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A New System of Multiple Emulsions with Lamellar Gel Phases from Vegetable Oil

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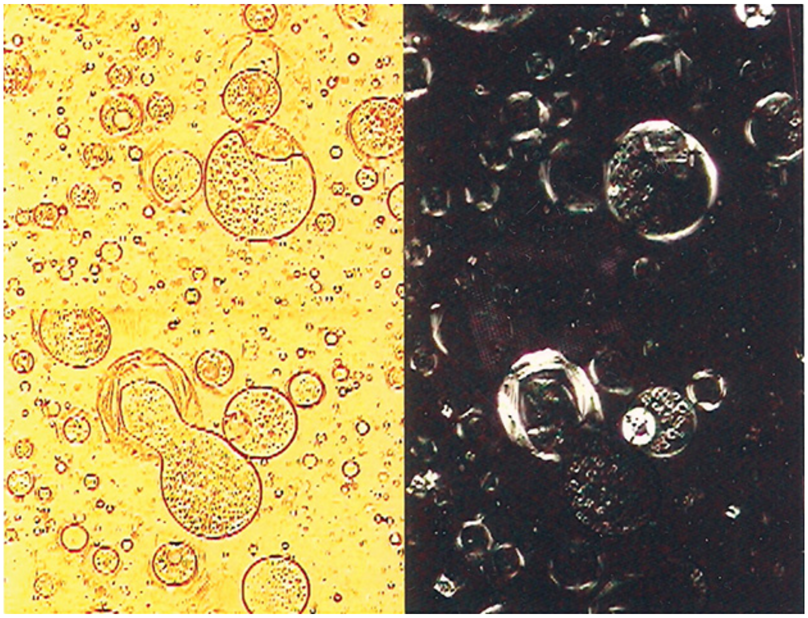
GRAPHICAL ABSTRACT



Emulsions are excellent pharmaceutical vehicles used in both the pharmacy and cosmetic industries. Vegetable oils have several effects/benefits on skin and can be used in emulsions to release principal active components for cosmetic purposes. Herein, multiple W/O/W emulsions were formulated in a one-step emulsification method, and the resulting anisotropic structures were characterized by x-ray diffraction measurements. The multiple emulsions obtained were stable and maintained their anisotropic structures over 2 years. WAXS (wide-angle x-ray scattering) measurements of these emulsions suggested that the carbon chains of the surfactant around the globules are disposed in a gel network phase. Furthermore, SAXS (small-angle x-ray scattering) measurements indicated that the surfactant is organized in lamellar layers around the globules. Thus, for the first time, we demonstrated that stable lamellar gel phase multiple emulsions can be made from vegetable oils. In addition to having the advantage of being prepared in one step, these emulsions have desirable characteristics that can be used in the cosmetic industry as natural active principles with low surfactant concentration and the unique features of multiple emulsions with gel phases.

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Keywords Double emulsions, formulation, liquid crystals, pseudo-ternary diagram, sunflower (*Helianthus annuus* L.) oil

INTRODUCTION

Multiple emulsions are heterogeneous polydispersed systems that, in the presence of hydrophilic and lipophilic stabilizing surfactants, coexist simultaneously both water-in-oil (W/O) and oil-in-water (O/W) emulsions.[1,2] One- or two-step emulsification methods can be used to obtain multiple emulsions. One-step methods produce multiple emulsions that are shear and temperature sensitive, present low yield of multiplicity, have reduced reproducibility, potentially convert into simple O/W emulsions, and lack long-term stability.[2] One-step multiple emulsions can be obtained via catastrophic inversions. Typically, a catastrophic inversion experiment is performed by adding a known volume of one phase to another phase under continuous agitation. Subsequently, the added phase is dispersed as droplets, and the droplet population increases until inversion takes place. Critical conditions can trigger sudden changes in the morphology of droplets. However, when continuous evolution takes place, the morphology of droplets gradually changes, which commonly occurs through multiple emulsions.[2]

Chains of surfactant can form lamellar bilayers of surfactant separated by water in emulsions comprised of non-ionic surfactants. Carbon chains of surfactants can be organized in the following two physical states: ordered (gel phase network) and disordered states (liquid crystalline state). These physical states are defined by the physical–chemical characteristics of the surfactant and temperature of the system due to the phase transition between both states.[3,4] These two states create a phenomenon called anisotropy, which is a birefringent pattern observed when formulations are submitted for analysis under cross-polarized light on a microscope.

Despite the large number of studies on multiple emulsions,[5–10] special patterns, such as multiple emulsions with lamellar gel phases or liquid crystals, have been poorly described. Some authors only report liquid crystal formation as a possible mechanism of stabilizing multiple emulsions.[11,12] Kavaliunas and Frank[13] produced multiple emulsions W/O/W with liquid crystals based on a ternary diagram using a two-step emulsification process. Although the authors claimed that the liquid crystals stabilized the multiple emulsions, after 14 days these multiple emulsions showed signs of gradual phase separation. Vaziri and Warburton[14] reported the production of multiple emulsions W/O/W with liquid crystals using two-step emulsification methods. However, they did not show any pictures of these emulsions. In addition, their emulsions were very unstable as they separated soon after the production. Genty et al.[15] produced a "complex dispersion of multilamellar vesicles" and compared this system to multiple emulsions W/O/W. When they observed the vesicles under crossed polarized light, birefringence was observed, but the stability of these systems over time was not discussed. Macierzanka et al.[16] produced "W/O+O/W/O" systems (only identified by optical microscopy), which contained either liquid crystals or lamellar gel phases, using a one-step emulsification method. These emulsions were stable over 6 months, but the authors did not state whether the emulsions maintained their multiple structures after 6 months. Other researchers produced multiple emulsions with nematic liquid crystals. However, they studied the defective structures of the liquid crystals and did not state whether these multiple emulsions were stable or pharmaceutically important.[17,18]

Hence, the aim of this study is to create a new system of multiple emulsions with lamellar gel phases using vegetable oil. These multiple emulsions were determined to be stable and can be used as possible pharmaceutical vehicles.

EXPERIMENTAL SECTION

Materials

Steareth 2 (HLB = 4.9) and Steareth 20 (HLB = 15.3) surfactants (Oxiteno, Brazil) and sunflower (*Helianthus annuus* L.) oil (cosmetic grade) (Lipo do Brasil, Brazil) were used as oil phases. All chemicals were kindly donated, and they were used as received from the manufacturers. Freshly purified water was used as the aqueous phase in all experiments.

Emulsion Preparation Using the Phase Diagram Method

One phase diagram was prepared by varying the concentrations of the three components by 10% intervals to cover the whole area of the triangle followed by 5% intervals to better define the limits of each zones (Figure 1).[19] Emulsions were prepared by the emulsion phase inversion method. Two surfactants were used, Steareth 2 associated with Steareth 20 with a previously determined critical hydrophilic–lipophilic balance (HLB) of 6.5. The oily and aqueous phases were heated to 75.0 ± 2.0°C. The heated aqueous phase was shed under constant stirring (Mechanic Mixer Fisatom Mod. 713 D) at 600 rpm until room temperature (25.0 ± 2.0°C) was reached. A total of 57 formulations were prepared (Figure 1). Selected stable emulsions were stored at 25.0 ± 2.0°C for additional experiments.

Macroscopic Analysis

Macroscopic analyses were conducted for all formulations 24 hours after preparation to observe any sign of

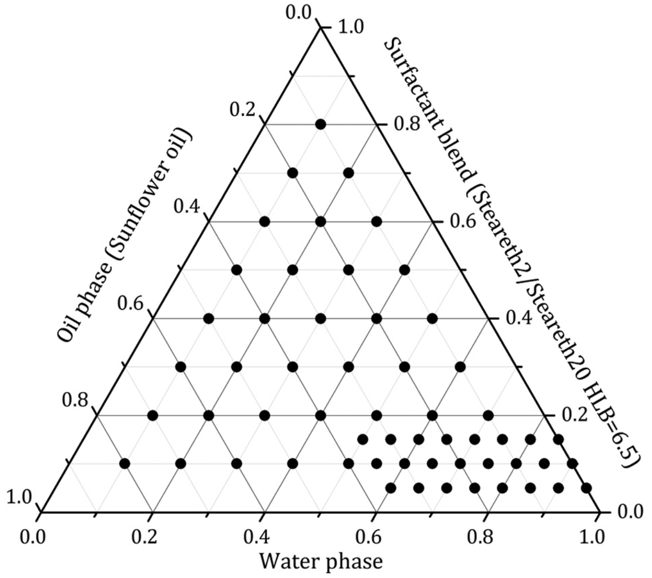


FIG. 1. Pseudo-ternary phase diagram constructed using sunflower oil, water, and Steareth 2/Steareth 20.

macroscopic instability, such as creaming or coalescence (phase separation).

Preliminary Stability Test

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

Thermal Stress Test

After centrifugation tests, stable emulsions were subjected to a thermal stress test. All measurements were conducted over a temperature range of 40.0–75.0°C with a temperature rate increment of 5.0°C/30 min in a thermostatic bath (NT 281, Nova Tecnica, Piracicaba-SP, Brazil).

Accelerated Stability Test

An emulsion consisting of Steareth 2 (4.23%, m/m), Steareth 20 (0.77%, m/m), sunflower oil (10%, m/m), and water (85%, m/m) was used in stability tests. Emulsions were stored at 25.0 ± 2.0, 4.0 ± 1.0, and 40.0 ± 1.0°C over 90 days, and freeze/thaw cycles of 4.0–40.0°C were performed by changing the temperature every 24 hours over the course of 60 days. Samples were removed from storage conditions at predetermined times (days 1, 8, 15, 22, 29, 60, and 90) and allowed to warm to room temperature (25.0 ± 2.0°C) prior to evaluating the following in triplicate: pH, electrical conductivity, viscosity, and micro- and macroscopic analyses.[19]

Optical Microscopy

An optical microscope (Olympus BX 50, Olympus Optical Co., Tokyo, Japan) with a camera (Olympus DP71, Olympus Optical Co., Tokyo, Japan) was used. Photomicrographs of the multiple emulsions were taken at predetermined times (see section "Accelerated Stability Test") without any dilution.[20]

Determination of pH Values and Electrical Conductivity

Electrodes (PM608 model, Analion, Ribeirão Preto-SP, Brazil) were directly inserted into solutions, and their pH values were determined. Electrical conductivity measurements were made using a conductivimeter (model mCA 150, Tecnopon, Brazil).[21]

Viscosity

Samples were analyzed by a Rate/Stress Rheometer (Brookfield R/S plus Rheometer) with cone-plate geometry (spindle C-50) operated by Rheo V2.8 software. The measuring device is equipped with a temperature unit (Brookfield PTR-I) for temperature control (25.0 ± 0.1°C). To characterize the system, steady-state flow properties were investigated by subjecting the system to a rate stress of 10–100 s^{-1} and later 100–10 s^{-1} over the course of 60 seconds per step. A plot of shear stress (D/cm^2) versus shear rate (s^{-1}) was constructed to determine the hysteresis area.[19]

X-ray Diffraction

Using x-ray diffraction, emulsions composed of 5% (m/m) surfactants (4.23% Steareth 2 and 0.77% Steareth 20), sunflower oil (10%, m/m), and water (85%, m/m) were chosen to investigate the state of the carbon chains within the lamellar layers and determine the lamellar repetition distance. Using WAXS (wide-angle x-ray scattering), the hydrocarbon chains can be determined to be either in gel or in liquid crystal phases within the lamellae. SAXS (small-angle x-ray scattering) can be used to determine the lamellar repetition distance. Samples were placed in glass capillary tubes with a 1.5 mm internal diameter and submitted for SAXS and WAXS measurements at room temperature. Sample–detector distances were 650 mm and 47.5 mm for wide- and small-angle measurements, respectively. Calibration was performed using SLES (sodium lauryl ether sulfate) in powder, which presents diffraction peaks in both wide- (4 Å) and small (~30 Å)-angle regions. Measurements were made at 25.0°C under a potency of 40 KV/30 mM on a Nanostar (Bruker, Atibaia-SP, Brazil) (Cu Kα radiation, $\lambda = 1.5418$ Å) located at the Laboratório de Cristalografia of IFUSP at the Instituto de Física da Universidade de São Paulo (São Paulo, Brazil). Systems were examined 7 days and 3 months after being prepared. The positions of the diffraction peaks obey the following Bragg equation:

$$n\lambda = 2d_L \cdot \sin\theta \tag{1}$$

where n is an integer (the reflection order), λ is the wavelength of the incident x-rays, θ is the Bragg angle of reflection, and, in the case of a lamellar structure, d_L is the repetition distance in the direction perpendicular to the lamella.

RESULTS AND DISCUSSION

Emulsions Attained Using the Phase Diagram Method

Figure 2 shows the following phase behavior of the Steareth 2/Steareth 20 (HLB = 6.5)/sunflower oil/water system, including lamellar gel phase multiple emulsions (W/O/W), in which the lamellar gel phase multiple emulsions zone narrowed for the interval between 5/10/85 and 10/35/55 (w/w/w) for surfactant, sunflower oil, and water, respectively.

Multiple emulsions were observed in the pseudo-ternary diagram (Figure 2, area A) between 5/10/85 and 10/35/55 (m/m/m). This area is narrower than that reported by Morais et al.[2] between 2.5/2.5/95 and 15/55/60 (m/m/m) but is enclosed. Our results demonstrate that the formation of multiple emulsions in one step occurs in a constrained pseudo-ternary area of the phase diagram and is related to the variation of oil composition and surfactant systems. Furthermore, these results are in agreement with Salager and coworkers[22,23] who claim that multiple emulsions obtained in one step (abnormal emulsions) can be obtained at low concentrations of surfactants (i.e., located at the base of ternary diagram) and, in the case of emulsions type W/O/W, with a high proportion of water (in our work, more than 50%). Interestingly, in the ternary diagram of Morais et al.[24] which uses Steareth 2/Ceteareth 5 (HLB = 9.0)/annatto, coffee, and tea tree oils/water (8:1:1, m/m/m), a single point [5% (m/m) surfactant's system; 15% (m/m) oily phase; and 80% (m/m) purified water] was observed to correspond to area (A) in Figure 2, forming anisotropic multiple emulsions. However, the emulsion of Morais et al.[24] was not stable during the centrifugation test. Maybe the stirring (600 rpm) speed used was too high to promote the formation of that multiple emulsions containing a lamellar gel phase.[2] Additionally, in this work, when 600 g of emulsion comprised of 5% (m/m) surfactants, 10% (m/m) sunflower oil, and 85% (m/m) water was produced, a speed of 400 rpm was used because there was no formation of multiple emulsions at 600 rpm, indicating that this type of emulsion is sensitive to stirring speed.

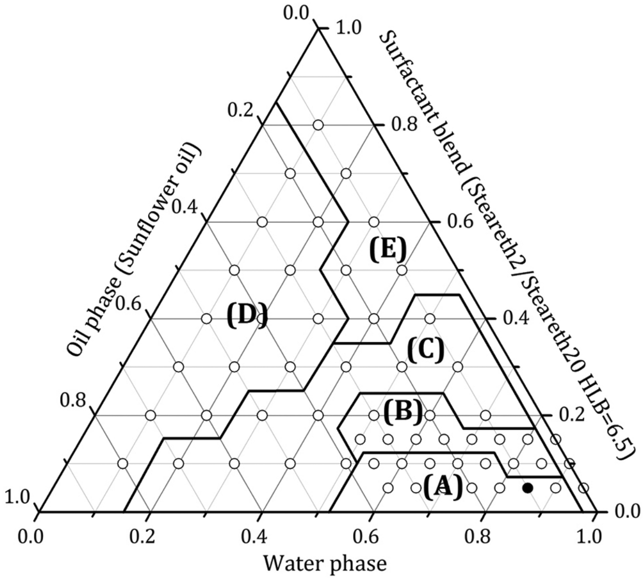


FIG. 2. Pseudo-ternary diagram showing areas in which different types of emulsions are observed. (A) Lamellar gel phase multiple emulsions (W/O/W). (B) Lamellar gel phase emulsions. (C) O/W emulsions. (D) Phase separation within 24 hours. (E) Amorphous systems that appear to be emulsions, but the droplets could not be identified by microscopy. The black circle represents a system consisting of 5% (m/m) surfactants (4.23% Steareth 2 and 0.77% Steareth 20), sunflower oil (10%, m/m) and water (85%, m/m).

Regarding the presence of anisotropy (lamellar gel phase), an area with concentrations of surfactant/oil/water (m/m/m) between 5/10/85 and 20/35/50, respectively, was obtained (areas A and B in Figure 2), whereas Zanatta et al.[19] obtained a common region of anisotropy between 5/10/85 and 30/35/60 for two slightly different systems (Steareth 2/Ceteareth 5, HLB = 7.25; and Steareth 2/Ceteareth 20, HLB = 7.25). The difference in the anisotropy area of the lipophilic surfactant Steareth 2 used by Zanatta et al.[19] (also used in the present work) may be due to the oil used for the oily phase (buriti oil) or different HLB (HLB = 7.25 for that both systems). In contrast, Andrade et al.[25] had a common region between 5/5/90 and 25/20/50 for two different systems (andiroba oil and silicone) with ionic surfactant. This region is quite similar to that found in the present study. Comparing the three works, we speculate that despite differences in the concentration limits, an area favorable to the formation of anisotropic structures in emulsions that can be well defined as concentrations of surfactant/oil/water (m/m/m) between 5/10/85 and 20/20/50 exists, even by using different oils and surfactants. Friberg et al.[26] and Engels and Von Rybinski[27] also found anisotropic areas in their ternary diagrams within the same region corresponding to areas A and B in Figure 2.[26,27] The formulator can target these structures, preventing time, oil, and surfactants from being wasted. Regarding the type of surfactant, non-ionic surfactants are more suitable in cosmetic formulations because they are less irritating to the skin and exhibit less incompatibilities when loaded with drugs and excipients compared to ionic surfactants.[28]

We also observed that phase separation (area D in Figure 2) typically occurs for emulsions formed from high oil and low surfactant concentrations and water, which was also observed by Bernardi et al.[29] when they used a surfactant blend comprised of sorbitan monooleate (Span® 80) and PEG-30 castor oil. Andrade et al.[25] made similar observations when using a self-emulsifying base containing ionic surfactant, demonstrating that the phase separation in this region is a trend, regardless of the type of surfactant used. In this case, there is not enough surfactant to stabilize the amount of oil in the form of globules.

We found simple emulsions in area C (Figure 2) and amorphous systems in area E (Figure 2), where it is not possible to observe droplets under a microscope, although macroscopic aspects of an emulsion are present. Andrade et al.[25] also observed this type of dispersion in their pseudoternary diagram using cetearyl alcohol, dicetyl phosphate, and ceteth-10 phosphate/andiroba oil/water.[25] In addition, on the top of the ternary diagram reported by Salager et al.[22] these systems, which were dominated by multiphase regions, were observed. According to these studies, other emulsions could be produced due to a lower concentration of surfactant that would be sufficient to disperse oil and water. Thus, the oil–water proportion is the true variable composition and not the concentration of surfactant.[22]

Macroscopic Evaluation

The emulsions evaluated from areas A, B, C, and E in Figure 2 were creamy, white-colored, with characteristic odors and no sign of macroscopic instability, such as creaming or coalescence. Phase separation was observed for emulsions from area D (Figure 2).[30]

Preliminary Stability Test via Centrifugation

The emulsions from areas A, B, C, and E in Figure 2 remained stable after the centrifugation test. According to Latreille and Paquin,[31] if stability is assumed to be directly proportional to the gravitational force, then the long-term behavior of emulsions can be assessed by centrifugation at moderate speeds. Centrifugation accelerates the destabilization of the product, simulating aging. The shelf-life under normal storage conditions can be rapidly predicted by observing the separation of the dispersed phase due to either creaming or coalescence upon centrifugation.[30] Based on our results, the emulsions proved to be physically stable after this preliminary test.

Thermal Stress Test

The emulsions from areas A, B, C, and E in Figure 2 show changes (phase separation or creaming) at temperatures up to 55.0°C. However, this should not be considered to be a sign of instability because the emulsions are normally not stored at these temperatures.

Accelerated Stability Test

As indicated in the pseudo-ternary diagram (black circle) in Figure 2, the emulsion composed of surfactants (4.23% Steareth 2 and 0.77% Steareth 20, m/m), sunflower oil (10%, m/m), and water (85%, m/m) presented differentiated characteristics with a greater quantity of droplets compared to other emulsions. These emulsions were subjected to the accelerated stability test. Samples were analyzed 24 hours (*t*0) and 8, 15, 22, 29, 60, and 90 days after being prepared.

Optical Microscopy

The emulsions maintained their properties of multiplicity and anisotropy over the time in which they were analyzed (16 months for emulsions stored at 25.0 ± 2.0°C). Anisotropic structures and multiple globules did not undergo modifications that could be observed by optical microscopy (Figure 3).

Determination of pH Values and Electrical Conductivity

Considering the pH values, only emulsions stored at 40.0 ± 2.0°C and 4.0/40.0 ± 2.0°C (freeze/thaw) dairy cycles underwent significant changes on the 60th day (Figure 4).

After 1 week, the values of electrical conductivity varied significantly (Figure 5). Despite this variation, the emulsion remained stable over 1 year (Figure 3). The conductivity initially decreased for all emulsions and then increased for emulsions stored at 40.0 ± 2.0°C. The conductivity of only the sample stored at 4.0 ± 2.0°C did not differ statistically during the first week. Masmoudi et al.[32] reported that when an emulsion is stable, no changes in conductivity are observed. However, they presented stable emulsions stored at 50.0 ± 2.0°C with increased conductivity, and destabilization could not be observed on a macroscopic

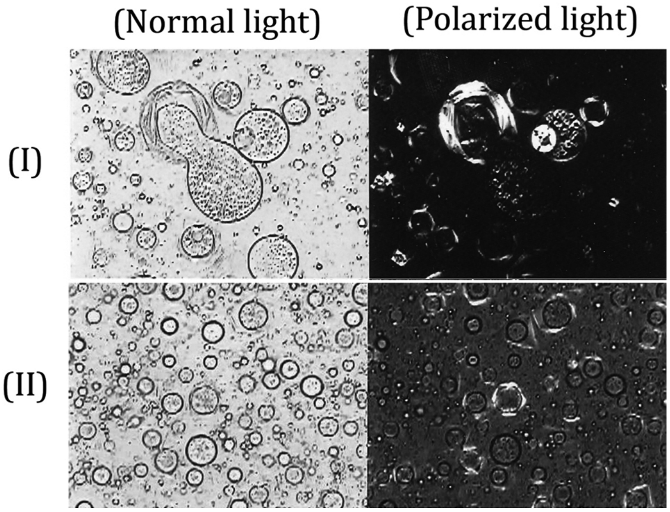


FIG. 3. Photomicrograph of emulsions prepared from Steareth 2/Steareth 20 (HLB = 6.5)/sunflower oil/water stored at 25.0 ± 2.0°C. Normal and polarized light after (I) 8 days, (II) 16 months.

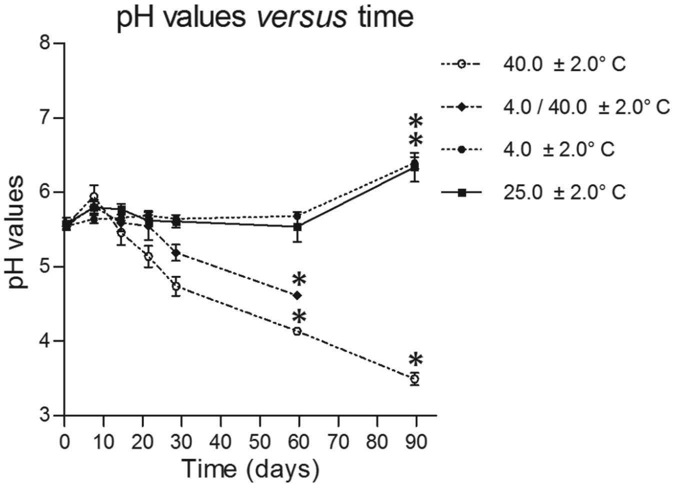


FIG. 4. Variation of the pH values of samples during the accelerated stability study. The colored marks (*) above each measure indicate the pH of samples on that day is different when compared to that of the first day. For statistical analysis, one-way ANOVA and the Tukey's Multiple Comparison post test were used. Statistical differences were considered to be significant when $P < 0.05$.

scale.[32] We found emulsions macroscopically stable after 16 months (stored at $25.0 \pm 2.0°C$) (Figure 3), despite statistical differences in their conductivity after 1 week. In fact, Masmoudi et al.[32] reported that emulsions with the highest conductivity are the most unstable, which is in agreement with our results because the emulsions have lower conductivity due to the use of non-ionic surfactants and absence of electrolytes.

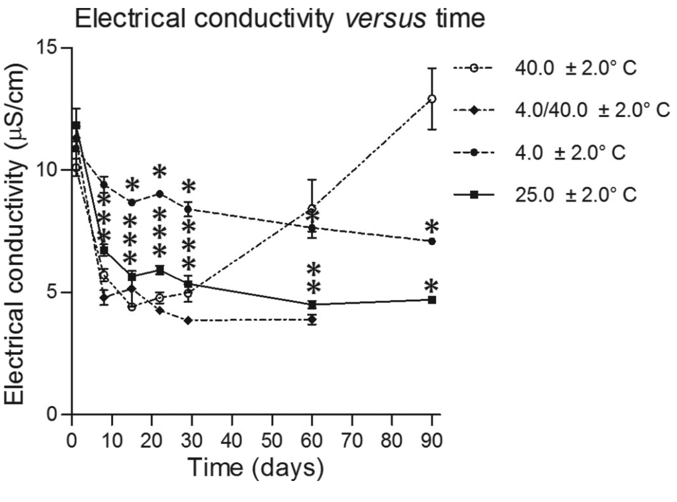


FIG. 5. Variation of electrical conductivity of samples during the accelerated stability study. The colored marks (*) above each measure indicate the conductivity of samples on that day is different when compared to that of the first day. For statistical analysis, one-way ANOVA and the Tukey's Multiple Comparison post test were used. Statistical differences were significant when $P < 0.05$.

Viscosity

Based on the computational analysis of the graphs of shear stress versus shear rate, we concluded that the most accurate model of studied emulsions is the Herschel–Bulkley model. In this model, a minimum value of shear stress must be overcome to deform the fluid (Figure 6). If emulsions are pseudoplastic, the rheogram of the axes will pass through the origin (i.e., the fluid would not provide any resistance to deformation). The largest variations occurred in samples stored in freeze/thaw cycle. Figure 6 shows that the profile was always thixotropic, with the descending flow curve always in the lower position than the ascending flow curve.

A slight increase in the apparent viscosity over the time of storage (Table 1) was observed. These results are consistent with those reported by Andrade et al.[25] who also noted an increase in the apparent viscosity between 1 and 15 and associated this increase with the formation of a gel phase in the external phase of the emulsions (the organization of the microstructure of the emulsion).[33] Santos et al.[34] used a surfactant system very similar to the surfactant system used in this work and also found an increase in viscosity between days 1 and 7.

The emulsions were determined to exhibit pseudoplastic behavior (or shear thinning) based on Table 1, which shows the values of flow index (n) being less than 1 ($n < 1$) for all tested times. This pseudoplastic behavior is suitable for products intended to be used in topical applications. For example, after emulsions are stressed, the initial resistance to the flow decreases, facilitating a good application of product.[35] Similar results were reported by Izidoro et al.[36] who studied the influence of green banana pulp on the rheological behavior of emulsions (mayonnaise). In addition, Andrade et al.[25] and Ferrari et al.[37] observed similar results in O/W cosmetic emulsions and cosmetic

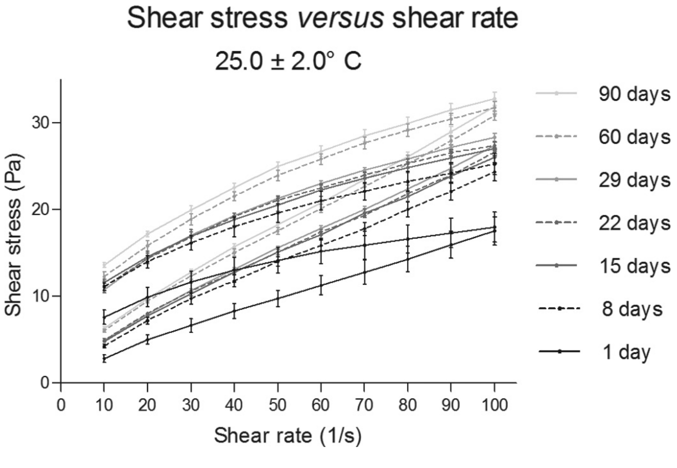


FIG. 6. Variation of samples shear stress with shear rate over the course of the accelerated stability test.

TABLE 1
Values of apparent viscosity, flow, and consistency index of the formulations after 24 hours and 30, 60, and 90 days of preparation as determined by the accelerated stability test

Stress condition	Apparent viscosity (Pa · s)	Flow index (ε)	Consistency index (K)
25°C			
1 day	18.0 ± 3.0	0.37 ± 0.02	3.3 ± 0.8
30 days	28.3 ± 0.8*	0.43 ± 0.01	3.8 ± 0.3
60 days	31.7 ± 1.3*	0.45 ± 0.04	3.8 ± 0.8
90 days	32.8 ± 1.3*	0.47 ± 0.02	3.3 ± 0.4
4°C			
1 day	17.1 ± 4.8	0.42 ± 0.01	2.2 ± 0.6
30 days	19.0 ± 1.1	0.60 ± 0.09	1.5 ± 0.5
60 days	20.7 ± 1.1	0.56 ± 0.05	2.0 ± 0.7
90 days	21.2 ± 0.7	0.67 ± 0.06	1.1 ± 0.3
40°C			
1 day	15.0 ± 3.1	0.40 ± 0.04	2.7 ± 1.1
30 days	28.2 ± 5.3*	0.39 ± 0.02*	3.0 ± 0.2
60 days	27.1 ± 0.8*	0.41 ± 0.02	3.0 ± 0.5
90 days	24.0 ± 4.5*	0.39 ± 0.02*	3.6 ± 0.4
4°C/40°C			
1 day	13.5 ± 1.3	0.38 ± 0.02	2.3 ± 0.4
30 days	27.9 ± 3.8*	0.47 ± 0.05	3.0 ± 0.9
60 days	33.1 ± 1.6*	0.51 ± 0.04	2.6 ± 0.0
90 days	ND	ND	ND

ND: not determined.

multiple emulsions $O_1/W/O_2$, respectively.[25,37] Morais et al.[24] also associated a lower flow index (more pronounced pseudoplastic behavior) of emulsions with a lower amount of surfactant.

The consistency index did not show changes during the entire test, indicating that this formulation is stable with respect to that parameter (Table 1).

X-ray Diffraction

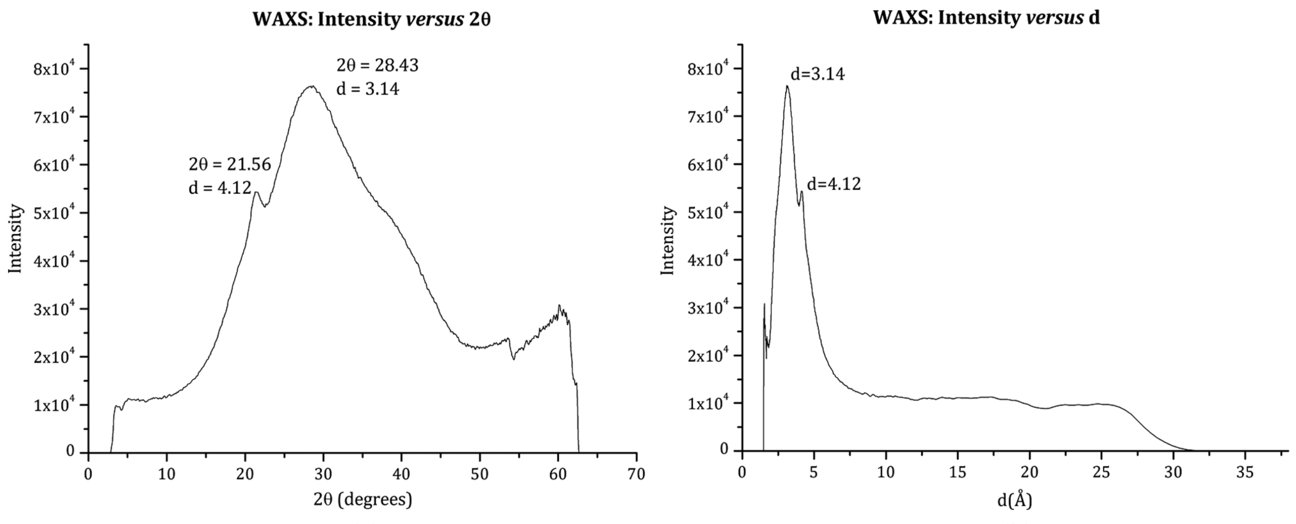
Because the difference between the ordered gel phase network and liquid crystalline phase is due to the organization of carbon chains, many anisotropic emulsions are referred to as *gel phase lamellar emulsions* or *liquid crystal emulsions* without further confirmation. X-ray diffraction is one of the most direct methods for obtaining information about the swelling of lipids and surfactants in bilayer structures because it can differentiate between the two forms of carbon chain packing (gel phase network and liquid crystalline phase).[38] Using this technique, the stability of globules by a monomolecular or multilamellar layer can be determined.[39]

Carbon chains within a lamellar layer were investigated by WAXS measurements. A broad band at approximately 4.5 Å indicates the presence of liquid crystals.[4] Figure 7 shows that this band does not exist, indicating that these emulsions do not have liquid crystalline phases as we expected because the surfactants used in these emulsions were solids at 25.0°C, the temperature at which the measurements were made. However, two other bands are observed at 3.14 and 4.12 Å, representing the water and gel phases, respectively.

To quantitatively characterize the lamellar structure, SAXS measurements were performed. The Bragg spacings, which are characteristic of lamellar structures, were in the ratios of 1:1, 2:1, 3:1, and 4:1.[4] Figure 8 shows that the interplanar distances associated with the first (149.7 Å) and the second peaks (73.75 Å) have a ratio close to 2, indicating a lamellar structure with repetition distance of approximately 150 Å. The overlaid lines indicate that the lamellar distances did not change after 3 months of storage. Thus, the emulsion is stable with respect to this parameter.

Our results are more extensive than many other studies described in the literature. The majority of reports describe the development of multiple emulsions using liquid crystals (or lamellar gel phases), and the authors assume that these structures stabilize multiple emulsions[11,12] or identify these structures by crossed polarizers. However, these emulsions were not characterized a reliable method.[13,14]

Genty et al.[15] measured synchrotron x-ray diffraction by covering the small-angle and the wide-angle diffraction regions of emulsions. However, they did not present the



(a) (b)

FIG. 7. Wide angle X-ray scattering (WAXS) measurements of the lamellar layer. (A) Intensity (a.u.) versus 2θ (degrees). The "d" peaks are labeled. (B) Intensity (a.u.) versus d (Å). Measurements were made after one week of storage. The bands at 3.14 and 4.12 Å represent the water and gel phases, respectively.

WAXS graphic indicating the bands that represent the water and gel phases. Actually, the authors focused on the lamellar structures of their "complex dispersed systems," and these lamellar structures were shown through freeze–fracture electron micrographs. However, it is possible that their system consists of lamellar gel phase multiple emulsions.

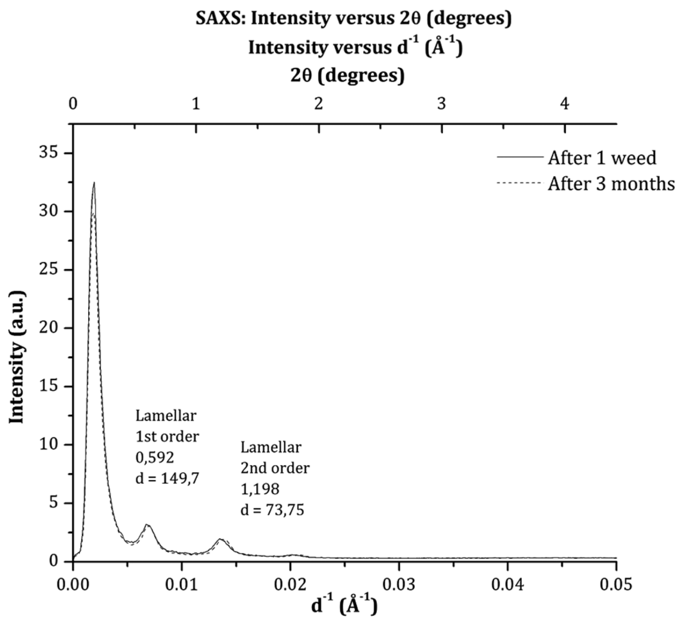


FIG. 8. Small angle X-ray scattering (SAXS) measurements for lamellar structures in storage after one week (in black) and three months (in red). Based on equation (1), "d" represents the lamellar interplanar spacings. Note that the decay near 0 does not correspond to a peak. The decay is due to the beam stopper, which avoids the detection of the direct beam.

Macierzanka et al.[16] reported that multiple emulsions with lamellar liquid crystals can be made, but the emulsifiers used in their formulations began to crystallize approximately 40°C. In the liquid crystalline phase, the carbon chains of emulsifiers are in the liquid form, which have a temperature above of the melting temperature of the hydrocarbon chains. Below this temperature, (e.g., at room temperature), the carbon chains crystallize and liquid crystals transform into α-gel (highly hydrated structure of the emulsifiers bilayers with ordered hydrocarbon chains).[40] In addition, Luzzati and Husson[4] reported that the liquid-like structure of hydrocarbon chains is an essential feature of the liquid crystalline phases of all lipids. Thus, the word "liquid crystal" is not appropriate for systems at temperatures below the order–disorder phase transition of hydrocarbon chains.

Based on our analysis of the literature, we conclude that our system is unique due to having multiple droplets, a gel phase network, and long-term stability. These three characteristics together were not found in any work in the literature. To date, this is the only reported system that uses vegetable oil in the oil phase.

CONCLUSION

This study has an original finding: we developed a new formulation strategy to obtain stable lamellar gel phase multiple emulsions successfully. Furthermore, these stable multiple emulsions were obtained from vegetable oils and by one-step emulsification method. Notably, as an outlook for future research and applications, these emulsions have desirable characteristics for the cosmetic industry, such as natural active principles, low surfactant concentration, and unique features, such as the multiple nature and the presence of gel phases. We believe that multiple emulsions can be used as drug delivery systems for medicines and cosmetics.

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