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Physicochemical Characterization of Canola Oil/Water Nano-emulsions Obtained by Determination of Required HLB Number and Emulsion Phase Inversion Methods

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Depending on the emulsion preparation method, different droplet size distributions might be achieved. The EPI method (low-energy emulsification method) works with water volume fraction changes. Nanometer emulsions can be obtained by this method. The aim of this work was to evaluate the canola oil-water nano-emulsion physicochemical characteristics: zeta potential and droplet size values upon preparation and after stability tests. The obtained outcomes suggest that nano-emulsion yielded by EPI and required HLB methods is quite stable. This can support reported nanometer size droplets and high zeta potential values. Although the emulsions have been submitted to a range of temperatures, the evaluated parameters did not present significant change.

INTRODUCTION

An emulsion is a heterogeneous system consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets. These systems are thermodynamically unstable and are not formed spontaneously (Becher and Schick 1987). Their properties depend not only on thermodynamic conditions but also on preparation methods and the order of components addition (Forgiarini et al. 2001). Emulsions basically have three components: water, oil, and surfactant. Physicochemical properties of these components could affect the emulsion behavior (Sajjadi et al. 2003). Nanometer emulsions can be obtained by employing these properties, generally referred to as low-energy emulsification methods. These methods make use of changing the spontaneous curvature of the surfactant (Zerfa et al. 2001; Forgiarini et al. 2001; Fernandez et al. 2004).

Depending on the preparation method, different droplet size distributions might be achieved, explaining why the preparation route can have an influence on emulsion stability (Jeong et al., 2001; Zerfa et al. 2001; Fernandez et al. 2004).

Three methods may be applied for the preparation of nano-emulsions: use of high-pressure homogenizers, use of the low--energy emulsification method at constant temperature, or application of the phase inversion temperature concept (Sonneville-Aubrun et al. 2004; Tadros et al. 2004). Therefore, the smaller the droplet size, the more energy and/or surfactant is required, making this preparation route unfavorable for industrial applications when very small droplets are desired (Zerfa et al. 2001; Sajjadi et al. 2003; Fernandez et al. 2004).

Phase inversion is the process whereby an oil-in-water system (O/W) inverts to a water-in-oil system (W/O) and vice versa. Submicron emulsions can be obtained by this method (Marszal, 1987; Zerfa et al. 2001; Miller et al. 2001; Salager et al. 2004). There are at least two types of phase inversion. A catastrophic inversion occurs when the inversion take place because of a change in volume fraction (i. e., increasing the volume fraction of the dispersed phase). This type of inversion is not reversible and can occur over a wide range of volume fractions. A transitional inversion occurs when there is a change in the surfactant's affinity towards the two phases. This is induced by hydrophilic-lipophilic balance (HLB) changes of the emulsion system. In order to achieve a transitional inversion process, the surfactant concentration must be above the critical micelle concentration (CMC) values (Becher et al. 1987; Zerfa et al. 2001; Sajjadi et al. 2003; Salager et al. 2000, 2004; Xie et al. 2004).

PIT (phase inversion temperature) or HLB temperature is defined as the temperature at which a hydrophilic-lipophilic property of a surfactant just balances at the interface. The balanced surfactant exhibits several characteristic properties such as PIT in emulsion, a strong solubilizing power, and ultralow interfacial tension (Marszal 1987). The PIT concept has proved fruitful from both theoretical and practical points of view. This method works with a surfactant system that is temperature sensitive, generally a nonionic surfactant of the polyethoxylated type that becomes less hydrophilic as temperature increases and vice versa (Marszal 1987; Zerfa et al. 2001; Miller et al. 2001; Salager et al. 2004).

Another transition in the spontaneous radius of curvature can be obtained by changing the water volume fraction; this method is called emulsion inversion point (EIP). The EIP is the point at which the emulsion, composed of an oil, emulsifier, and water, changes from a W/O to an O/W system at constant temperature. Therefore, the EIP represents the number of cubic centimeters of added water per cubic centimeter of the oil present when phase inversion occurs. By successively adding water into oil, water droplets are initially formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a W/O emulsion to an O/W emulsion at the inversion locus (Lin et al. 1975; Marszal 1987; Fernadez et al. 2004; Salager et al. 2004; Tadros et al. 2004).

Tadros et al. (2004) reported some advantages for pharmaceutical and cosmetic application of the nano-emulsion system: (i) suitable for efficient delivery of active ingredients through the skin, the large surface area of the emulsion system allows rapid penetration of the nanopariticles actives, (ii) due to small size, they can penetrate through the "rough" skin surface, and this enhances penetration of actives, and (iii) the transparent nature of the system, where its fluidity as well as the absence of any thickeners may give it a pleasant aesthetic character and skin feel.

Emulsion stability is evidently controlled for the properties of the adsorbed layer formed in the surface of its globules. These properties are not only the interfacial tension but also the properties of the continuous solution near the interface. The layer structure depends on the composition and concentration of the surfactants and/or polymers used (Friberg et al. 1988; Wiacek and Chibowski 1999).

When a small oil droplet is dispersed in an immiscible aqueous solution, it may be charged and surrounded by ions of opposite sign from the solution. The arrangement of the electric charge on the oil droplet, together with the charge balance in the solution, called the electrical double layer of the droplet (Gu and Li 1997). The pattern of the electrical double layer allows us to visualize the ionic atmosphere in the region surrounding a charged globule and explains how the forces of electrostatic repulsion act. The repulsion from the electrical double layer is important because it played a decisive role in the theory for colloidal stability called

DLVO, after its originators Derjarguin, Landau, Vervey, and Overbeek. The DLVO theory relates the stability of emulsified droplets for two independent potentials that come into action when two droplets approach each other. The van der Waals potential is negative (attractive) and the potential from the electrical double layer is positive (repulsive) (Friberg et al. 1988; Wiacek and Chibowski 1999; Zeta-Meter, Inc. 1997). The net charge of these potentials, i. e., the electric superficial potential, cannot be directly measured, but this parameter can be obtained from the electrokinetic or zeta potential.

Therefore, zeta potential can be used to estimate the stability of an O/W emulsion since this determines the electrostatic repulsion between approaching droplets (Friberg et al. 1988; Wiacek and Chibowski 1999; Zeta-Meter, Inc. 1997).

The HLB number defines the polarity of nonionic surfactants in terms of an empirical quantity. It must be noted that each nonaqueous component has associated with it a required HLB number, i.e., the required HLB of an emulsifier for optimum emulsification, and that the required HLB will differ depending on whether water or oil will be the continuous phase (Becher et al. 1987). The best HLB number of an emulsifier for an emulsion is similar to the required HLB value of the oil (Marszal 1987). Braconi et al. (1995) have reported the feasibility of canola oil for cosmetics purposes.

Measurement of the globule distribution sizes initially and during storage provides an indication of the system stability: the faster the globules increase in size, the lower the stability (Friberg et al. 1988; Craig et al. 1995; Pather et al. 1995; Sontum et al. 1997).

Accelerated testing methods are need for characterization of long-range stability. This has been attempted by means of storage at elevated temperatures and centrifugation (Becher et al. 1987).

The aim of this work was to evaluate canola oil-water nano-emulsion physicochemical characteristics: zeta potential and size distribution upon preparation by required HLB number and EPI methods and after thermal stress and accelerated stability tests.

MATERIAL AND METHODS

Chemicals

Emulsions were prepared with different pairs of hydrophilic and lipophilic surfactants (Table 1), distilled water, and canola oil. The chemicals were used as received without further purification.

Determination of Required HLB Number for Canola Oil

The required HLB number of canola oil was determined by an HLB scan, involving the preparation of emulsions over a range of HLB (7.0 up to 12.0) (Gonçalves and Rocha-Filho 1999; Santos et al. 2005). Blends of Spans and Tweens, Unitol and Volpo, and Span 80 and Surfom R-400 in various

TABLE 1
Surfactants employed for production of O/W emulsions

Product	Chemical name	CTFA name	HLB
Span® 20 (S20)	Sorbitan monolaurate	Sorbitan laurate	8.6
Span® 60 (S60)	Sorbitan monooctadecanoate	Sorbitan stearate	4.7
Span® 80 (S80)	Sorbitan monooleate	Sorbitan oleate	4.3
Tween® 20 (T20)	PEG 6 sorbitan laurate	Polysorbate 20	16.7
Tween® 60 T60)	PEG 6 sorbitan stearate	Polysorbate 60	14.9
Tween® 80 (T80)	PEG 6 sorbitan oleate	Polysorbate 80	15.0
Unitol® O-100 (U100)	PEG 10 olelyl alcohol	Oleyl alcohol	12.4
Volpo® 3 (V3)	PEG 3 oleyl alcohol	Oleyl alcohol	6.6
Surfom® R-400 (S400)	PEG 40 hydrogenated castor oil	Hydrogenated castor oil	13.0

ratios were used (Table 2). The amount of surfactants was kept at 5.0%. The HLB number of the surfactant mixture (A/hydrophilic and B/lipophilic surfactants) was calculated using the weight fraction of the corresponding surfactants as shown in the following equation:

$$HLB(mixture) = HLB_A \times 0.01A + HLB_B \times 0.01B$$
$$A\% + B\% = 100\%$$

Preparation of Emulsions

Emulsions were yielded by the EPI method. Blends of surfactants (5.0% w/w) were dissolved in the oil phase (5.0% w/w). Oil and aqueous phases were separately heated to $70° \pm 2°C$. Water phase (90.0% w/w) was slowly added into oil phase. The mixer speed was set at 400 rpm (Mechanical Agitator Fisaton-702 R) to provide better mixing conditions and give a uniform distribution of the components of the emulsion. Emulsions were kept under mixing until reaching room temperature, $25° \pm 2°C$.

Centrifugation Test

A 5.0 g sample of each emulsion was submitted to three different rotations (1,000, 2,500, and 3,500 rpm) during 15

minutes for each cycle and at room temperature, $25° \pm 2°C$. Only those samples that did not show instability signs (flocculation, creaming, and breaking) were regarded as stable and subjected to the stability tests.

Thermal Stress Test

Samples were submitted to a range of temperatures (50° up to 80°C) during 30 minutes for each condition in a thermostatic bath (Nova Tecnica NT 281).

Accelerated Stability Test

Samples were submitted to three different temperatures (5°, 25° and 45°C) during 30 days. Evaluation of samples was carried out at 1, 7, 15, and 30 days after their preparation.

Determination of pH Values

Samples were diluted with distilled water (1 : 10), then electrodes (Digmed DMPH-2) were directly inserted into solutions and their pH values were determined.

Determination of Zeta Potential and Size Distribution Analysis

Zeta potential and size distributions were performed using a Coulter DELSA 440SX (Santos et al. 2005). This system

TABLE 2
Possible required HBL number for canola oil and weight fraction of surfactants pairs employed for O/W emulsions production

Required HLB (canola oil)	T20	S20	T60	S60	T80	S80	U100	V3	S400	S80
7.0	—	—	1.13	3.87	1.26	3.74	0.35	4.65	1.55	3.45
8.5	—	—	1.86	3.14	1.97	3.03	1.64	3.36	2.42	2.58
8.8	0.12	4.88	2.01	2.99	2.10	2.90	1.90	3.10	2.58	2.42
9.0	0.25	4.75	2.10	2.90	2.20	2.80	2.07	2.93	2.70	2.30
10.0	0.86	4.14	2.60	2.40	2.66	2.34	2.93	2.07	3.28	1.72
12.0	2.10	2.90	3.58	1.42	3.60	1.40	4.65	0.35	4.43	0.57
14.0	3.33	1.67	4.56	0.44	4.53	0.47	—	—	—	—

analyses zeta potential by electrophoretic particle and colloid mobility (0.02 to 30 μm diameter) in liquid dispersions. The system analyzes suspensions and emulsions by making independent laser Doppler measurements at four different angles (8.9°, 17.6°, 26.3°, 35.2°) simultaneously with 256-channel resolution each. Comparisons of simultaneous laser Doppler spectra from four angles allow the detection of very small particles. The size distribution of particles is based on photon correlation spectroscopic analysis. This obtains the size distribution of particles by illuminating them with laser. The samples were diluted in distilled water (1 : 20) before application in the device sample chamber.

Determination of Phase Inversion Temperature (PIT)

The PIT can be obtained by measuring the change in conductivity of an O/W emulsion upon heating. At the inversion point, a nonconductive W/O emulsion is formed, resulting in a sharp decrease in conductivity (Fernandez et al. 2004).

Determination of Liquid Crystalline Structure Existence

Samples were submitted to a microscopic analysis performed by a polarized light view microscope (Olympus BX50).

All measurements were obtained in triplicate.

RESULTS AND DISCUSSION

The HLB number at which maximum stability occurs can be determined, and this is the required HLB number for the oil in question (Becher et al. 1987). In this work, we have calculated the required HLB number for canola oil by an HLB scan, involving the preparation of emulsions over a range of HLB (7.0 to 12.0). Moreover, we have decided work with O/W emulsions. For the five pairs of surfactants only the pair of Surfom R-400 and Span 80 has showed stable emulsions in the range HLB of 8.0 to 9.0, even after they have been submitted to stability tests (except zeta potential and size distribution analysis). This may suggest a positive influence of required HLB number preparation methods on this feature. Thus, we have chosen to work with O/W emulsions with HLB number of 8.8. This was selected as the required HLB number for canola oil for the system in question. PIT for this system was obtained by measuring the change in conductivity of O/W emulsion upon heating. For the surfactant mixture (Surfom R-400 and Span 80), we could not observe any phase invertion until the temperature of 90° ± 2°C.

The size of emulsion droplets varies markedly with the temperature of preparation, and the droplet diameter is very small close to the EPI. In this work we can relate this characteristic, which is in accordance with literature (Lin et al. 1975; Marszal 1987; Forgiarini et al. 2001; Fernandez et al. 2004; Tadros et al. 2004). When a fairly hydrophilic surfactant was first placed in the oil phase, gradually added water was initially solubilized by the surfactant micelles dispersed into the oil phase as a result of the swelling of micelles. When

the swelling continued, a great number of water droplets may have been formed into the oil phase. This primary emulsion inverts to finally form O/W emulsions as the surfactant migrates into the aqueous phase. At the EPI minimum the inversion from W/O to O/W type emulsion occurs and produces emulsions with a very small diameter of droplets (Marszal 1987; Salager et al. 2000, 2004; Fernandez et al. 2004). Some researchers have proposed that the droplet sizes may be controlled by the structure of the bicontinuous or lamellar phase formed during the phase inversion (Forgiarini et al. 2001; Fernandez et al. 2004). In contrast to the above studies, we could not find any lamellar liquid crystalline structure in our samples. However, this feature is in conformity with Forgiarini et al. (2001). Their observations have suggested that a lamellar liquid crystalline phase is probably required but not sufficient to obtain nano-emulsions.

The magnitude of the zeta potential is directly correlated to the colloid and/or emulsion stability. Measurement of the globule size initially, during storage and after thermal stress and accelerated stability tests, provides an indication of the system stability (Friberg et al. 1987; Pather et al. 1995; Stachurski and Michalek 1996; Wiacek and Chibowski 1999; Hsu and Nacu 1999; Jeong et al. 2001; Zeta-Meter, Inc. 2003; Roland et al. 2003).

In this study we have used kinetic energy (high temperatures) to improve Brownian motion; this process would allow the droplets to overcome the energy barrier and, thus, they could approach each other. Some methods to create an energy barrier between two droplets should be considered such as the electrical double layer and steric repulsion, moreover this is the place where maximum energetic repulsion occurs. The high energy barrier is directly related to system stability (Friberg et al. 1988; Zeta-Meter Inc., 1997).

As a result, we should expect a fall in high energy barrier after thermal stress and accelerated stability tests are carried out. A decrease in the absolute value of zeta potential was correlated with a higher tendency to flocculate. Generally, lower negative or positive zeta potential values might not stabilize O/W emulsions; therefore these values indicate an inadequate electrostatic repulsion between droplet surfaces (Friberg et al. 1988; Jeong et al. 2001; Zeta-Meter Inc., 2003). Then phenomena such as flocculation and coalescence would become possible, and these processes can therefore increase droplet sizes.

Sample results of pH, zeta potential, and droplet sizes before and after the thermal stress test are shown in Table 3. Moreover, Table 4 and Figure 1 show the results of pH, zeta potential, and droplet sizes after the accelerated stability test was carried out.

Assessment of the droplet sizes just after preparation indicated nano-emulsion formation, their globule size from 215 to 233 nm. According to our results, nano-emulsions can be produced in the studied system by the chosen method (EPI). Note that an emulsification method by PIT can be excluded

TABLE 3

Sample results of pH, zeta potential (zp), and droplet sizes (mean of three values and their standard deviation) before and after thermal stress test

Before thermal stress test			After thermal stress test		
pH	zp (mV)	Size (nm)	pH	zp (mV)	Size (nm)
6.35 ± 0.19	-49.54 ± 5.69	214.7 ± 0.47	6.51 ± 0.02	-37.04 ± 0.87	232.33 ± 24.51

for these experiments, since emulsion systems showed an inversion temperature around $90^\circ \pm 2^\circ$C. Moreover, we did not employ high mechanical agitation. We can assume that the submicrometer-sized oil droplets were obtained by emulsion phase inversion. These systems still show narrow size distributions (data not shown) and a milky, bluish appearance.

Submitting samples to high (45°C) and low (4°C) temperatures even after 30 days did not demonstrate significant influence of droplet size emulsions (data not shown). Some authors have reported similar behavior between temperature and droplet sizes (Jeong et al. 2001; Kong et al. 2001).

We have noted significant negative zeta potential values for these emulsions in the study, although they had been obtained with a blend of nonionic surfactants. The observed charge on the emulsion droplets presumably arises as a result of hydrogen bonding at the ether-oxygen of the polyoxyethylene chain, with subsequent oxonium formation. Consequently, the charge must be proportional to the number of polyoxyethylene groups per chain and the surface concentration of the chains

(Friberg et al. 1988; Stachurski and Michalek 1996; Ho and Ahmad 1999; Hsu and Nacu 2003).

Shifts on emulsion droplet charge can be measured through of zeta potential. When zeta potential as such is known, it is often a limited value. However, related changes in the zeta potential with changes of conditions and/or constituents of emulsions provide significant information about the condition of the droplet interface (Stachurski and Michalek, 1996). However, we choose to keep the same constituted emulsions and change the sample storage conditions (temperature). We could observe that zeta potential values did not suffer significant shifts by temperature variations. A variance analysis test (ANOVA) (results with p values >0.05 were regarded as not significant) was used. For the thermal stress test the zeta potential results did not show significant variation ($p = 0.79269$). At 25°C, zeta potential values varied from -41.44 mV to -51.71 mV with $p = 0.96121$. For variations in zeta potential values at 4° and 45°C the p values were 0.14473 and 0.89379, respectively. For the same conditions cited above, pH

TABLE 4

Sample results of pH, zeta potential (zp), and droplet sizes (mean of three values and their standard deviation) after accelerated stability test

	Time			
	1 d	7 d	15 d	30 d
Accelerated stability test				
Temp. 25°C				
pH	6.35 ± 0.19	5.92 ± 0.04	6.73 ± 0.02	6.92 ± 0
zp (mV)	-49.54 ± 5.69	-41.44 ± 4.31	-51.71 ± 13.16	-46.18 ± 4.78
Size (nm)	214.7 ± 0.47	216 ± 0	216 ± 0	233.33 ± 24.51
Temp. 4°C				
pH	6.63 ± 0.01	6.0 ± 0.04	6.89 ± 0	7.26 ± 0
zp (mV)	-52.96 ± 7.23	-39.47 ± 6.18	-46.9 ± 10.02	-39.47 ± 6.18
Size (nm)	233.33 ± 24.51	268 ± 0	268 ± 0	268 ± 0
Temp. 45°C				
pH	6.61 ± 0.02	5.82 ± 0.03	6.71 ± 0.02	5.59 ± 0.32
pz (mV)	-45.81 ± 5.56	-49.03 ± 0.64	-45.55 ± 4.01	-56.97 ± 6.36
Size (nm)	216 ± 0	250.67 ± 24.51	233.33 ± 24.51	216 ± 0

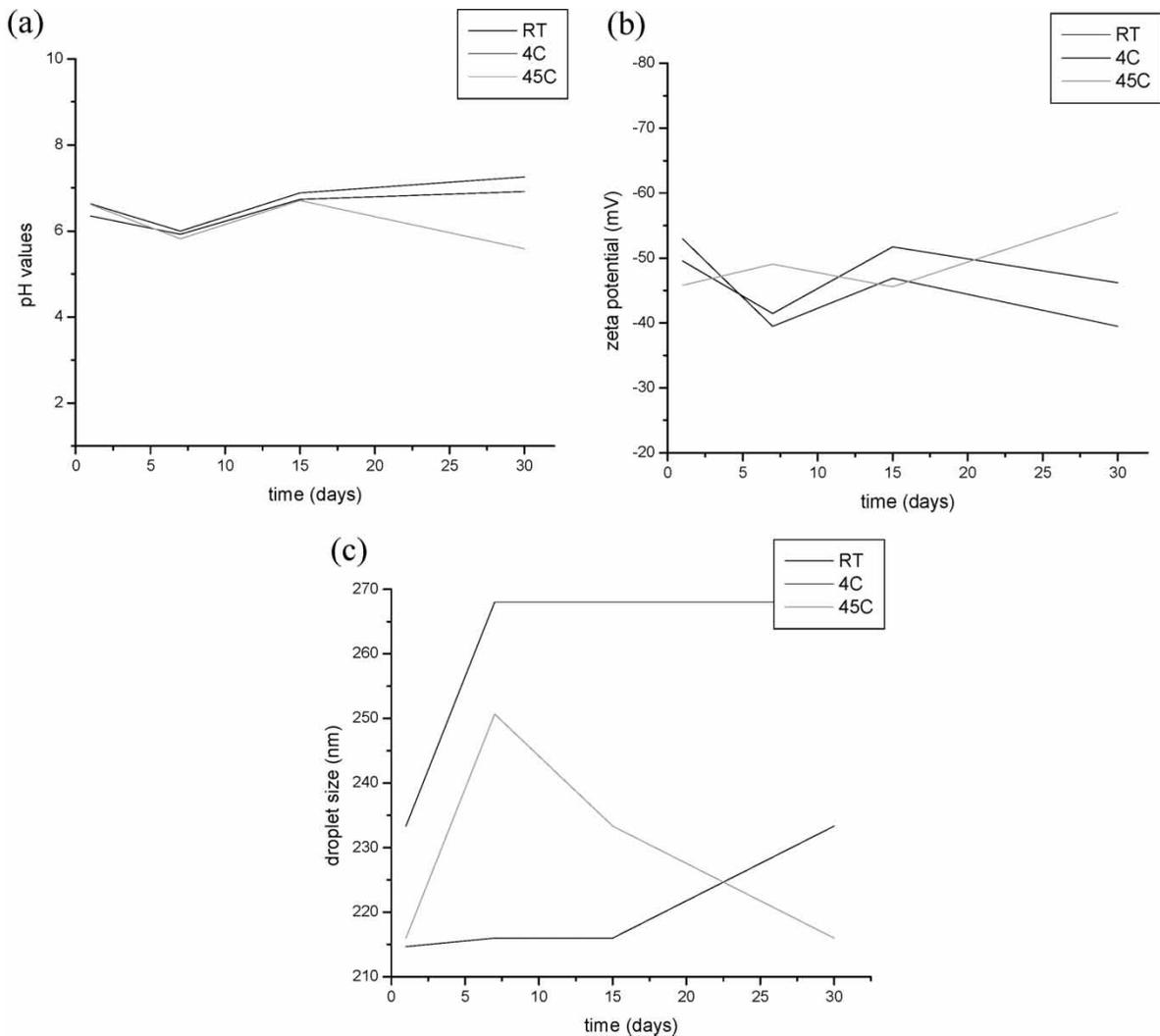Temperature (temp) and time in days (d).

FIG. 1.   Sample results of pH (a), zp in mV (b), and droplet sizes in nm (c) after accelerated stability test.

values did not show significant variations (data not shown). However, when we have compared the obtained zeta potential results at 4°, 25°, and 45°C we can observe at 25° and 4°C (7 days) p = 0.01602. Furthermore, all other zeta potential results did not demonstrate significant variations (data not shown). In addition, our study agrees with related literature of emulsions, such as Kong et al. (2001); zeta potential values were little affected by high temperatures applied to studied nano-emulsions.

CONCLUSION

Our results demonstrate that nanometer droplet sizes could be yielded by the chosen method (EPI) and suggest that the main factor for fine emulsion formation should be attributed to the kinetics of the emulsification process and consequently the change of the surfactant molecule curvature during this

process. Furthermore, the formation of liquid crystal structure and high mechanical energy seems to be a secondary factor for fine emulsion formation.

The results obtained after thermal stress and accelerated stability tests for zeta potential and globule sizes allows us to suggest that the yielded nano-emulsion system is quite stable. These results may be supported by the related literature. This study is currently being extended. Samples will be submitted to other parameters that could significantly influence zeta potential and droplet sizes.

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