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Rheology analysis can be added in thermal stability test for design microemulsion materials

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ABSTRACT

Microemulsion materials (ME) are a viable alternative for the vehicle of drugs and molecules that improve compartmentalization, dissolution and biocompatibility of the drug. However, the characterization of the stability of ME is a limiting factor in their application. The aim of the present study was to correlate the stability and the rheological behaviour of ME. A pseudoternary diagram method using Span 80 (SP), Tween 80 (TW), 1-butanol (BT), distilled water and capric/caprylic triglyceride was carried out. The ME region was delineated, and samples were subjected to rheological characterization and preliminary stability testing. The correlation of thermal stability and rheological analysis was evaluated using the Pearson's linear correlation test. Samples 1M, 2M, 3M, 4M, 5M, 6M, 7M, 8M, 9M, 11M, 12M and 13M showed Newtonian behavior, and a hysteresis area between +0.20 and -0.20 Pa/s was approved in testing preliminary stability. Samples 10M, 14M, 15M, 16M, 17M and 18M exhibited non-Newtonian behavior and phase separation and were deprecated in the stability tests. There was a linear correlation between the primary stability and the rheological behavior. The data show that the rheological behavior of the study is a viable tool for decreasing the analysis time for the development of new microemulsion materials.
Keywords: *microemulsion, rheology parameters, thermal stability.*

1. INTRODUCTION

Modified release systems have been used to improve the pharmacological properties of various drugs. In this perspective, the use of biomaterials has recently gained ground in pharmacology and medicine [1] to establish more efficient therapeutic alternatives and for improved bioavailability of biologically active molecules and drugs in specific sites of action, with relevant therapeutic levels and minimized side effects [2, 3].

Microemulsions (MEs) represent an interesting type of therapeutic molecule delivery. MEs are homogeneous dispersions of oil and water, which are translucent and thermodynamically stable. They are stabilized by an interfacial film composed of conjugated surfactants and co-surfactants [2, 4, 5, 6] and occur as droplets on the order of 100 nm in diameter [7]. They are capable of compartmentalizing molecules and protecting them from external agents [8].

Although the techniques for obtaining MEs are effective, they need further characterization and evaluation of their thermal stability. For this purpose, techniques such as centrifugation are employed, as well as measuring pH and conductivity [5, 9]. The study and use of hydrophilic-lipophilic balance (HLB) and pseudoternary diagrams phases as tools for obtaining new vehicles have proven to be very efficient for the formulation of stable dispersed systems [3, 7].

The process of characterization of these materials involves several steps, tests and statistical evaluations; thus, it is a time-consuming process.

However, rheology quantifies the flow and deformation of bodies under the action of a stress [10, 11], and provides an important source of information regarding their microstructures.

The rheological behavior of the study provides data such as viscosity, rates and shear stresses supported by the material and hysteresis area under isothermal or temperature variation. Because it is focused on a mechanical behavior, the rheology is directly linked to the form and structural stability and thermodynamics of the material [11, 12]. Thus, changes in the rheological behavior suggest variations in the structure and stability of the material [13].

A correlation between the stability test and rheological parameters can suggest a new method of evaluation of thermal stability to reduction steps during the characterization process. The present study intends to correlate thermal stability and the rheological parameters of microemulsion.

2. EXPERIMENTAL SECTION

2.1. System composition.

The microemulsions (ME) were formulated using distilled water, caprylic/capric triglyceride - Polymol 812®, HLB= 10.8 (Emfal®, Betim, Brazil), Sorbitan Oletado - Span 80® (SP), HLB = 4.3, (Emfal®, Betim, Brazil), Polysorbate 80 - Tween 80® (TW) - HLB = 15.0, (Vetec®, Rio de Janeiro, Brazil) and 1-butanol (BT), (Vetec®, Rio de Janeiro, Brazil), and the system was abbreviated as SP/TW/BT.

2.2. Determination of the Surfactant Mixing Ratio.

Study of the hydrophilic lipophilic balance (HLB) of a system can provide information concerning the appropriate proportions of surfactant necessary to form optically translucent systems with good stability. The surfactants that provided HLB values at or near the oily phase were chosen [14, 15].

To act as a co-surfactant, BT was used in a ratio equivalent to 10 % (Fc = 1) of the surfactant mixture. The HLB of the surfactant mixture and the composition that was required to achieve an HLB corresponding to the oily phase was calculated. The HLB of the SP and TW surfactants were taken into account when utilizing equations 1 and 2:

$$HLBr = \frac{HLBT1 \times FT1 + HLBT2 \times FT2}{10 - (Fc)} \quad \text{(eq. 1)}$$

$$FT1 + FT2 + Fc = 10 \quad \text{(eq. 2)}$$

Where HLB was required to be in the oil phase (HLBr), HLB of surfactant 1 (HLBT1), HLB of surfactant 2 (HLBT2), fraction of surfactant 1 (FT1), fraction of surfactant 2 (FT2) and fraction of the co-surfactant (Fc) [16].

2.3. Microemulsion Systems Development.

Development of 36 formulations utilized pre-established component quantities in which each component ranged from 10 to 80 %. The samples were visually rated after 72 hours at 25°C for ME liquid region (MEL), ME gelling (MEG), emulsion liquid (EL), gel emulsion (EG) and phase separation (PS) parameters.

2.4. Pseudoternary Phase Diagram Constructions.

Titrations were performed on aqueous mixtures with surfactant/oil phase mass ratios of 1:9 and 9:1 under agitation to obtain the delimiting points, area and classification of different systems to form the regions in the diagram. The titration was performed by adding distilled water in quantities between 0.05 mL and 0.20 mL. During this process, the mixture was agitated both manually and mechanically. After homogenization of each titrant, the formulations volumes were visually classified.

Pseudoternary diagrams were constructed from the sample and titration data using OringinPro 8.5 software. In the diagram, the superior vertex represents 100% surfactant/co-surfactant, the lower right represents 100% oil phase, and the lower left represents 100% aqueous phase.

2.5. System Pre-selection.

After pseudoternary diagram acquisition, composition of the systems that fall into in the MEL region can be determined. This region contained pre-selected points that were distributed in lines dividing the region to achieve representative samples of the studied systems. In order to better apparent viscosity for serving drug, points were selected distributed in straight cutting following the MEL region to be characterized and evaluated for physical and chemical stability.

2.6. Rheological Characterization.

Rheological parameters were measured using a Modular Compact Rheometer – MCR 102 (Anton Paar® Germany GmbH, Ostfildern, Germany) according to França et al, [17]. In all of the experiments, 600 µL microemulsions was added to the reading plate surface, and the excess sample was removed. Readings were taken with continuous control of the gap measurement with the supported TruGap™ in 0.099 mm. The measuring cell was a Toolmaster™ CP 50, and precise temperature control was achieved with T-Ready™. The data were compiled using Rheoplus V3.61 Software. Graphics were also obtained using Rheoplus Software. The flow and viscosity curves were based on the established parameters for the control of shear stress (τ) to 0 – 5 Pa for the upsweep and 5 – 0 Pa for the curve downward. These tests were conducted under isothermal conditions at 25°C, and 75 readings were taken for analysis.

The viscosity curve was taken using established parameters that were relative to temperature and based on fixed control shear stress (τ) to 1 Pa with variation in temperature from 5 ± 0.1 to 45 ± 0.1°C and a heating rate of 1°C min-1. Parameters were recorded every 0.5 ± 0.1°C, and 41 readings were taken for analysis.

2.7. Physicochemical characterization.

A method to verify physicochemical parameters was designed to test the suitability of the formulations to act as delivery vehicles and to perform the initial characterization of each sample. This method verified the physical and chemical parameters of the formulations 24 h after preparation and at the end of the stability study, the tests were performed in triplicate (n = 3).

Aliquots of some pre-selected formulations were centrifuged (BABY I FANEM®, São Paulo, Brazil) at 3600 rpm for 30 minutes at room temperature. After centrifugation, the samples with visual heterogeneity were excluded.

Formulations pH were determined with a pHmetro pH Meter (DEL Lab®, Araraquara, Brazil) that were calibrated with standard pH = 7 and pH = 4 solutions. The electrode was inserted directly into the sample to assess its stability against possible decomposition reactions such as hydrolysis [3, 8].

Electrical conductivity was measured using a conductivity meter (LIDA®, São Paulo, Brazil) calibrated with a 0.1 mol.L-1 KCl solution by inserting the electrode directly into the sample to identify the system type (water in oil (W/O) or oil in water (O/W)) and any tendency towards phase inversion.

2.8. Stability Studies.

Samples were divided into two groups according to temperature: those that were refrigerated at 5 ± 1ºC and those that were heated to 40 ± 1ºC. These systems were preliminarily subjected to alternating cycles of 5 ± 1ºC and 40 ± 1ºC for 24 hours each, and the cycles were completed on the 14th day. After the cycle, it was possible to identify the most thermodynamically stable formulations [3, 8].

2.9. Statistical Analysis.

Statistically significant differences in pH, conductivity and hysteresis area were used Analysis of Variance (ANOVA). The correlation of thermal stability and rheological analysis was evaluated used the Pearson's linear correlation test. Statistical significance was considered for a p-value lower than 0.05 [p<0.05].

3. RESULTS SECTION

3.1. Surfactant Mixing Ratio Determination.

The concentration of co-surfactant BT was found to be 10%, and the percentages of surfactant SP and TW were calculated according to equation 1 to achieve the required Caprylic/Capric Triglyceride HLB. A surfactant mixture (SP/TW/BT) of 3.5:5.5:1.0 was used to achieve the required HLB, which was the same as the oily phase.

3.2. Microemulsion System Development.

According to the levels that were pre-established by the diagram proportions of the system SP/TW/BT (3.5:5.5:1.0), we achieved 36 formulations (Figure 1) with different levels of stability.
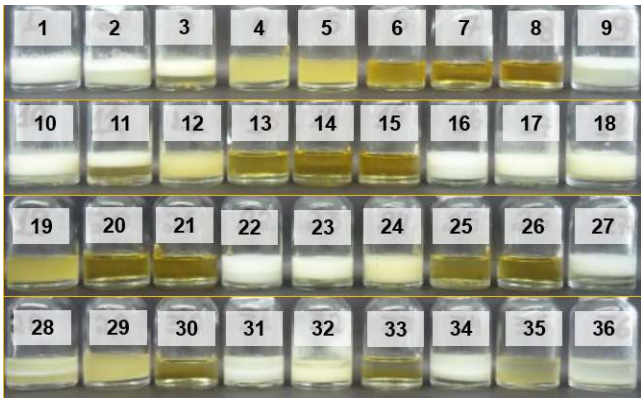

Figure 1. Formulations of study system SP/TW/BT (3, 5:5, 5:1, 0) of 36 points with various equilibrium characteristics.
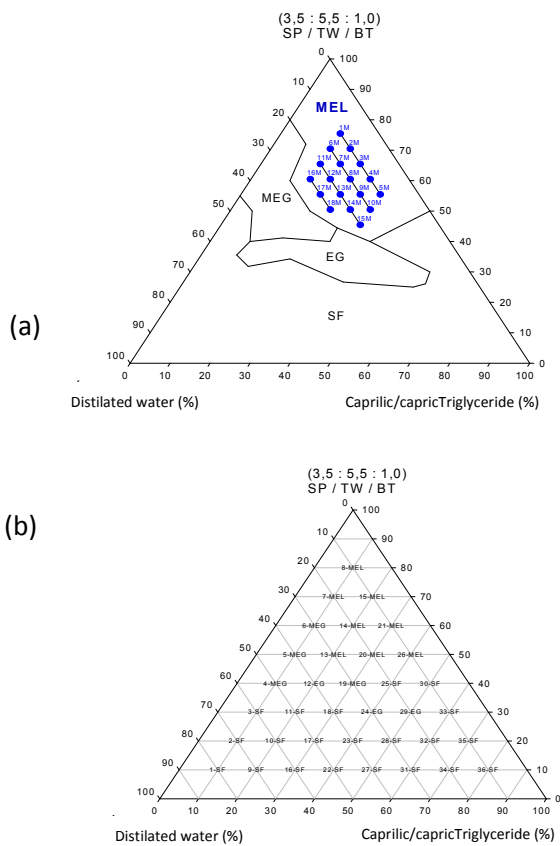

(a)

(b)

Figure 2. Diagram of the pseudoternary classification of all points (a) and domains with the points selected for the study system SP/TW/BT (b). Microemulsion liquid (MEL), microemulsion gel (MEG), emulsion gel (EG), and phase separation (PS).

Figure 2a represents data resulted of 36 points with divergent equilibrium characteristics and their visual aspects (MEL, MEG, EG and PS). Figure 2b shows the regions of the areas pseudoternary diagram delimited from the 36 formulations and titrations. Analysis of the distribution diagram of the domains revealed that the surfactant system has promoted a wide range of stable materials (MEG and MEL). However, the material located in regions below 45% of surfactants present instability and phase

separation. Samples were selected in the MEL region for the study of the SP/TW/BT system.

3.3. Rheological Characterization.

Figure 3 shows the differences between the curves for flow of Newtonian and non-Newtonian fluids from the material selected for the study of the system SP/TW/BT.

We observed that the flow curve began at the origin and then exhibited linear ascending and descending behavior (Figure 3) and did not exhibit differences between formulations. Figure 3b shows the observed non-Newtonian behavior and the thixotropic behavior for pseudoplastic samples 14M, 16M, 18M and 17M. The samples showed significant variation (Figure 3b).
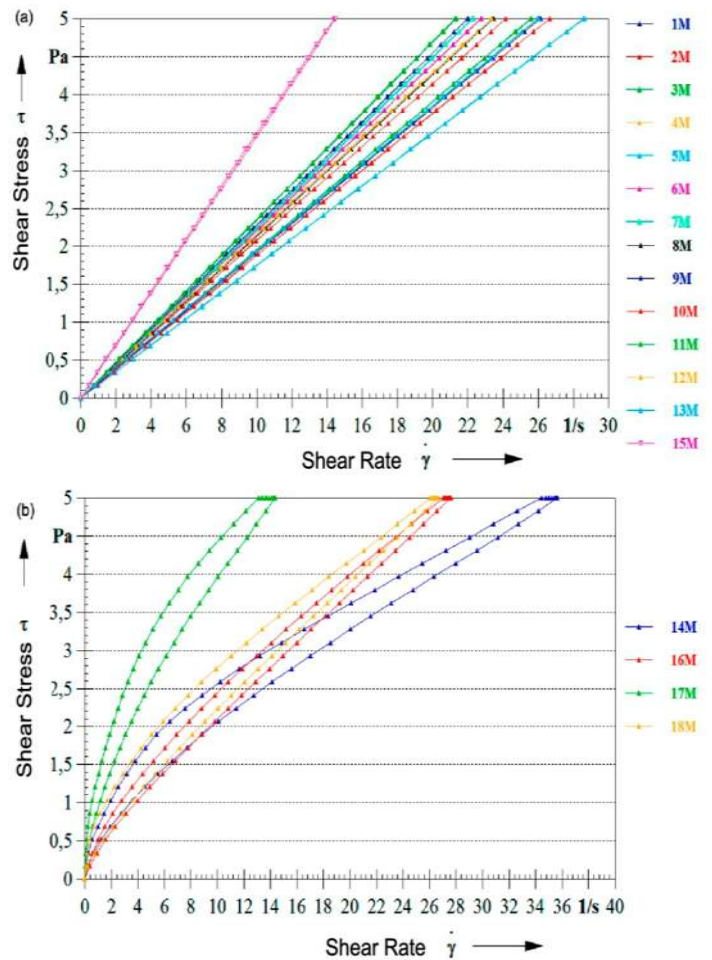

Figure 3. Flow curves (a)non- Newtonian fluids and (b) Newtonian fluids thixotropic/ pseudoplastic materials selected system SP/TW/BT $(25°(25°C - n=3))$.

Table 1. Hysteresis area before (1) and after (2) alternating cycles of temperature and reclassification of formulations (n = 3).

Point	Hysteresis area (Pa/s)*		Flow rate
	1	2	
1M	-0,10 ± 0,09	-0,02 ± 0,01	Newtonian
2M	+0,06 ± 0,02	-0,10 ± 0,02	Newtonian
3M	+0,05 ± 0,02	-0,02 ± 0,01	Newtonian
4M	-0,05± 0,05	-0,46 ± 0,03	Newtonian
5M	-0,01 ± 0,03	-0,02 ± 0,01	Newtonian
6M	-0,06 ± 0,04	-0,18 ± 0,1	Newtonian

Point	Hysteresis area (Pa/s)*		Flow rate
	1	2	
7M	-0,08 ± 0,02	-0,07 ± 0,03	Newtonian
8M	-0,03 ± 0,01	0,16± 0,04	Newtonian
9M	-0,16 ± 0,08	+0,02 ±0,01	Newtonian
10M	-0,20 ± 0,01	+8,54 ± 0,2	Non-Newtonian: thixotropic
11M	-0,09 ± 0,05	-0,12 ± 0,02	Newtonian
12M	-0,07 ± 0,02	-0,17 ± 0,03	Newtonian
13M	+0,07 ± 0,03	+0,13 ± 0,01	Newtonian
14M	+7,87 ± 0,04	+12,96 ± 0,2	Non-Newtonian: thixotropic
15M	+0,21 ± 0,01	+0,68 ± 0,4	Newtonian

*$p > 0.05$ (ANOVA) Comparing the before and after of alternating temperature cycles.

The hysteresis area values between - 1 Pa/s + 1 Pa/s demonstrate the Newtonian behavior of the materials (Table 1). The materials showing a hysteresis area value greater than 1 Pa/s and less than - 1 Pa/s and were classified as non-Newtonian. According to the sample behavior (Figure 3) and the values of the hysteresis area (Table 1), the SP/TW/BT system had a predominance of Newtonian fluids. Samples 10M and 15M had the highest hysteresis value ranges between Newtonian fluids, between 0.20 Pa/s and 0.21 Pa/s.

The apparent viscosity from the Newtonian material curves (Figure 4a) did not significantly vary from the apparent viscosity with increasing shear rate and remained between 0.16 and 0.25 Pa/s. The formulation did not show linearity for 15M when the apparent viscosity with increasing shear rate varied (Figure 4a).

The non-Newtonian materials showed non-linearity of the curves with apparent viscosity change (Figure 4b). It was seen that 17M had a steady decrease in apparent viscosity, accompanied by an increased shear rate.

All of the formulations were classified as O/W because they had electrical conductivity values above distilled water (>1.3 µS.cm-1). The pH values obtained before and after the test remained between 6.5 and 7.1 (Table 2). The formulations 10M, 14M, 15M, 16M, 17M and 18M displayed significant changes in the electrical conductivity values (Table 2).

3.4. Physicochemical analysis.

The results of a physicochemical analysis after temperature cycling for the stable formulations after drug incorporation are demonstrated in Table 2. Formulations 1M and 14M displayed macroscopic stability after centrifugation and were clear and homogeneous, while the others showed phase separation.

After the alternate cycles of temperature, materials 10M and 14M changed their MEL aspects to PS. The formulation 15M did not show relative stability and showed phase separation in the centrifuge test (Table 2).

The formulations had similar Newtonian flow curves, and linear alternating after temperature cycling (Figure 5a) did not significantly vary the values of the hysteresis area. Although

classified as Newtonian, 15M presented in Figure 5b demonstrated thermal instability similar to the materials 10M and 14M, which also showed a statistically significant variation.
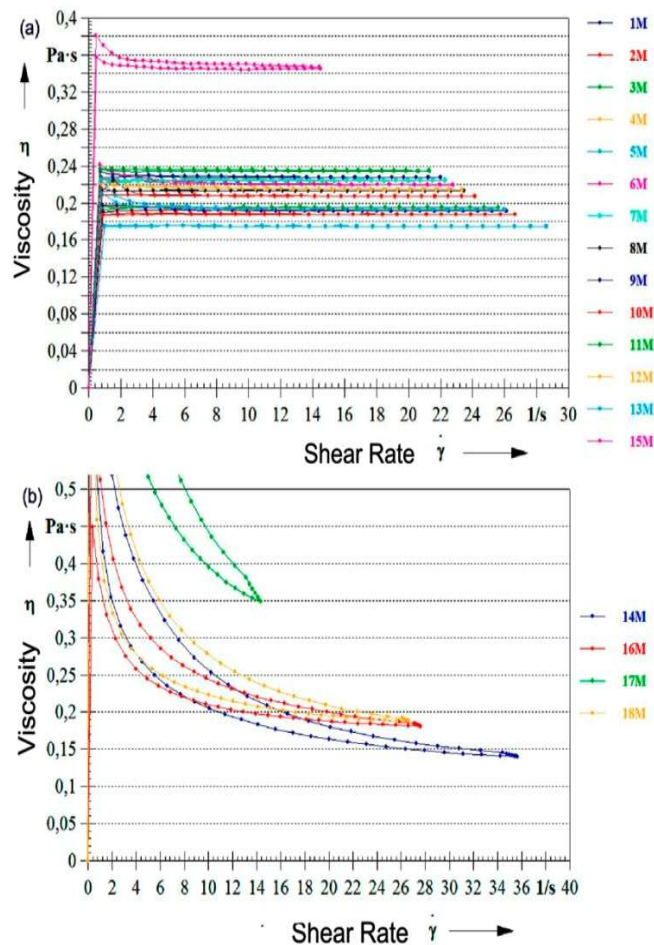

Figure 4. Curve viscosity of Newtonian materials (a) and non-Newtonian (b) (25°C n=3).

Statistical analysis of the data showed that there were no significant changes in the value of the hysteresis area for the formulations 1M, 2M, 3M, 4M, 5M, 6M, 7M, 8M, 9M, 11M, 12M and 13M, which kept their Newtonian classification, also adopted in all physical and chemical stability testing (Tables 1 and 2).

Table 2. Physicochemical analysis for the preliminary stability testing of the formulations. SP/TW/BT (n = 3).

Formulations	Centrifugation analysis	Form		Conductivity		pH	
		Before	After	Before	After	Before	After
1M	N	MEL	MEL	8,9 ± 1,7	5,8 ± 1,0	7,1 ± 0,1	7,0 ± 0,0
2M	N	MEL	MEL	8,0 ± 1,0	7,1 ± 0,6	7,0 ± 0,2	6.9 ± 0,1
3M	N	MEL	MEL	7,6 ± 0,7	6,4 ± 2,1	7,0 ± 0,3	7,0 ± 0,4
4M	N	MEL	MEL	8,2 ± 0,9	4,5 ± 1,5	6,9 ± 0,2	6,9 ± 0,1
5M	N	MEL	MEL	9,0 ± 0,3	7,9 ± 1,7	7,0 ± 0,3	6,7 ± 0,2
6M	N	MEL	MEL	18,8 ± 0,9	7,3 ± 1,5	6,8 ± 0,1	6,6 ± 0,4

Formulations	Centrifugation analysis	Form		Conductivity		pH	
		Before	After	Before	After	Before	After
7M	N	MEL	MEL	20,0 ± 2,3	18,1 ± 1,0	6,9 ± 0,2	6,9 ± 0,1
8M	N	MEL	MEL	23,0 ± 1,1	10,0 ± 2,0	6,9 ± 0,3	6,8 ± 0,4
9M	N	MEL	MEL	24,9 ± 3,4	11,3 ± 2,1	7,0 ± 0,2	7,2 ± 0,4
10M	N	MEL	PS	33,4 ± 5,2	0,2*± 0,06	6,5 ± 0,9	7,6 ± 1,3
11M	N	MEL	MEL	43,4 ± 3,4	33,9 ± 3,5	6,7 ± 0,1	6,8 ± 0,1
12M	N	MEL	MEL	50,4 ± 5,6	24,9 ± 3,6	6,8 ± 0,2	6,8 ± 0,1
13M	N	MEL	MEL	57,6 ± 2,9	22,0 ± 6,1	6,8 ± 0,1	6,9 ± 0,4
14M	N	MEL	PS	30,9 ± 1,4	7,2*± 10,8	6,9 ± 0,1	8,2 ± 0,7
15M	A	PS	PS	10,52± 2,3	3,5*± 2,8	6,8 ± 0,3	5,7 ± 1,3
16M	A	PS	PS	19,23± 1,5	2,9*± 9,9	7,4 ± 0,2	6,1 ± 1,2
17M	A	PS	PS	36,40± 2,6	5,6*± 18,0	6,7 ± 0,2	6,7 ± 0,3
18M	A	PS	PS	108,27±19,1	10,2*±19,1	6,7 ± 0,1	7,5 ± 0,7

Notes: N=Normal, A=Altered, MEL=Microemulsion Liquid, PS=Phase Separation.
*p<0,05 (ANOVA) Comparing before and after of alternating temperature cycles.
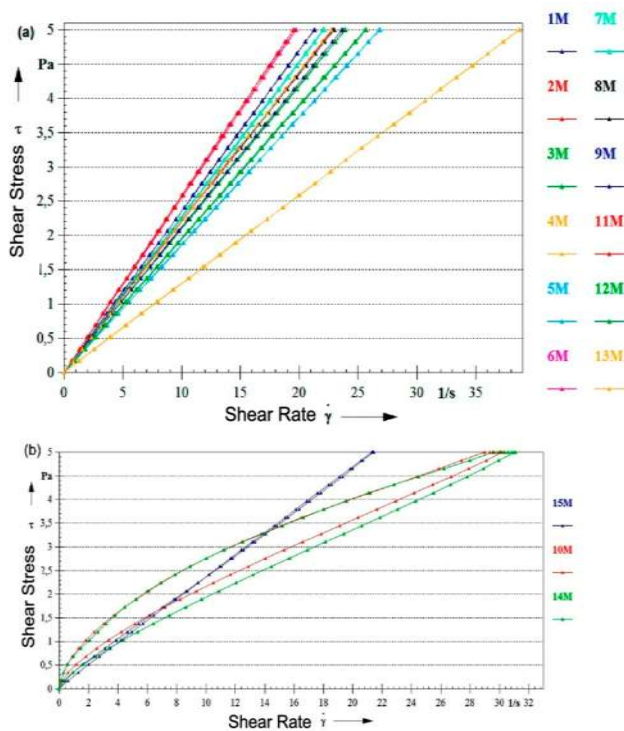

Figure 5. Flow curves of the materials (a) that remained normal and (b) who were rheological changes after the alternating temperature cycles (25°C, n=3).

According to the results, the formulations 10M previously showed Newtonian rheological character and a hysteresis área of -0.20 Pa/s, and after cycles of alternating temperature, it showed non-Newtonian behavior and a hysteresis área of + 8 54 Pa/s

(Table 1). The formulation 14M maintained non-Newtonian thixotropic behavior but showed an increased hysteresis area (Table 1).
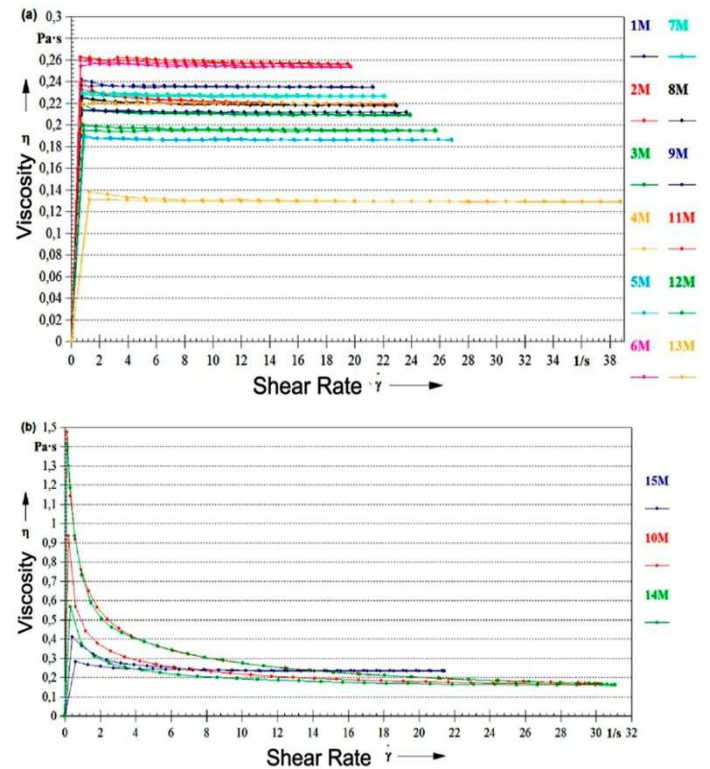

Figure 6. Viscosity curves of the materials (a) that remained normal and (b) who were rheological changes after the alternating temperature cycles (25°C, n=3).

Table 3. Mean viscosity of the samples before after the thermal stability tests (25°C, n = 3).

Formulation	Viscosity medium	
	Before	After
1M	0,2205 ± 0,08	0,2277 ± 0,06
2M	0,2010 ± 0,06	0,2151 ± 0,09
3M	0,1885 ± 0,04	0,2035 ± 0,08
4M	0,1852 ± 0,02	0,1255 ± 0,09
5M	0,1692 ± 0,08	0,1803 ± 0,02
6M	0,2145 ± 0,8	0,2463 ± 0,04
7M	0,2169 ± 0,07	0,2195 ± 0,03
8M	0,2063 ± 0,09	0,2121 ± 0,02
9M	0,1855 ± 0,07	0,2050 ± 0,06
10M	0,1817 ± 0,08	0,1867 ± 0,09
11M	0,2273 ± 0,09	0,2497 ± 0,04
12M	0,2087 ± 0,03	0,1889 ± 0,05
13M	0,1884 ± 0,05	0,2126 ± 0,07
14M	0,3091 ± 0,07	0,2402 ± 0,01
15M	0,3378 ± 0,06	0,2395 ± 0,03
16M	0,2488 ± 0,03	-
17M	1,0356 ± 0,04	0,3237 ± 0,01
18M	0,3605 ± 0,06	0,2956 ± 0,09

The apparent viscosity of Newtonian curves of the samples remained linear and assumed values between 0.19 and 0.27 Pa/s (Figure 6a).

The sample 15M, although characterized as a Newtonian material, after stability tests showed atypical rheological behavior

for other formulations of system SP/TW/BT, in terms of both the flow curve (Figure 5b) and the apparent viscosity curve (Figure 6b).
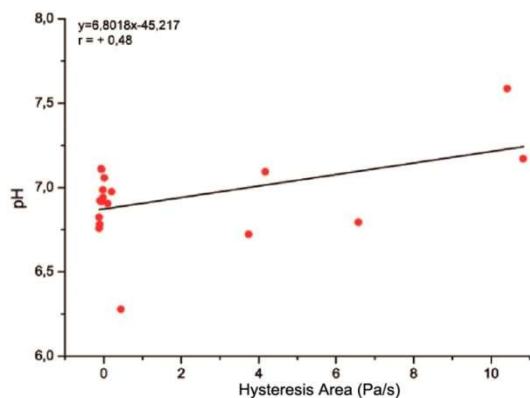

Figure 7. Graphic representation of the linear correlation between the pH values and the hysteresis area.

The hysteresis area increased from 0.21 Pa/s to 0.68 Pa/s. Statistical analysis of the data showed that there were no significant changes in the value of the hysteresis area for the formulations 1M, 2M, 3M, 4M, 5M, 6M, 7M, 8M, 9M, 11M, 12M and 13M, which kept their Newtonian classification, also adopted in all physical and chemical stability testing (Tables 1 and 3).
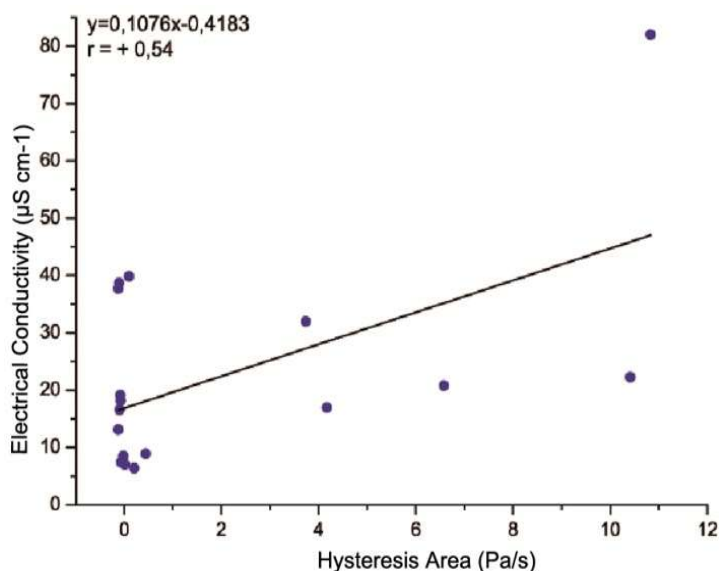

Figure 8. Graphic representation of the linear correlation between the electrical conductivity values and the hysteresis area.

3.5. Pearson's linear correlation.

Pearson's linear correlation showed that pH and electrical conductivity presented directly proportional correlations with hysteresis area, $r = 0.48$ and 0.54, respectively, as shown in Figures 7 and 8.

3.6. Discussion.

In the present study, microemulsion liquid materials were developed with the purpose of being assessed for thermal stability and rheological behavior, from sorbitan oletato, polysorbate 80, 1-butanol, capric/caprylic triglycerides, and distilled water.

An important factor in the development of thermodynamic colloidal dispersions is the proper determination of the proportion of surfactants. The contents of the hydrophilic and lipophilic groups of the surfactant are determined by the HLB value, which is an important material for microemulsion formulation [2].

According to the HLB value of the mixture of surfactants and/or co-surfactants, it is possible to predict the predominance of systems on classification stages, O/W or W/O, in the preparation of stable systems. The correct combination of the proportions of surfactants and co-surfactants provides HLB values closest to the required HLB of the oil phase. The closer these values are the more stable the system will therefore be, resulting in a more rigid and stable film on the oil/water interface. The HLB is dependent on the molecular geometry and arrangement among surfactants [18].

In this work, the mixture of surfactants used for the development of SP/TW/BT in the ratio 3.5:5.5:1.0 provided an HLB value equal to that required by the triglycerides of capric/caprylic. Studies indicate that a high HLB value indicates more hydrophilic properties and, additionally, provides a slower growth and reduced core of the final particle size [19, 20]. This reduction in particle diameter provides materials with greater thermal stability [21].

The use of combined non-ionic surfactants favours the formation of stable systems, which can be seen in the regions of the phase diagram [22]. The combination of surfactants, such as polysorbate 80 and others is more effective in providing microemulsion materials [23].

Mixtures of triglycerides are capable of forming large regions of thermodynamically stable systems because they can act by assisting the co-surfactant [24]. Other studies have shown the use of the phase diagram as a tool to study the levels of stability of different regions according to the variation of the formulation components [2, 8, 9, 25]. Through the study of these regions, you can obtain better material, in terms of stability, which can aid in achieving the intended purpose [2].

The rheological profile of the formulations aimed to serve as a means for monitoring the physical and chemical stability of the microemulsion because it allows for the observation of the structural changes that result from the destabilization processes [26, 13].

The rheological behavior of the microemulsion depends on the material type, shape, density and number of components present in the formulation and the structures and arrangements that can be formed. Thus, microstructural changes caused by destabilizing agents such as time, heat, humidity, pressure, sedimentation, coalescence and chemical reactivity are reflected in the rheology of the microemulsion [27]. The MEL typically exhibits Newtonian behavior with constant viscosity and a wide shear range [27, 28].

The non-Newtonian components can be characterized [13, 29]; that is, the apparent viscosity decreases over time when these materials are subjected to shearing. This is due to the existence of an unstable structure in the material, which can be broken by applying a specific energy [11, 30]. This analysis showed most MEL showed Newtonian rheological behavior and steady shear viscosity over a wide range as previously described in the literature. The formulations 14M, 16M and 18M showed non-Newtonian rheological behavior with thixotropy. The 17M sample showed non-Newtonian and pseudoplastic behavior. The thixotropic non-Newtonian character, as evidenced by a hysteresis

area greater than 1 Pa/s, suggests the occurrence of a structural break under shearing action [31, 32]. This result gives a degree of thermodynamic instability to the material [32, 33]. Other studies have shown that the oscillation of the viscosity indicates the occurrence of structural changes in the material occurring during the shear and increased temperature, with evidence of thermodynamic instability [32]. The pseudoplastic character is characterized by a viscosity curve in which apparent viscosity decreases as the shear rate gradually increases [31, 32].

Combined with the rheological behavior, the physical and chemical tests provided information on the levels of stability of materials when subjected to extreme conditions. Through this work, it was possible to relate the rheological behavior of the material and its thermal stability.

The thermal stability is linked directly to the particle size and the translucent material. Therefore, a stable change can be assumed when there is an increase in the proportion of the oil phase, resulting in the transition from translucent to opaque phase system and phase separation systems. Increasing the oil concentration may cause an increase in particle size, which accelerates the destabilizing of the material [34].

We observed that in the SP/TW/BT system, the oil phase percentage influenced the stability of the microemulsion systems not yet in the MEL domain, between 15 and 35% oil phase. This initially did not have direct influence on stability because stable samples (1M, 5M, 6M, 10M and 11M) were found in all ranges.

Regarding the aqueous phase, previous research has shown that proportions near 80% are considered critical because they are not able to produce the original thermodynamically stable systems [9]. This principle has proved true because translucent materials were observed with a maximum water percentage of 45% and microemulsion oil with a maximum percentage of 30% in the aqueous phase.Centrifugation is a screening test that consists of accelerating the natural process of collision between the particles through centrifugal force. This increases the size of the colliding particles, which promotes destabilization of the formulation [16, 26, 34].

The materials 14M and 1M remained unchanged after centrifugation. Formulations 15M, 16M, 17M and 18M showed no macroscopic stability after centrifugation with the visible phase separation process.

The evaluation of the pH during the stability tests provide information on the chemical stability of the material. Chemical instability can occur in these materials by oxidation of the oil phase with the formation of oxidized chains or by hydrolysis of triglycerides with the formation of free fatty acids [3, 26]. Changes in conductivity during heat stress are normal and can be explained by absorption or evaporation of water present in the interstitial phases of the formulation [35].

Studies indicate that the pH values of our organism vary between 4.5 and 7.0, which is also a determinant of biocompatibility [36]. According to our results, of the 14 approved materials in the test centrifugation, and when subjected to analysis by temperature stability, two formulations (10M and 14M) were modified for stability, showing phase separation when retested with centrifugation. The other formulations showed results that are

classified as stable and did not significantly vary in pH after heat stress. The pH fluctuated between 6.5 and 7.2, indicating biocompatibility with human tissue.

The electrical conductivity was greater than 1.3 µS.cm-1, indicating systems of the type O/W [35]. The values found for electrical conductivity of the formulations was higher than 1.3 µS.cm-1, and thus, the materials were characterized as O/W. After the thermal stability tests, the samples varied significantly in their electrical conductivity values. In the material 10M, was observed a drastic reduction in the value generated by a phase inversion process was observed. The hysteresis area between two curves represents the work done per unit time and volume to break the bonds in a structural material [37, 38]. This area is represented on the graph when the material undergoes any breakage in structure under the action of shearing [31]. With the hysteresis volume, the area denotes the shear volume of related work, and it is possible to draw conclusions regarding the structural stability of the materials through a qualitative analysis [33, 38]. Studies also indicate that the smaller the area of the hysteresis, the higher the dispersion efficiency of the material [39]. A positive hysteresis area indicates thixotropic material.

The thermal stability analysis showed that after the thermal stability test, for the formulation 10M, which showed Newtonian behavior and hysteresis area -0.20 Pa/s, the area started to change to + 8.54 Pa/s, similar to non-Newtonian thixotropic materials. Moreover, an increase in hysteresis area was observed with phase separation when it was again submitted to centrifugation. The change of state of the formulation to 14M after a thermal stability test is compatible with the non-Newtonian behavior.

In the present study, it was observed that the variations in stability, as demonstrated by physicochemical analyses, during the stability tests as a function of time and temperature are directly linked with the rheological behavior of the material. A study of microemulsion has related increase in viscosity with increase in temperature [40]. In this study has also been found that the pH has a direct correlation with the hysteresis area (r = 0.48).

The electrical conductivity and the hysteresis area presented a directly proportional correlation for these systems (r =0.54); increasing the electrical conductivity also increases the hysteresis area value. The reduction in conductivity is observed in the experiments from the decreased concentration of water in the material after the alternating temperature cycles. It is likely that this reduction has not been promoted as an increase in particle size for Newtonian materials, as their thermal stability was not compromised. As previously mentioned, unstable systems have high particle size distribution and large hysteresis areas [34].

For non-Newtonian materials, conductivity was significantly decreased (p <0.05), and the change was accompanied by an increase in the hysteresis area of the phase inversion process. This behavior is a sign of thermal instability, which causes the separation of water and oil phases. When this occurs, the system loses its characteristics and properties. This behavior was observed in the materials 10M, 14M, 15M, 16M, 17M and 18M. This significant variation in the values of electrical conductivity observed in non-Newtonian materials demonstrates a

close relationship between the rheological behavior and the thermal stability.

In other dispersed systems the stability study by rheological behaviour is not only linked to the nature of flow (Newtonian or non-Newtonian) but also the magnitude of the hysteresis area [38]. The study demonstrated that the liquid microemulsion materials with Newtonian rheological behavior and hysteresis area between +0.20 and -0.20 Pa/s, showed higher thermal stability, confirming the physical and chemical stability test. The MEL, with non-Newtonian behavior, demonstrated thermal instability during

stability testing. Thus, rheological analysis is a good parameter for predicting instability formulations. Although the hysteresis area values were initially classified as Newtonian, formulations 10M and 15M presented instability in the physical and chemical tests. These materials had the highest hysteresis área values between the Newtonian ranges +0.20 and +0.21 Pa/s, respectively. This suggests that hysteresis area values close to 0.20 Pa/s can be considered critical for the physical and chemical stability of the microemulsion materials.

4. CONCLUSIONS

In conclusion, this study on the evaluation of thermal stability and the rheological behavior of materials demonstrated that microemulsion formulations with non-Newtonian behavior and thixotropy did not resist preliminary stability testing. It was also apparent that a direct correlation exists between pH and electric conductivity and the hysteresis area.

The Newtonian behavior with hysteresis area between +0.20 Pa/s and -0.20 Pa/s is indicative of a thermodynamically stable system such as MEs. Values outside of this range are

indicative of an unstable system, even with Newtonian characteristics.

The rheological behavior studies are an important tool that can decrease analysis time during the development of new materials. The study demonstrated that there is a correlation between rheological and thermal stability analyses, and thus, the rheometer can be incorporated in thermal stability tests for microemulsions.and represent an important biological application for drug design.

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6. ACKNOWLEDGEMENTS