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Micro-emultocrit Technique: A Valuable Tool for Determination of Critical HLB Value of Emulsions

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Janus P. F. Macedo,[1] Leonardo L. Fernandes,[1] Fábio R. Formiga,[1] Michael F. Reis,[1] Toshiyuiky Nagashima Júnior,[1] Luiz A. L. Soares,[1] and E. Socrates T. Egito[1]

[1]Universidade Federal do Rio Grande do Norte - Departamento de Farmácia - Programa de Pós Graduação em Ciências da Saúde - Laboratório de Sistemas Dispersos (LASID) - Natal/RN, Brazil

ABSTRACT

The aim of this work was to develop a methodology for rapid determination of the critical hydrophilic-lipophilic balance (HLB) value of lipophilic fractions of emulsions. The emulsions were prepared by the spontaneous emulsification process with HLB value from 4.3 to 16.7. The preparations were stored at 2 different temperatures (25ºC and 4ºC) and their physicochemical behavior was evaluated by the micro-emultocrit technique and the long-term stability study. The experimental data show a reverse relationship between HLB values of the surfactant mixtures and emulsion stability. A close correlation between the results for both stability procedures was observed, suggesting the use of micro-emultocrit to predict stabilities of such systems. In addition, it was found that the critical HLB of the Mygliol 812 was 15.367.

KEYWORDS: emulsions, hydrophilic-lipophilic balance, spontaneous emulsification process, micro-emultocrit, creaming index, stability.

INTRODUCTION

Emulsions are thermodynamic unstable dispersed systems defined as microscopic dispersions of liquid droplets contained within another liquid with a diameter ranging from 0.5 to 100 μm.[1] Most emulsions basically consist of 2 liquids; however, various systems may contain solid particles within them.[1]

An emulsion is formed when 2 immiscible liquids (normally, one being of a lipophilic nature, oil, and the other one of a hydrophilic nature, water) are mechanically stirred.[2] During the stirring process, both liquids tend to form phases. If a surfactant element is added to the system, it tends to stabilize, forming a continuous and a dispersed phase, with the latter presenting a droplet shape. During the above-mentioned stirring process, droplets are formed in both phases, the continuous phases being formed as a result of the great instability within their droplets. For example, if water and oil are mixed to generate oil-in-water (O/W) system, as many drops will be formed within the water as those formed within the oil. However, due to the quick coalescence possessed by the water drops, they will yield the continuous phase. This continuous phase is known as the external phase and surrounds the dispersed (internal) phase in the system.[1]

In general, emulsions are of great importance for the pharmaceutical and cosmetic industries since they enable the use of immiscible ingredients within the same preparation. They also permit a perfect control of their rheological properties owing to the shifting of relative proportions and the dispersion levels of the lipophilic and hydrophilic phases contained within their formulation. In addition, a variation in the concentration of their constituents does not affect the thermodynamic activity significantly, enabling therefore the stability and effectiveness of the active compounds contained in their composition.[3]

A parameter of utmost importance in the development of pharmaceutical emulsions is the evaluation of their critical hydrophilic-lipophilic balance (HLB). This system, developed by Griffin in the 1950s,[4,5] attempted to provide a partial answer to the search for an ideal surfactant for the stabilization of a given system. In the HLB system each surfactant is classified according to its hydrophilic-lipophilic tendency, the HLB value. Hydrophilic surfactants have a high HLB value (generally over 10), whereas lipophilic surfactants have values ranging from 1 to 10.[4,5] Surfactants with self-balance between their lipophilic and hydrophilic portions are extremely efficient as emulsifying agents because they tend to concentrate at the oil/water interface.

On the other hand, the HLB system never provides information concerning the quantity of emulsifying agents that an emulsion must contain. After determining the correct mix of surfactants required to generate the ideal HLB for an emulsified system (called critical HLB [cHLB]), different formulations must be prepared with the same cHLB, but with a varied percentage concentration of the components of the formula. The system chosen must be the one combining higher stability and a smaller amount of surfactants.[6]

Corresponding Author: E. Socrates T. Egito, Departamento de Farmácia, Rua Praia de Areia Branca, 8948 Natal-RN, 59094-450, Brazil. Tel: 55 84 9431 8816; Fax: 55 84 3215 4355; E-mail: socrates@digi.com.br

Table 1. Standard Formula for the Emulsified Systems

Components	$\%_{(wt/wt)}$	Final Weight (g)
Mygliol 812N	5	3
Surfactant system	2	1.2
Distilled water	100	60

Several techniques of preparation can be used to obtain an emulsion. The spontaneous emulsification method is rather efficient for the production of highly homogeneous systems with reduced granulometric sizes, increasing its stability.[7]

The stability analysis of an emulsified system may be performed by 2 methods: long-term and short-term stability studies. The long-term analysis consists of observing the visual and microscopic variations during a preestablished long period.[8] The short-term analysis, however, consists of submitting the emulsions to centrifugation tests at different revolution speeds.[9,10] Recently, we showed that this stability analysis can be performed by the micro-emultocrit technique,[11] a derivation of the microhematocrit technique, which is widely used in clinical hematology and which consists of submitting blood to a revolution of 11 500*g* for 10 minutes.

The aim of this work was to apply the micro-emultocrit technique for the determination of critical HLB of the Mygliol 812N, a short-chain triglyceride, using 24 fluid emulsions prepared by a mix between 2 surfactants, one with a hydrophilic nature (Tween 20, polyoxyethylene 20 sorbitan monolaurate) and the other of a lipophilic nature (Span 80, sorbitan monooleate). The use of this technique intends to foster a broader comprehension of the emulsion stabilization process by means of tools from other fields of knowledge such as the clinical analysis domain.

Materials and Methods

Materials

The surfactants (Tween 20 and Span 80) were purchased from Sigma (St Louis, MO); Mygliol 812N was kindly donated by Condea (Houston, TX); and the ethyl alcohol, analytical grade, was supplied by Vetec (Natal, Brazil).

Methods

The standard formula applied to all emulsions is presented in Table 1.

Hydrophilic-lipophilic Balance Spreadsheet Design

The emulsions were prepared following the spreadsheet design shown in Tables 2 and 3. This spreadsheet includes 2 surfactants: one of a lipophilic nature (Span 80, HLB = 4.3) and the other of a hydrophilic nature (Tween 20, HLB = 16.7). The final HLB value of each system varied according to the individual percentage of each surfactant. Therefore, the variation among the HLB total values comprises one unit (Table 2). In a second step, the 3 more stable emulsion systems generated a second HLB spreadsheet design with HLB values adjusted to a significant figure of 2 decimals (Table 3).

Preparation of the Emulsions

The emulsions were prepared by the spontaneous emulsification method,[7] which consisted of the injection of an organic solution into an aqueous solution. The oil-phase components were diluted in ethyl alcohol and the water-phase components in distilled water. The ingredients were

Table 2. Hydrophilic-Lipophilic Balance Value Spreadsheet Design in Accordance With Individual Surfactant Percentages*

	Tween 20		Span 80		Final HLB Value of the
Formulation (F)	$(\%_{wt/wt})$	HLB Contribution	$(\%_{wt/wt})$	HLB Contribution	Formulation
F1	100.0	16.7	0.0	0.0	16.7
F2	91.9	15.4	8.1	0.3	15.7
F3	83.9	14.0	16.1	0.7	14.7
F4	75.8	12.7	24.2	1.0	13.7
F5	67.7	11.3	32.3	1.4	12.7
F6	59.7	10.0	40.3	1.7	11.7
F7	51.6	8.6	48.4	2.1	10.7
F8	43.5	7.3	56.5	2.4	9.7
F9	35.5	5.9	64.5	2.8	8.7
F10	27.4	4.6	72.6	3.1	7.7
F11	19.4	3.2	80.6	3.5	6.7
F12	11.3	1.9	88.7	3.8	5.7
F13	3.2	0.5	96.8	4.2	4.7
F14	0.0	0.0	100.0	4.3	4.3

*HLB indicates hydrophilic-lipophilic balance.

Table 3. Hydrophilic-Lipophilic Balance Value Spreadsheet Design Comprising 2 or More Decimals

Formulation (F)	Tween 20		Span 80		Final HLB Value of the Formulation
	($\%_{wt/wt}$)	HLB Contribution	($\%_{wt/wt}$)	HLB Contribution	
F15	100.000	16.700	0.000	0.000	16.700
F16	98.208	16.401	1.792	0.077	16.478
F17	96.416	16.101	3.584	0.154	16.256
F18	94.624	15.802	5.376	0.231	16.033
F19	92.832	15.503	7.168	0.308	15.811
F20	91.040	15.204	8.960	0.385	15.589
F21	89.248	14.904	10.752	0.462	15.367
F22	87.456	14.605	12.544	0.539	15.145
F23	85.664	14.306	14.336	0.616	14.922
F24	83.872	14.007	16.128	0.694	14.700

*HLB indicates hydrophilic-lipophilic balance.

then measured and separated according to their solubility properties (lipophilic or hydrophilic). The components of the oil phase were dissolved in 28 mL of ethyl alcohol. In a different beaker, the hydrophilic components were mixed in 56 mL of distilled water. The spontaneous emulsification process occurred immediately when the oil phase was slowly incorporated into the water phase using a syringe and slight magnetic stirring. At the end of the oil-phase incorporation, the organic cosolvent was removed by reduced pressure at 50°C, using an evaporator (Fisatom, São Paulo, SP, Brazil). The final volume was then established and the newly prepared emulsions were stored under 2 different conditions: 20 mL at 25°C in a test tube and 20 mL at 4°C in a test tube. This methodology produce emulsions with a negligible amount of residual alcohol.[7]

Characterization of the Emulsions

Mean diameter evaluation

For the mean diameter calculation, the diameters of 500 droplets of emulsion were counted, following Ferret's method,[12] by using an optical microscope (model Axioscop 50, Carl Zeiss, Oberkochen, Germany) equipped with a calibrated eyepiece micrometer (1 unit = 1.6 µm at 400×).

Morphological analysis

Morphological examination of the emulsions was performed using an optical microscope following blue staining from a methylene blue solution at $2\%_{(wt/wt)}$. The preparation was observed by 4×, 10×, and 40× ocular.

Macroscopic aspect

The color of the emulsions, as well as their stability variation (presence of creaming, coalescence, or separation of phases), was verified through visual examination of the 2 storage conditions. The creaming was followed by the measurement of the creaming index (CI). The CI value was obtained by the ratio between the cream layer and the total emulsion layer according to Equation 1[13]:

$$\%CI = \left(\frac{H_C}{H_O}\right) \times 100, \qquad (1)$$

where H_c is the numeric value of the height of the cream layer and H_o is the numeric value of the total height of the emulsion.

pH Evaluation

The pH measurements of the emulsions were performed at both storage temperatures. For measurements at 25°C, a precalibrated pH meter (model Checker, Hanna Instruments, Vila do Conde, Portugal) was used, while a pH paper (pH 0-6 and 6-7.2) (Sigma) was used for those stored at 4°C.

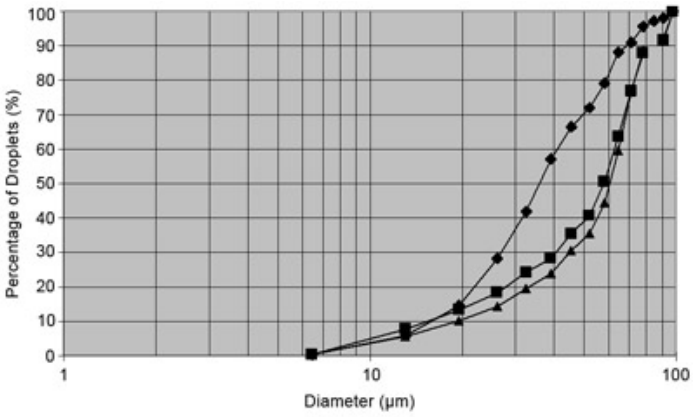

Figure 1. Mean diameter size of the Formulations F1 (■), F2 (▲), and F3 (◆).

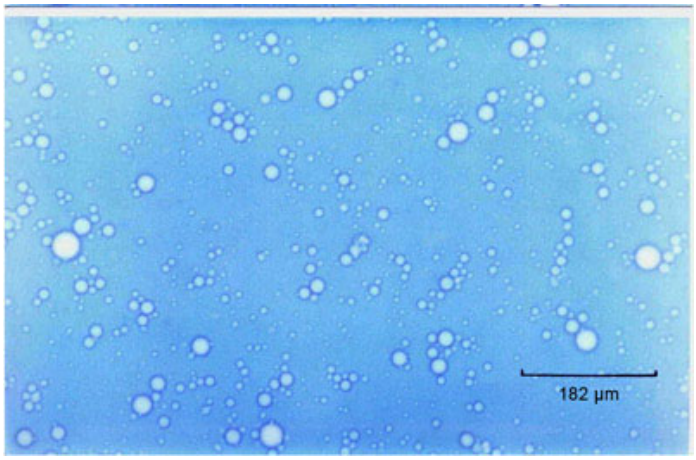

Figure 2. Microscopic droplets of the emulsified systems.

Stability studies

The emulsions were analyzed by 2 stability parameters:

Long-term stability

The macroscopic aspect, creaming rate, and pH were determined on storage days 1, 3, 5, 9, 15, 30, 60, 90, 120, and 180.

Short-term stability - stability after centrifugation

For this study, the micro-emultocrit technique was used.[11] The micro-emultocrit was performed by filling 75% of a heparin-free capillary tube with each formulation and placing it in a microcentrifuge (Fanen, São Paulo, SP, Brazil) at 11 500*g* for 10 minutes. This procedure simulates the microhematocrit technique, largely used for evaluating the percentage of erythrocytes in human blood. After the centrifugation cycle, the capillary tubes were placed against the microhematocrit scale, and the CI was directly measured. The visual aspect was evaluated in order to investigate phase separation. For those preparations, which were not broken, CI was measured by the microhematocrit reading scale.

Results and Discussion

The emulsions produced using the presented method were stable, and their mean droplet size was found to be ~40 µm (Figure 1), which was confirmed by the optical microscopic evaluation (Figure 2).

cHLB Calculation

As can be seen below, from the first set of emulsions (F1-F14; Table 2), the short- and long-term stability study reveals that the cHLB for the Mygliol 812N ranged from 14.7 to 16.7, normal for an emulsion with a water external phase.[4,5] From the second new series of emulsions (F15-F24; Table 3), which presented HLB values from 16.7 to 14.7, it was established that the final cHLB for Mygliol 812N was 15.367. Therefore, the improved stability of Formulations 1 to 3 concerns the localization of the couple of surfactants in the interfacial layer.[14] In fact, for emulsion systems with a water external phase, an HLB value between 8 and 14 is mandatory.[5,6] Higher or lower HLB values will induce the solubility of the surfactant in the water or oil phase, respectively.

Long-term Stability Study

Despite the large range of the HLB values of the systems, the spontaneous emulsification process was able to produce emulsions that were stable with a milky aspect and white color, and that remained stable on the first day (D_0) of preparation (Table 4). Starting from the third day (D_3), emulsions

Table 4. Microscopic Aspect of Emulsions Stored at 25ºC and 4ºC*

Formulation	D_0	D_1	D_3	D_5	D_9	D_{15}	D_{30}	D_{60}	D_{90}	D_{120}	D_{180}
F1	M	M	M	M	M	M	M	M + CR	M + CR	M + SP	M + SP
F2	M	M	M	M + CR	M + CR	M + CR	M + CR	M + CR	M + CR	M + SP	M + SP
F3	M	M	M	M + CR	M + CR	M + CR	M + CR	M + CR	M + CR	M + SP	M + SP
F4	M	M	M + CR	M + CR	M + CR	M + CR	M + CR	M + CR	M + CR	M + SP	M + SP
F5	M	M	M + CR	M + CR	M + CR	M + CR	M + CR	M + CO	M (Y) + CO	M (Y) + SP	M (Y) + SP
F6	M	M	M + CR	M + CR	M + CR	M + CR	M + CR	M + CO	M (Y) + CO	M (Y) + SP	M (Y) + SP
F7	M	M	M + CR	M + CR	M + CR	M + CR	M + CO	M + CO	M (Y) + CO	M (Y) + SP	M (Y) + SP
F8	M	M	M + CR	M + CR	M + CR	M + CO	M + CO	M + CO	M (Y) + CO	M (Y) + SP	M (Y) + SP
F9	M	M	M + CR	M + CR	M + CO	M + CO	M + CO	M + CO	M (Y) + SP	M (Y) + SP	M (Y) + SP
F10	M	M	M + CR	M + CR	M + CO	M + CO	M + CO	M + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP
F11	M	M	M + CR	M + CR	M + CO	M + CO	M + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP
F12	M	M	M + CR	M + CR	M + CO	M + CO	M + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP
F13	M	M	M + CR	M + CR	M + CO	M + CO	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP
F14	M	M	M + CR	M + CR	M + CO	M + CO	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP	M (Y) + SP

*D indicates day; M, milky aspect; CR, creaming; CO, coalescence; SP, separation of phases; (Y), yellowish aspect; and M (Y), milky and yellowish aspect.

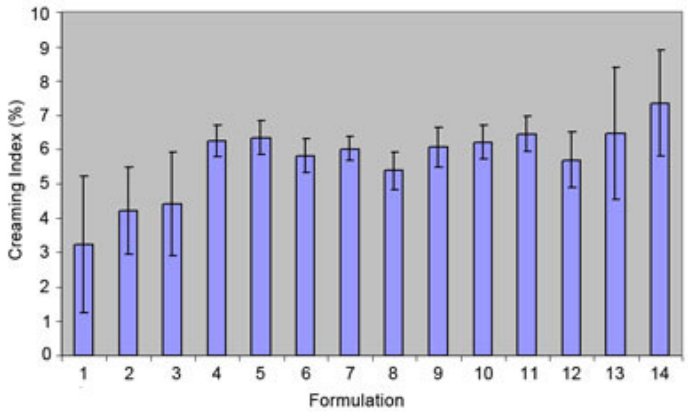

Figure 3. Creaming index (%) of the Formulations stored at 25ºC for 180 days.

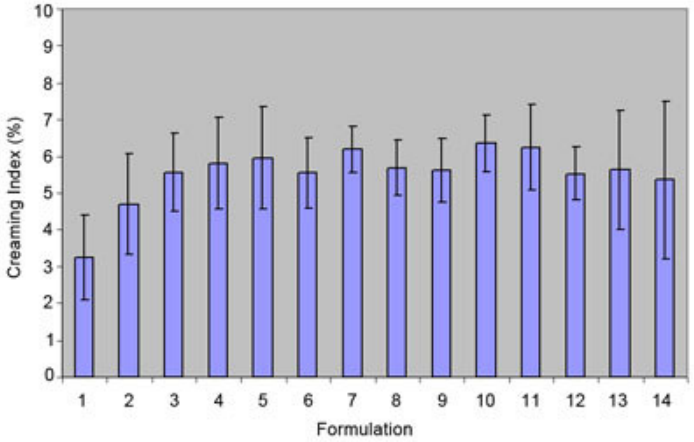

Figure 4. Creaming index (%) of the Formulations stored at 4ºC for 180 days.

with HLB systems lower than 14.7 (starting from Formulation F4) presented a cream layer that increased over time. The coalescence was noticed only on the ninth day for Formulations F9 to F14. This phenomenon was observed for the systems stored at both 25°C and 4°C. Therefore, the storage temperature had no influence on the evaluation of the macroscopic aspect of the emulsions.

After $D_{30,}$ the emulsions tended to have a greater instability, and separation of phases took place (Table 4). Formulations F1 to F4 presented creaming until D_{90}. On D_{60}, the coalescence took place in Formulations F5 to F9. After D_{90}, Formulation F9 started to present separation of phases. Later, all formulations presented separation of phases.

Except for Formulations F1, F2, and F3, all emulsions maintained at 25°C presented an important value on the CI rate (Figure 3 and Table 5). After D_5, most systems (Formulations F6-F14) tended to change their creaming rates, owing mainly to the beginning of the coalescence. Again, these results suggested that the emulsifying systems used on Formulations F1, F2, and F3 had provided more stable emulsions, probably owing to their localization on the interface layer of the emulsion droplets.[15,16]

The creaming rate of emulsion systems maintained at 4°C varied toward the same CI as those maintained at 25°C (Figure 4). However, the emulsions maintained at the lower temperature presented a more stable CI for Formulations F1, F2, and F3 and a less stable CI for Formulations F13 and F14. In fact, the CI reduction during this period for these formulations (F13 and F14) was the result of the appearance of coalescence in such systems (Table 4). It is important to point out that after D_{60}, Formulations F12 to F14 started to present separation of phases. Therefore, during this period, the CI was not calculated.

Table 5. Correlation Between the Creaming Index Values From the Micro-emultocrit Assay and the Long-term Stability of the Emulsions at 25ºC*

Micro-emultocrit		Period of Time and CI value (%)								
Formulation (F)	CI value (%)	D_1	D_3	D_5	D_9	D_{15}	D_{30}	D_{60}	D_{90}	D_{180}
F1	3	2.4	2.0	2.5	2.5	2.0	2.0	4.8	7.6	7.6*
F2	3	3.1	3.2	3.5	4.0	4.0	4.2	4.6	7.1	7.1*
F3	3	3.1	3.5	3.5	4.0	4.2	4.5	4.5	7.9	7.9*
F4	6	6.0	6.3	6.5	6.3	6.0	6.0	5.7	7.2	7.2*
F5	7	6.0	6.5	6.8	6.3	6.2	6.0	5.7	7.2†	7.2*
F6	7	5.8	6.0	6.5	6.0	6.0	6.0	6.0	6.0†	6.0*
F7	9	5.8	6.2	6.5	6.0	6.2	5.9	6.2	6.2†	6.2*
F8	9	4.8	5.5	6.0	6.0	6.0	6.0†	6.0†	6.0†	6.0*
F9	9	5.8	6.0	6.3	6.8	6.8	6.8†	6.8†	6.8†	6.8*
F10	11	6.1	6.5	6.7	6.2	6.7	6.7	6.7†	6.7†	6.7*
F11	11	6.0	6.5	6.8	7.0	7.0†	7.0†	7.0†	7.0†	7.0*
F12	11	5.0	6.5	6.7	6.7†	6.7†	6.7†	6.7†	6.7†	6.7*
F13	13†	8.0	8.0	8.5	8.5†	8.5†	8.5†	8.5†	8.5†	8.5*
F14	13†	8.5	8.5	8.8	8.8†	8.8†	8.8†	8.8†	8.8†	8.8*

*CI indicates creaming index; and D, day.
†indicates presence of separation of phases.

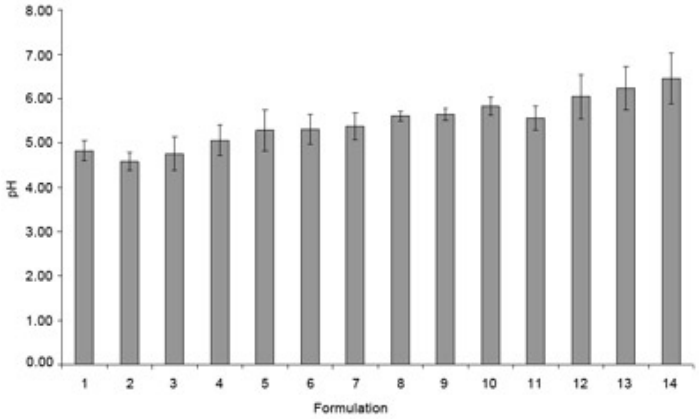

Figure 5. pH Evolution of the Formulations stored at 25ºC for 30 days.

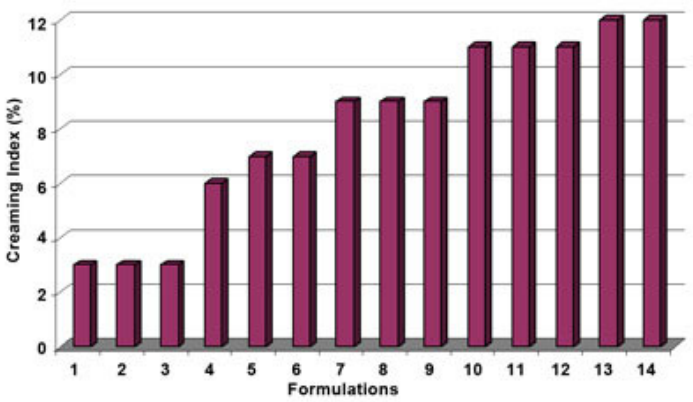

Figure 7. CI analysis of the formulation performed by the micro-emultocrit technique.

Based on the pH analysis data for both storage conditions of the emulsions (25°C and 4°C), it was verified that the pH tends to increase in the systems with higher amounts of Span 80 and to diminish in the ones with higher amounts of Tween 20 (Figures 5 and 6). These results are likely owing to the destruction of the binding between the polar and apolar groups of the surfactants. This causes either a pH increase (in the case of an increasing amount of Span 80) or decrease (in the case of an increasing amount of Tween 20), according to the pH of the respective surfactant, which constitutes the micelles or lamellas of the interfacial layer.

Short-term Stability Study

The centrifugation studies reveal not only that the emulsion stability was highly influenced by the gravity acceleration,[9,10] but also that the HLB value of the surfactant system played an important role (Figure 7). In fact, an inverse correlation between the HLB value of the surfactant system and the CI variation was found. Moreover, while formulations F1, F2, and F3, which have HLB values of 16.7, 15.7, and 14.7, respectively, presented a CI of ~3%, Formulations F13 and F14, which have HLB values of 4.3 and 4.7, respectively, showed a CI value of 12%.

Because these results were quite similar to those found for the long-term stability study, they show how efficient the micro-emultocrit technique is in the analysis of emulsion stability.

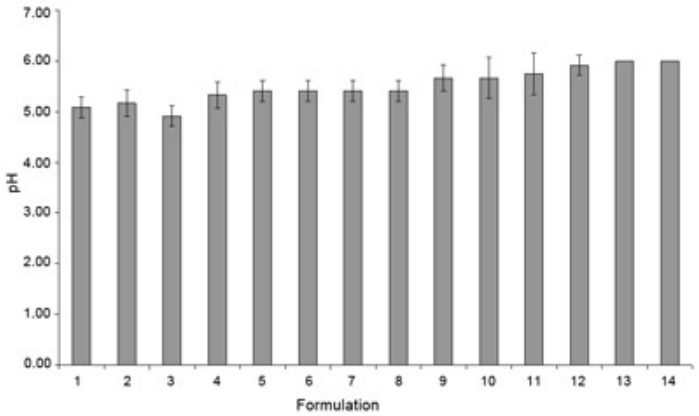

Figure 6. pH Evolution of the Formulations stored at 4ºC for 30 days.

Correlation Between Creaming Index Value and the Long-term Stability Study (Tables 5 and 6)

For the emulsion systems stored at 25ºC, no close correlation between their CI value and their long-term stability study was found on any specific day. In fact, because of the stress induced for all systems, individually, each day during the long-term stability analysis, this relationship could not be seen. However, the micro-emultocrit was an important tool to predict the stability behavior of the studied formulations throughout the days. It could be observed that the range of the lowest CI for the micro-emultocrit test was correlated to the most stable formulations. Likewise, higher CI values for the micro-emultocrit test indicated unstable systems. This behavior concurs with the long-term stability results. Therefore, those formulations with lower CI for the micro-emultocrit test presented maximum stability up to 60 days. The systems with higher CI values were stable only for a few days. Thus, the stability behavior of the emulsified systems could be distributed in independent blocks (Table 5). The boldface values represent the intermediate block and are the biggest CI values before the beginning of the broken formulations. The values marked with an asterisk are part of the less stable represented block in the table, which contains formulations with separation of phases and high values of CI. Based on the micro-emultocrit results, the time of stability of the emulsified systems can be predicted.

The same correlation was found for the emulsion systems stored at 4ºC (Table 6). However, these systems presented

Table 6. Correlation Between the CI Values From the Micro-emultocrit Assay and the Long-term Stability of the Emulsions at 4ºC*

Micro-emultocrit		Period of Time and CI value (%)								
Formulation (F)	CI value (%)	D_1	D_3	D_5	D_9	D_{15}	D_{30}	D_{60}	D_{90}	D_{180}
F1	3	2.8	2.8	2.5	2.5	2.7	2.5	5.1	5.1	5.1
F2	3	3.8	4.0	4.2	4.2	3.8	3.9	6.2	7.5	7.5
F3	3	4.7	5.0	5.2	5.3	5.0	4.8	7.2	7.3	7.5†
F4	6	4.8	5.2	5.7	5.71	4.8	4.8	7.7	7.8	7.8†
F5	7	4.8	5.2	5.8	5.9	4.8	5.0	7.8	8.4	8.4†
F6	7	4.8	5.5	5.7	5.7	4.8	4.8	7.5	7.5	7.5†
F7	9	5.8	6.0	5.8	6.7	5.8	5.9	7.4	7.5†	7.4†
F8	9	5.8	6.3	6.7	5.0	5.0	4.8	6.2	6.2†	6.2†
F9	9	4.8	5.5	6	5.8	5.0	4.9	7.3	7.3†	7.3†
F10	11	6.4	6.5	6.5	5.5	5.9	5.8	7.9†	7.9†	7.9†
F11	11	5.5	6.0	6.5	5.5	5.9	5.7	8.8†	8.8†	8.8†
F12	11	5.6	6.0	6.5	6.5†	6.5†	6.5†	6.5†	6.5†	6.5†
F13	13	7.0	7.2	7.2†	7.0†	7.0†	7.0†	7.0†	7.0†	7.0†
F14	13	7.0	7.5†	7.5†	7.5†	7.5†	7.5†	7.5†	7.5†	7.5†

*CI indicates creaming index; and D, day.

†indicates presence of separation of phases.

only a little instability due to the lower kinetic energy, which contributes to speeding up the instability process that the molecules suffer, induced by the low temperature.[1-3]

CONCLUSION

The micro-emultocrit technique in this study showed a close correlation with the long-term stability assay, suggesting that it can be a valuable appropriated tool for a fast determination of the cHLB of lipid fractions of emulsions. In conclusion, this methodology not only decreases the time needed for emulsion stability studies but also allows the use of a very small sample volume that reduces the final cost during emulsion analysis and its cHLB determination.

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