85.1	0.34 ± 0.01	15,741.5 ± 504.2
F3B-LT	820.8 ± 128.6	0.365 ± 0.02	13,087.5 ± 3085.1
F3B-HT	879.8 ± 378.9	0.37 ± 0.01	13,661.5 ± 6391.5
F3B-CT	887.15 ± 34.7	0.37 ± 0.01	13,713 ± 212.1
12 days			
F3A-CT	1674.8 ± 64.3	0.375 ± 0.01[c]	25,779.5 ± 1262.2[c]
F3B-CT	1533.5 ± 83.4[c]	0.345 ± 0.01	25,932 ± 1982.7[c]
15 days			
F3A-RT	1802.6 ± 95.6	0.35 ± 0.01	3,0331 ± 3249.9
F3A-LT	1577.75 ± 232.9	0.345 ± 0.01	27,694 ± 4149.3
F3A-HT	1371.3 ± 187.7	0.37 ± 0.01	21,490 ± 3982.4
F3B-RT	1265.6 ± 34.8[b]	0.3 ± 0.04	27,803.5 ± 4727
F3B-LT	796.2 ± 163.3	0.37 ± 0.01	12,388 ± 3292.3
F3B-HT	1410.6 ± 330.2	0.365 ± 0.02	27,879 ± 629.3

[a]RT, room temperature (25°C); LT, low temperature (4°C); HT, high temperature (40°C); CT, cycling temperature (40°C and 4°C).
[b]Mean and SD of triplicate. [c]Presented significant difference when compared to 24-hours measure.

After the period of 15 days under stress conditions or the temperature cycling test, the emulsions subjected to centrifugation did not show macroscopic changes and no flocculation signs were observed during microscopic analysis. No changes were noticed in the color or smell of the emulsions.

No significant differences were observed in the pH values measured after 24 hours (5.47 for F3A and 5.62 for F3B), 12 days of temperature cycling (5.51 for F3A and 5.67 for F3B), or 15 days under stress conditions (5.4 for F3A and 5.65 for F3B). The pH values were measured to detect any preliminary sign of oxidation of the oily phase by the heat, as ketones, aldehydes, and epoxides are sub-products of this reaction and promote a decrease in the pH.

The main objective of the accelerated stability test is to accelerate any probable sign of instability latent in the products to estimate their shelf-life. The results obtained showed that within 15 days, the emulsions F3A and F3B were stable to the stress conditions applied and even being a preliminary assay, based on the short interval of time, these emulsions could be considered as promising systems.

Conclusions

The phase diagram showed to be an efficient method to develop stable emulsion using Buriti oil and nonionic surfactants. Associated to preliminary stability centrifugation tests, the analyses of zeta potential and rheological behavior evaluation were considered to be important tools to discard nonviable systems and to select feasible ones to be investigated. Even being a preliminary assay, based on the short interval of time, the accelerated stability test performed showed that the emulsions obtained could be considered promising systems. Moreover, this work confirmed the successful application of Buriti oil to produce stable delivery systems for pharmaceutical applications.

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Declaration of interest

The authors report no conflicts of interest.

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