Journal of Dispersion Science and Technology

ISSN: 0193-2691 (Print) 1532-2351 (Online) Journal homepage: https://www.tandfonline.com/loi/ldis20

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To cite this article: Erika Cristina Vargas de Oliveira , Naira Rezende Maciel , Lia Queiroz do Amaral & Pedro Alves da Rocha-Filho (2013) Natural Brazilian Raw Material to Develop O/W Emulsions Containing Lamellar Gel Phase (Development and Analysis of Emulsion with Vegetable Oils), Journal of Dispersion Science and Technology, 34:12, 1656-1662, DOI: 10.1080/01932691.2012.742787

To link to this article: https://doi.org/10.1080/01932691.2012.742787

Journal of Dispersion Science and Technology, 34:1656–1662, 2013

ISSN: 0193-2691 print/1532-2351 online
DOI: 10.1080/01932691.2012.742787

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Natural Brazilian Raw Material to Develop O/W Emulsions Containing Lamellar Gel Phase (Development and Analysis of Emulsion with Vegetable Oils)

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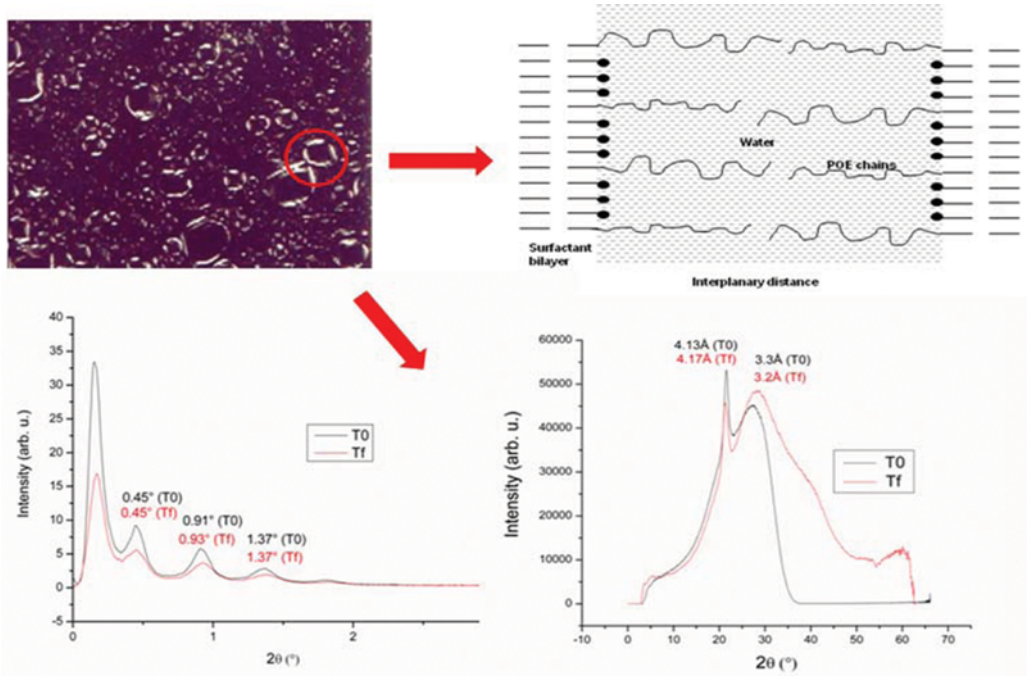
GRAPHICAL ABSTRACT



Oil-in-water emulsions were developed employing the HLB system and emulsion phase inversion (EPI) method. X-ray diffraction revealed that the anisotropic structures around the inner phase globules were lamellar gel network phases. The calculated distances between the lamellae made after preparation and 3 month latter showed that there was no swelling of the lamellar gel network indicating good stability and few changes during storage. The developed emulsions were stable and have potential to be employed for cosmetic and pharmaceutical purposes. The gel phase network and vegetal components seemed to be contributing factors.

Keywords Gel network phase, O/W emulsions, vegetable raw material, x-ray diffraction

1. INTRODUCTION

The employment of vegetable raw material in topical formulations for their rich chemical composition follows the tendency of using renewable resources as a sustainable alternative. Oils, butters and vegetable waxes have emollient properties, are able to maintain skin's softness and flexibility.[1] The incorporation of vegetable emollients in

Received 24 September 2012; accepted 24 September 2012.
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topical formulations provides reposition and protection of skins lipid matrix. Vegetable oils can further have advantages such as low viscosity and low molecular weight, therefore being less occlusive than mineral oil. They yet provide good cutaneous penetration and controlled active's delivery.[1–4] The andiroba oil (*Carapa guyanensis* seed oil) has a high percentage of fatty acids such as palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic acids and presents anti-rheumatic, anti-inflammatory, and healing activities.[5] Copaiba oil (*Copaifera sp* seed oil) has analgesic, antiallergic and anti-inflammatory activities, and is rich in tri and tetraterpenes, alkaloids and limonoids.[3] Cocoa butter is the natural fat of the cacao bean (*Theobroma cacao* L) and due to physical and chemical properties such as low melting point (±35°C), emollient and antioxidants activities, biocompatibility and low toxicity, has widespread use in food and cosmetic industries.[6] Cupuassu butter (*Theobroma grandiflorum* L seed butter) also has low melting point (±30°C) and high content of saturated and unsaturated fatty acids that confers emollient and moisturizing properties which are improved by its capability of water absorption approximately 240% higher than lanolin. All these raw material are widely used in Brazilian folk medicine mainly in the Amazon region.[3]

In some emulsions the surfactant's molecules on the interfacial film are arranged in bi-layers separated by water layers. This conformation confers to the emulsion advantages like (i) actives protection against photo and thermo degradation and (ii) water retention and consequent hydration enhancement of the *stratum corneum*.[7] The hydrocarbon chains of these bilayers can exist in different physical states named as (i) ordered or gel state, and (ii) disordered or liquid crystalline state, which are determined by the surfactants' physical chemical properties and the temperature of the system. However, emulsions are thermodynamically unstable systems where the presence of lamellar structure improves their stability.[8]

The aim of this work was to develop biocompatible O/W emulsions with cosmetic and pharmaceutical potential, presenting lamellar structures using Brazilian vegetable raw material. The lamellar structures were characterized by x-ray diffraction. Their behavior facing water evaporation and their physicochemical characteristics were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Carapa guyanensis seed oil, *Copaifera* oil, *Theobroma grandiflorum* (cupuassu) seed butter and steareth-2 (Hidrophyle-Lipophile Balance-HLB = 4.9) furnished by Beraca, Brazil. *Theobroma cacao* (cocoa) seed butter was provided by Croda do Brasil and PEG-80 sorbitan laurate (HLB = 17.8) by Oxiteno, Brazil. Recently purified water was obtained from reverse osmosis equipment (GEHAKA–OS 1OLX) purchased from GEHAKA Brazil. Preservative BHT (Butylated Hydroxytoluene) was purchased from Labsynth Brazil.

2.2. Methods

2.2.1. Emulsions' Development

Choice of Surfactants. Six hydrophilic and three lipophilic surfactants were combined in pairs in ratios calculated based on the HLB values of each oil and a 2:1 blend of andiroba:copaiba oils.

Butters Addition. Cocoa and cupuassu butters were added to the formulation together or separately at a concentration of 3 wt%.

2.2.2. Emulsions' Attainment

Emulsions were prepared by the emulsion phase inversion (EPI) method. Aqueous and oily, plus surfactants, phases were heated separately until they reached 75 ± 5°C. Then, the aqueous phase was gently spilled over the oily one under constant stirring (600 rpm) until the system reached room temperature (25 ± 5°C).

2.2.3. Required HLB Study

Equations 01 and 02 were used to calculate the HLB value required to produce stable oil-in-water emulsions with the oil blend andiroba:copaiba oils (2:1).

$$\text{HLB req.} = (\text{HLBA} \cdot \%\text{A} \cdot 0,01) + (\text{HLBB} \cdot \%\text{B} \cdot 0,01) \quad [1]$$

$$\%\text{A} + \%\text{B} = 100\% \quad [2]$$

where

HLB req. = Final HLB value required by the emulsion;
HLBA = HLB value of the lipophilic surfactant;
HLBB = HLB value of the hidrophilic surfactant;
%A = percentage of lipophilic surfactant to be used in the formulation
%B = percentage of hydrophilic surfactant to be used in the formulation.

2.2.4. Emulsions Characterization

Macroscopic Evaluation. Twenty four hours after the manipulation emulsions were visually observed in order to determine organoleptic characteristics and identify instability processes such as creaming and/or phase separation.

Microscopic Evaluation. The microstructure of o/w creams was studied with an Olympus BX50 optical microscope (Olympus Optical Co., Ltd., Tokyo, Japan) in bright field and under polarized light. Micrographs were made at a magnification of ×200.

Centrifugation. Each emulsion was weighted in graduated vials and processed in a centrifuge (Fanem model 206 R, Excelsa Baby II II-440 W, Brazil) at three speeds: 1500, 2500, and 3500 rpm (70, 440, and 863 G, respectively), standing for 15 minutes on each rotation. The procedure was carried out at room temperature ($25 \pm 2°C$).

Thermal Stress. Samples were submitted to water bath heating (Nova Tecnica Ltd., Brazil model 281 NT). Temperature were increased by 5°C at a time beginning at $40 \pm 2°C$ and maintaining each temperature for 30 minutes up to reach $80 \pm 2°C$.

Long-Term Stability. Samples were stored under three different temperatures ($25 \pm 5°$, $4 \pm 5°C$, and $45 \pm 5°C$) during 90 days and had their lamellar structures microscopically evaluated and pH values determined (Digimed-mod. DM20). For that, samples (1 g) were diluted in water (9 g) and the probe was immerged in the dilution.

2.2.5. Lamellar Structure Evaluation after Water Loss

Approximately 1 g of sample was placed on a glass slide over a delimited area. The sample was then spread to obtain a uniform layer of approximately 0.2 mm thick. Water evaporation was measured by a scale equipped with infrared light heating the sample at 70°C. Every 10% weight loss a photomicrograph was taken under polarized light (Olympus microscope model BX 50) and a new slide was prepared for further testing.

2.2.6. X-Ray Diffraction

The experiments were carried out using a Nanostar (Bruker) equipment at the Laboratory of Crystallography in the Institute of Physics of the University of São Paulo, São Paulo-Brazil. This device allows measurements of high and low angles (SAXS and WAXS) and consists of an x-ray tube with Cu Kα radiation ($\lambda = 0.15418$), 1.5 kW, collimated by a system of Gobel mirrors and a system of three slots.

Samples (Table 1) were placed in glass capillary tubes with 1.5 mm of inner diameter and underwent Small Angle X-Ray Scattering (SAXS) and Wide Angle X-Ray Scattering (WAXS) measurements 3–7 days after preparation and after 90 days of aging under room temperature ($25 \pm 2°C$). The sample-detector distance was 650 mm for SAXS and 47.5 mm for WAXS. Calibration was performed with powder sodium dodecyl sulfate (SDS) which shows diffraction peaks at both low angle (~30 Å) and high angle regions (4 Å). Measurements were performed with 40 KV/30 mA of potency.

The interplanary distances were calculated using the Bragg equation showed below.

$$n\lambda = 2d \cdot \sin\theta \quad [3]$$

where: n is the integer (the reflection order), λ is the wavelength of the incident x-rays. In the case of a lamellar structure the repetition distance, here called dL, is the sum of the lamellar thicknesses plus the water thickness, and corresponds to the first order reflection. θ is the Bragg angle of reflection.

Only samples containing 80% of water (samples 1 and 4) were submitted to WAXS measurements.

TABLE 1
Composition of samples analyzed by x-ray diffraction

	Samples	Oil (%w/w)	Surfactants* (%w/w)	Water (%w/w)
HLB 7	1	10	10	80
	2	20	10	70
	3	05	10	85
HLB 9	4	10	10	80
	5	20	10	70
	6	05	10	85

*PEG-80 sorbitan laurate/steareth-2.

3. RESULTS AND DISCUSSION

Stable oil-in-water emulsions containing anisotropic lamellar structures were developed using oils and butters from Brazilian flora. The lamellar structures revealed to be in the gel network phase and remained stable after emulsions' water loss.

3.1. Emulsions' Development

3.1.1. Choice of Surfactants

Previous studies on development of emulsions[7,9,10] shows that a formulation containing 80% water, 10% surfactant and 10% oil can present great stability and form lamellar structures, being therefore selected for this study. The various surfactants and their ratios are described in Table 2. Microscopic examination of the emulsions prepared with those components showed anisotropic lamellar phases in samples 07 and 08 only with andiroba oil and the oil blend. All formulations prepared with copaiba oil alone presented phase separation probably due to its resinuous composition and from the fact that it is collected from several individuals of the same genus, contributing to chemical variation. Sample 08 with oil blend was selected for further studies for presenting more evident and clearer lamellar structures (Figure 1) and having good stability.

3.1.2. Butters Addition

According to previous data,[11] cocoa and cupuassu butters employed solely as oil phase showed great amount of lamellar structures. When butters were added (5%) together they decreased the sharpness of lamellar structures. It was then necessary to determine the optimal ratio

TABLE 2
Surfactant pairs tested and their proportions to the employed oils and blend of oils*

Formulation	Surfactants pairs	Proportions (wt%) for Andiroba oil and for blend of oils (2:1) (*)	Proportions (wt%) for Copaíba oil
1	PEG-75 lanolin/steareth-2	15.04/84.96	85.84/14.16
2	PEG-75 lanolin/ceteth 2	15.04/84.96	85.84/14.16
3	Ceteareth 20/PEG lauryl alcohol	8.42/91.58	92.63/7.37
4	Steareth-20/PEG lauryl alcohol	8.42/91.58	92.63/7.37
5	Ceteareth 5/PEG lauryl alcohol	21.05/78.95	92.63/7.37
6	Ceteth 20/PEG lauryl alcohol	8.42/91.58	77.6/22.4
7	PEG-80 sorbitan laurate/ceteth 2	13.6/86.4	77.6/22.4
8	PEG-80 sorbitan laurate/steareth-2	13.6/86.4	–

*Andiroba oil and blend of andiroba:copaiba (2:1) required HLB = 7; copaiba oil required HLB = 15.

between them (Tables 3 and 4) that could improve the appearance or increase the formation of anisotropic structures.

Formulation D3b was chosen for further studies considering the greater formation, appearance and maintenance over time of anisotropic lamellar structures. The resulting emulsion had the appearance of white solid cream. Characterization tests revealed stability after centrifugation and thermal stress test not showing any modification until 60°C.

3.2. Required HLB Study

The interval studied, HLB 5 to HLB 17.8, was determined according to the required HLB values by the pair PEG-80 sorbitan laurate/steareth 2. Results show that emulsions requiring HLBs 5, 12, and 17.8 presented phase separation in the first 24 hours after preparation being therefore excluded. All the other emulsions showed anisotropic structures but only those with HLB 7, 8, and 9 remained stable after thermal stress test (stable until 65, 65, and 55°C, respectively). Macroscopically, emulsions with HLB 7 and 8 were very similar, with the appearance of firm, shiny, white cream being the final HLB 7 chosen for further study. Emulsion with final HLB 9 was slightly yellow and shiny being therefore included in further work.

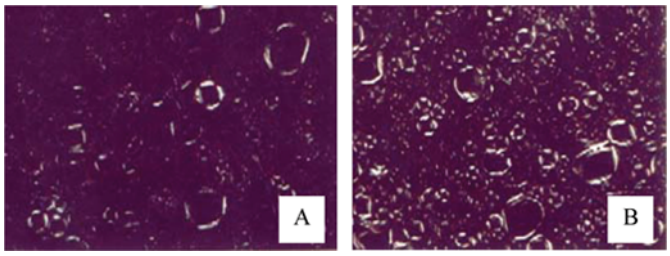

FIG. 1. Polarized photomicrograph (200X) of O/W emulsion from (A) PEG-80 (sorbitan laurate/ceteth-2) and (B) PEG-80 (sorbitan laurate/steareth-2). (Figure available in color online.)

3.3. Lamellar Structure Evaluation after Water Loss and Long-Term Stability

We found that even after 70% of water loss and under extreme storage conditions anisotropic structures could still be observed (Figure 2). This may occur because the water that evaporated during the test or during storage is bulk water. The water trapped between the lamellae, considered as bound water, need more energy to be removed. Some nonlamellar anisotropic observed areas could be due to recrystallized surfactant.[12,13]

For the pH values (Figure 3), we observed a decrease in all samples probably due to free radicals generating reactions such as oxidation and hydrolysis which are very common in raw material with high fatty acids content such as vegetable oils and butters. After statistical analysis (one-way analysis of variance with 0.05 level of significance) a significant difference was observed between emulsions with final HLB 7.0 and 9.0, the latter being less stable. The decrease in pH values observed in samples containing the preservative was not statistically significant indicating that a simple and regular preservative was able to stop degradation processes.

TABLE 3
Composition of formulations varying the cocoa and cupuassu butters' percentage in relation to oil and surfactants*

	Samples' composition (wt%)			
Components	D1	D2	D3	D4
Andiroba:Copaíba (2:1)	9.0	8.0	7.0	6.0
Cocoa butter	0.5	1.0	1.5	2.0
Cupuassu butter	0.5	1.0	1.5	2.0
Surfactant	10.0	10.0	10.0	10.0
Water	80.0	80.0	80.0	80.0

*PEG-80 sorbitan laurate = 16.28% and steareth-2 = 83.72%.

TABLE 4
Composition of formulations varying the cocoa and cupuassu butters' percentage difference*

Components	Samples' Composition (wt%)			
	D3a	D3b	D3c	D3d
Andiroba:Copaíba (2:1)	7.0	7.0	7.0	7.0
Cocoa butter	2.5	2.0	1.0	0.5
Cupuassu butter	0.5	1.0	2.0	2.5
Surfactants	10.0	10.0	10.0	10.0
Water	80.0	80.0	80.0	80.0

*PEG-80 sorbitan laurate = 16.28% and steareth-2 = 83.72%.

3.4. X-Ray Diffraction

We produced samples varying the water and oil contents because it is known that the amount of these components, especially water, influences the formation of anisotropic structures.[14–16]

3.4.1. WAXS Measurements Results

The wide angle measurements results (Table 5 and Figure 4A) reveal a sharper peak around 4.1 Å, attributed to the hydrocarbon chains, and a broad one around 3.3 Å which might be attributed to water. This is observed in both tested samples and in both times of analysis. These results indicate the presence of a gel phase network. According to Luzatti, 1962,[16] systems formed by water and lipids in which the carbon chains are rigid have a sharper peak at ~4.1 Å while chains organized as liquid crystals show a broad peak at 4.5 Å when submitted to x-ray diffraction measurements. Literature data[12] indicate that emulsions containing more than 47% of water present a peak at 4.04 Å, indicating the presence of lipid bilayers in the gel phase, according to the gel-network theory, developed by Barry and Eccleston. The gel phase is formed in emulsions composed by nonionic surfactants and water.[8] During the manufacturing process components are heated (±75°C) and water is added under stirring. When the system cools down, amphiphilic substances become less water-soluble and go to this phase at the water/oil interface. Initially, spherical micelles (amphiphile monolayer with oil inside) are formed and, as the systems continues to cool down, multilayers are formed around these micelles, with water in between (onion type structures). The lipidic fractions of the surfactant will be in a disordered form, but chains are on the average perpendicular to the layer, so they are considered to be liquid crystals. When the temperature reaches 40–50°C, which is approximately the temperature of the surfactant solidification, the unorganized form becomes organized and the gel network is formed. This change depends on the nature of the surfactant, its concentration on the system and on the production process.[18]

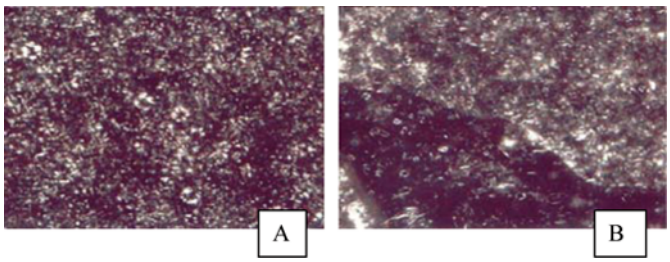

FIG. 2. Polarised photomicrograph (200×) of O/W emulsion after 70% of water loss showing the maintenance of anisotropic lamellar structures: (A) Emulsion with final HLB = 7 and (B) Emulsion with final HLB = 9. (Figure available in color online.)

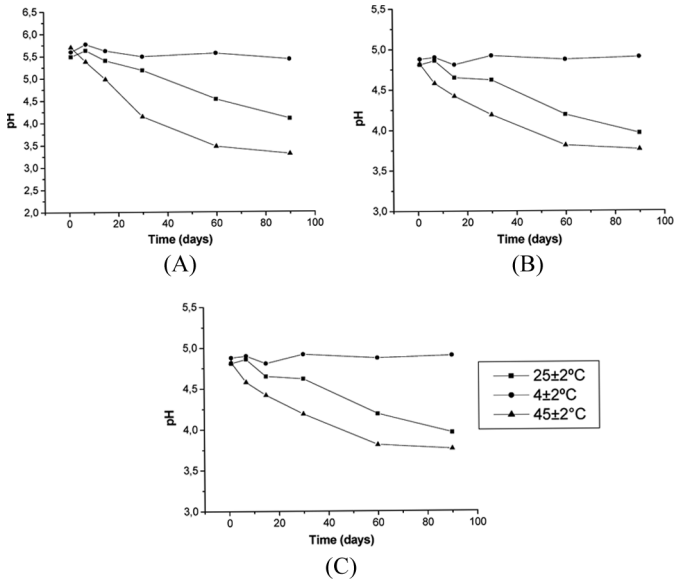

FIG. 3. pH values of O/W emulsions under different storage conditions with HLB = 7 (A), HLB = 9 (B), and HLB = 7.0 containing BHT (C).

3.4.2. SAXS Measurements Results

The small angle data is illustrated in Figure 4B and Table 6 in which one can observe that the anisotropic structures formed around the globules are lamellar since

TABLE 5
Wide angle x-ray diffraction data at initial (T0) and final (Tf) times of analyses

Samples	T0		Tf	
	2θ (°)	d (Å)	2θ (°)	d (Å)
1	21.5	4.13	21.3	4.17
	27.3	3.3	28.1	3.2
4	21.3	4.1	21.4	4.15
	28.0	3.2	28.0	3.2

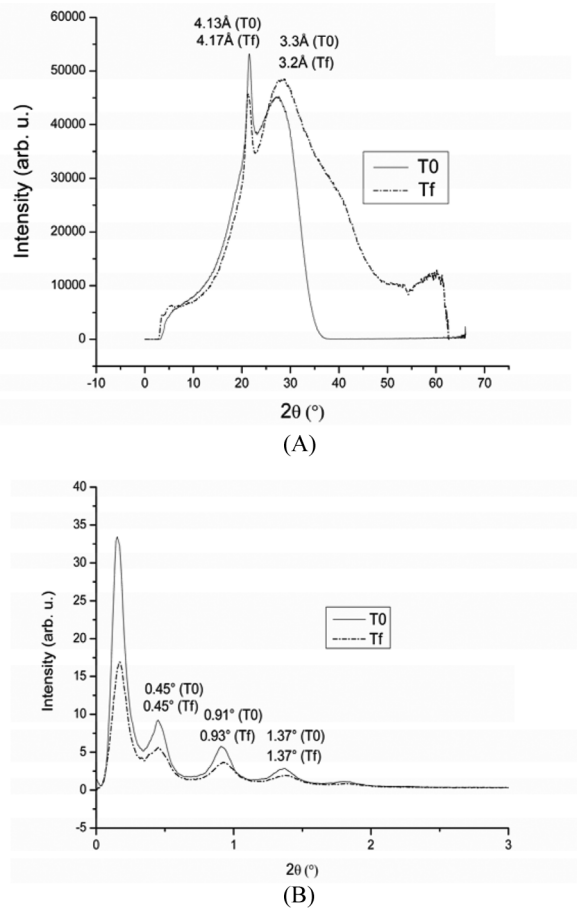

FIG. 4. Wide (A) and small angle (B) x-ray diffraction pattern of sample 1.

TABLE 6
Small angle x-ray diffraction data at initial (T0) and final (Tf) times of analyses

	T0		Tf			
Samples	2θ (°)	d_L (Å)	2θ (°)	d_L (Å)	Ratio	Structure
1	0.45	195.16	0.45	195.16	1:1/1:2/	Lamellar
	0.91	194.18	0.93	192.70	3:1	
	1.37	193.50	1.37	193.50		
2	0.46	192.70	0.39	226.73	1:1/1:2/	Lamellar
	0.93	190.30	0.85	208.30	3:1	
	1.40	189.50	1.30	211.20		
3	0.43	205.50	0.46	192.70	1:1/1:2/	Lamellar
	0.83	212.70	0.92	191.50	3:1	
	1.36	195.20	1.37	193.50		
4	0.43	205.5	0.43	205.5	1:1/1:2/	Lamellar
	0.87	202.9	0.89	198.9	3:1	
	1.31	202.0	1.33	199.4		
	1.75	202.2	1.78	198.3		
5	0.43	205.5	0.46	192.7	1:1/1:2/	Lamellar
	0.88	200.2	0.93	190.3	3:1	
	1.32	201.1	1.40	189.6		
	1.75	202.2	1.86	189.76		
6	0.43	205.50	0.43	205.50	1:1/1:2/	Lamellar
	0.85	208.30	0.87	207.40	3:1	
	1.30	211.20	1.28	207.40		
	1.69	209.10	1.75	202.20		

the results are in agreement with those of Luzatti,[16] The proportion of 1:1/2:1/3:1 is observed in all samples tested and maintained through time.

Eccleston[8] showed that an increase on the interplanary distances of emulsions containing cationic surfactans, submitted to SAXS and WAXS measurements, was directly proportional to the increase in the water content of the emulsion and determined by electrostatic repulsion. In emulsions with nonionic surfactants the interplanary distance is determined by the hydration of the surfactant's polyoxyetylene (POE) chain.[17] The distance values observed for samples 2 & 3 and 5 & 6, those with lower and higher water content respectivelly, did not present any difference between them. This result can be due to the small water content variation between the samples or indeed to existence of some 'excess water' in the system, staying between the globules instead of being between the bilayers. However, the interplanary distances are in agreement with those found in the literature, which were around 110 Å for emulsions with surfactants containing 18–20 POE groups and 500 Å with ionic surfactants. The surfactants employed in this study contain 80 POE groups, contributing to the larger interplanary distances found.

When comparing samples with final HLB 7 and 9 one can observe a slight increase in the interplanary distances of the latter. They have the greater amount of surfactant with more POE chains (PEG-80 sorbitan laurate = 31.78%, against 16.28% in emulsions with final HLB 7) therefore, requiring a greater water amount to hydrate them. No great variation could be observed between initial and final time.

During storage time the system tend to undergo structural changes. New lipidic bilayers can be formed while the chemical organization proceeds and it implies in reorganization of the bulk water.[8] Our results suggests that emulsions produced with natural oils and butters have not undergone structural changes contributing to the systems stability and equilibrium.

4. CONCLUSION

The results allow signal natural raw material as potential alternative to currently available preparations formulated with petroleum derivatives. Products containing vegetable

oils and butters seem to present equal or even better conditions to be employed for cosmetic and pharmaceutical purposes.

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