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Characterization and Evaluation of Electrolyte Influence on Canola Oil/Water Nano-Emulsion

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Emulsion stability is controlled by the physicochemical properties of the adsorbed layers formed on the surface of the droplets. Zeta potential and droplet size measured initially and during storage can estimate O/W emulsion stability. The aim of this study was to characterize and evaluate the effects of different hydrolyzable compounds employed in pharmaceutical and cosmetic preparations on the zeta potential and droplet size of canola O/W nano-emulsions and, consequently, the emulsion stability. The samples containing additives demonstrated significant change in zeta potential, but in spite of that, no macroscopic instability was observed. Yet the droplet size values did not undergo significant change.

INTRODUCTION

Nano-emulsions consist of very fine emulsions with droplet sizes between those of conventional emulsions and microemulsions, i. e., with a typical size range of 20–500 nm.[1] Emulsions are well accepted as carriers for both hydrophilic and lipophilic drugs and have been widely used in the pharmaceutical field. In recent years, progress made in controlling the size distribution and in understanding the stabilization mechanisms has resulted in renewed attention for these liquid dispersion systems.[2] Submicron emulsions and nano-emulsions have gained increasing attention as drug carriers not only for topical and/or cosmetic usages[3–5] but also for parenteral,[6,7] ocular,[8,9] and transdermal applications.[10] Some advantages have been reported for pharmaceutical and cosmetic applications of nano-emulsion systems: they are suitable for efficient delivery of active ingredients through the skin; the large surface area of the emulsion system allows rapid penetration of actives; and due to their small size, they can penetrate through the "rough" skin surface and this enhances the penetration of actives.[3] Some researchers have demonstrated the potential of nano-emulsions as reliable vehicles for skin care products.[4]

Emulsion stability is evidently controlled by the properties of the adsorbed layers formed on the surface of the droplets. These properties include not only interfacial tension, but also the properties of the continuous solution close to the interface. The layer structure depends on the composition and concentration of the surfactants and/or polymers employed.[8,11–13]

Stabilization of emulsions with nonionic surfactants occurs mainly via steric mechanisms, although an electrostatic mechanism may be considered.[14] Electrosteric stabilization arises when steric and electrostatic mechanisms occur in parallel. The steric and electrostatic components may not necessarily be equal in magnitude, and one or the other can dominate.[15,16] Possible influences on the interfacial electric charge should be studied even for nonionic stabilized emulsions. Some researchers have reported that to efficiently assess the stability of an emulsion, zeta potential and size measurements should be performed.[6,8,9,17] In current pharmaceutical and/or cosmetic usages, emulsions are commonly used as vehicle of many active ingredients. In some situations, these additives may be hydrolyzable, thus they may influence the interfacial film and may promote instability. So, there is an increasing interest in understanding the factors that control interfacial film interactions and emulsion stability.

The arrangement of the electric charge on the oil droplet associated with the charge balance in solution is called the electrical double layer of the droplet. The layer surrounding

the particle is made up of two parts, an inner region (Stern layer) where the ions are strongly bonded (counterions) and an outer (diffuse) region where they are less firmly associated (co-ions).[11,18,19] The DLVO theory (so named from its originators, Derjarguin, Landau, Vervey, and Overbeek) 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).[11,12,18,19] The surface potential cannot be directly determined, therefore the calculation of the electrostatic interaction is replaced by electrokinetic or zeta potential, which is a difference of potential between a point situated at a sufficiently large distance from the droplet surface and a point situated on the surface of the shear, i.e., the plane in which the relative movement of the phases occurs.[19,20] If the electrical double layer contribution is the main stabilizing mechanism, a surface potential of about 30 mV (in module) is necessary to prevent flocculation and coalescence.[6,9,11,19-22]

Addition of electrolytes to the continuous phase of an oil-in-water (O/W) emulsion increases the ionic strength. Some electrolytes bind to oppositely charged groups on the emulsion droplet surfaces, reducing their zeta potential (in module), thereby decreasing the electrostatic repulsion and simultaneously system stability.[22] Electrostatic stabilization is very sensitive to the ionic strength; as the electrolyte concentration increases, the electrical double layer is compressed (reduction of its thickness) and the distance over which electrostatic repulsion extends is reduced.[21-24]

The electrolyte effects on zeta potential of emulsion droplets have already been studied in many previous articles;[20,21,24-26] however, there have not been many studies to provide insight into the effect of hydrolyzable additives and cosurfactants on the stability of cosmetic and/or pharmaceutical emulsions frequently obtained with vegetable oils.

Three methods may be used for the preparation of nano-emulsions: high-pressure homogenizers; low-energy emulsification at constant temperature; or application of the phase inversion temperature concept.[1,3,4] In our previous study, we obtained nano-emulsion systems by the emulsion phase inversion (EPI) method using nonionic surfactants and evaluated their physicochemical characteristics. The EPI 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.[1,3,27-29] It was observed that the droplet size was about 215 nm, the zeta potential was -49.2 ± 5.7 mV, and pH was 6.3 ± 0.2,[5] indicating a nanometer-size distribution and high negative zeta potential, within the values of ideal electrostatic stability in accordance with reported results.[17] The aim of this work was to evaluate the effects of hydrolyzable raw materials, frequently used in pharmaceutical and/or cosmetic preparations, on the

emulsions' zeta potential and droplet size distribution. The emulsion stability was evaluated in the presence of these additives, by characterizing the steric and electrostatic contribution in the stabilization of nonionic emulsions.

MATERIAL AND METHODS

Chemicals

Emulsions were prepared using as surfactants PEG-40 castor oil (Surfom R 400®), a hydrophilic surfactant with hydrophile-lipophile balance (HLB) number 13.0, and sorbitan oleate (Span 80®), a lipophilic surfactant with HLB number 4.3; canola oil; and distilled water. Surfactant samples were purchased from Oxiteno®. Additives used that could influence zeta potential and droplet size were monovalent, divalent, and trivalent cations (NaCl, $CaCl_2$, and $AlCl_3$, respectively), monovalent and divalent anions ($NaHCO_3$ and Na_2CO_3, respectively), ionic surfactants (sodium dodecyl sulphate (SDS) and cetyltrimethylammonium chloride (CTAC)), and an amphoteric surfactant (cocamidopropyl betaine (CPB)). All chemicals were used as received without further purification.

Preparation of Emulsions

Samples were obtained by the emulsion phase inversion (EPI) method. A surfactant blend containing PEG-40 castor oil at 3.58% (w/w) and sorbitan oleate at 2.42% (w/w) was employed. The total amount of surfactants was kept at 5.0% (w/w) resulting in a HLB value of 8.8. This is the value of required HLB number for canola oil for the surfactant system in question and was previously determined.[5,30] Both surfactants were dissolved in the oil phase (5.0% w/w). Oil and aqueous phases were separately heated up to $70° \pm 2°C$. The water phase (90.0% w/w) was slowly added into the oil phase. The stirrer speed was set at 400 rpm (Mechanical Agitator Fisatom® 702 R) to provide better mixing conditions and a uniform distribution of the emulsion components. Emulsions were kept under mixing until reaching room temperature $25° \pm 2°C$. For the surfactant blend (Surfom R-400® and Span 80®), PIT was previously obtained by measuring the change in conductivity of O/W emulsion upon heating, which happens above $90°C$.[5] PIT (phase inversion temperature) or HLB temperature is defined as the temperature at which a hydrophilic-lipophilic property of surfactant just balances at the interface.[28] The additives were added into the O/W emulsion, in the following concentrations: 0.1%, 0.5%, and 1.0% (except for the cationic surfactant, employed also at 0.25% to try identifying the charge reversal point). All the additives were added to the emulsions immediately after preparation and the analyses were performed after 48 hours, providing enough time for the dissociated ions in the aqueous solution adsorbed on the O/W interface to reach equilibrium.

Determination of Zeta Potential and Size Distribution Analysis

Zeta potential and size distributions were performed using the Coulter Counter DELSA 440SX®. This system analyses zeta potential employing the electrophoretic mobility of particles and colloids in liquid dispersions. The size distribution of particles is based on photon correlation spectroscopic analysis. The samples were diluted in distilled water (1 : 20) before application in the device sample chamber. All measurements were carried out in triplicate.

RESULTS AND DISCUSSION

The zeta potential magnitude is directly correlated to the colloid and/or emulsion stability. Measuring globule size initially and during storage provides an indication of the system stability.[8,11,12,17,19−21,31,32]

Hydrolyzable compounds (electrolytes) were added to O/W nano-emulsions to evaluate possible influence on pH, zeta potential, and droplet size and thus to assess the stability of the emulsions. Analysis of variance (ANOVA) was used to evaluate significant differences in each parameter (results with p value >0.05 were regarded as not significant).

The pH, zeta potential, and droplet size for the emulsion (without any additive) after 48 hours from its preparation were 6.6 ± 0.1, -44.0 ± 5.1 mV, and 268 ± 0 nm, respectively. High negative zeta potential, were observed for the emulsions in this study, which could be explained by the existence of 40 ethylene oxide (EO) groups (PEG-40 castor oil) capable of creating hydrogen bonds with the OH^- groups, hydrogen bonds at the ether-oxygen of the polyoxyethylene chain, with subsequent oxonium formation.[11,14,24,26,32] Usually oil droplets stabilized by different nonionic surfactants are negatively charged at pH higher than 4.[26] Zeta potential alone is often a limited value. However, changes in the zeta potential related to altered conditions and/or ingredients provide us with information about the status at the droplet interface.[32]

It was observed that the zeta potential values undergo significant changes by the different types and concentrations of the electrolytes at each pH investigated.

Nano-emulsions spiked with the anionic surfactant SDS showed an increasingly negative zeta potential as the concentration of anionic surfactant was increased (p = 0.00226) (Figure 1 and Table 2). The increase in the absolute value of the zeta potential can be attributed to the sulphate group of the surfactant molecules adsorbed at the interface.[33] pH did not demonstrate any relevant changes (Table 1; variance data are not shown for all pH values). On the other hand, samples with the cationic surfactant CTAC showed a relevant change in zeta potential (p = 8.1349E^{-7}) (Figure 1 and Table 2). In this case, a reversal in charge from negative to positive was observed at a concentration around 0.25%. A small decrease in pH was also observed (Table 1). For the emulsions containing the amphoteric surfactant CPB, no significant change in pH
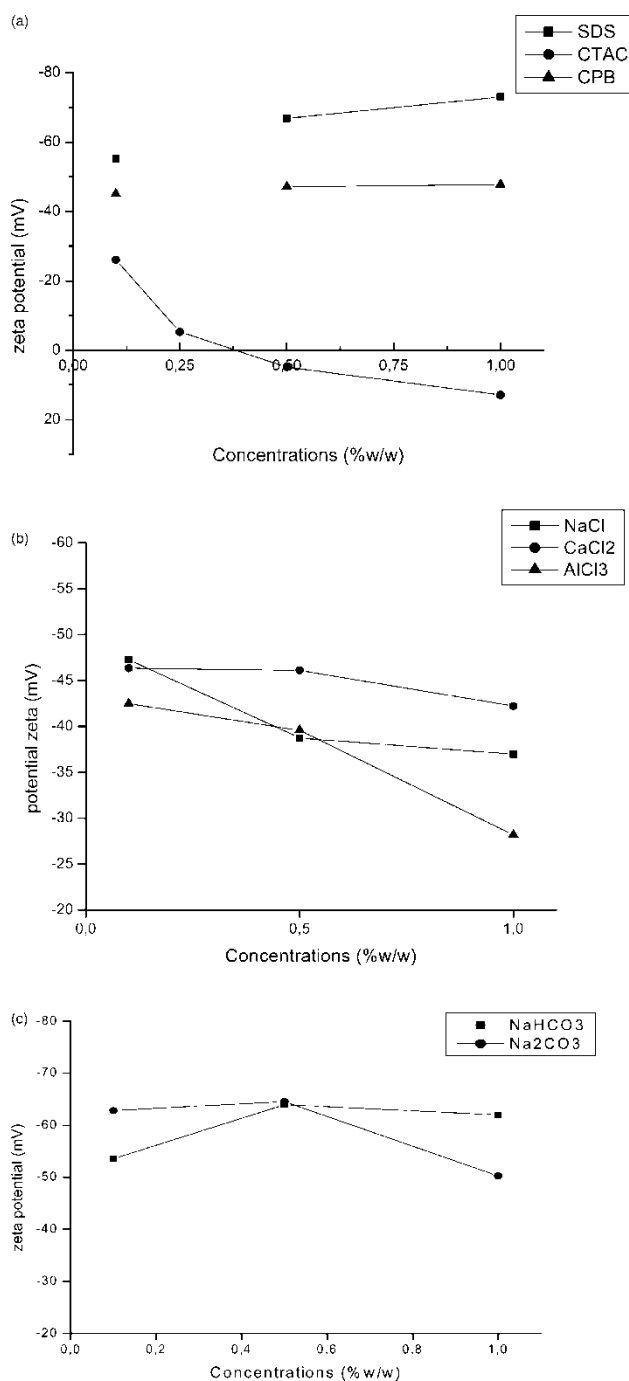
FIG. 1. Influence on zeta potential values (mV) of the samples containing (i) surfactants, (ii) salts/cations, and (iii) salts/anions at concentrations of 0.1, 0.5, and 1.0% (w/w) (except also at 0.25% for cationic surfactant/CTAC); mean of three values obtained and their standard deviation, 48 hours after their addition.

or zeta potential (p = 0.8404) was detected (Figure 1 and Tables 1 and 2), so we could conclude that these samples should be neutrally charged for their pH values; and therefore any change on droplet charges could be detected.

TABLE 1
pH value of the samples containing electrolytes (surfactants/salts)

	0.1% (w/w)	0.5% (w/w)	1.0% (w/w)
SDS (anionic)	7.6 ± 0.0	7.6 ± 0.1	7.5 ± 0.1
CTAC (cationic)	6.0 ± 0.1	4.6 ± 0.0	3.8 ± 0.0
CPB (amphoteric)	6.5 ± 0.1	6.3 ± 0.0	6.2 ± 0.1
NaCl (Na^+)	6.7 ± 0.1	6.4 ± 0.0	6.4 ± 0.0
CaCl$_2$ (Ca^{+2})	6.5 ± 0.0	6.5 ± 0.0	6.5 ± 0.1
AlCl$_3$ (Al^{+3})	4.1 ± 0.0	3.6 ± 0.1	3.3 ± 0.0
NaHCO$_3$ (CO_3^-)	8.9 ± 0.1	8.9 ± 0.1	9.0 ± 0.0
Na$_2$CO$_3$ (CO_3^{-2})	9.5 ± 0.1	10.0 ± 0.0	10.1 ± 0.1

Results of the influence on pH value (mean of three values obtained and their standard deviation) of the samples containing surfactants and salts as additives, 48 hours after their addition.

For the samples containing mono-, di-, and trivalent cations, only the one containing a trivalent cation (AlCl$_3$) resulted in a significant change in zeta potential (Figure 1 and Table 2). A decrease in pH was also observed (Table 1). The zeta potential for the samples containing NaCl and CaCl$_2$ are given in Figure 1 and Table 3. For their pH we could not observe any significant change either (Table 1).

Nevertheless, for emulsions containing mono- and divalent anions, we could observe an increase in zeta potential for NaHCO$_3$ (p = 0.00145) and for NaCO$_3$ (p = 0.00755), both with significant shifts; these values are depicted in Figure 1 and Table 3. An increase in pH can be observed in Table 1.

The electric charge does not depend only on the concentration, but also on the ionic valence of electrolytes added to the solutions. More specifically, divalent and trivalent cations, such as Ca^{+2} and Al^{+3}, are more readily adsorbed on the O/W interface, and thus their effects on the zeta potential are more pronounced than those of monovalent cations.[24,32] According to our results and related literature,

TABLE 3
Droplet size of the samples (nm) containing electrolytes (surfactants/salts)

	0.1% (w/w)	0.5% (w/w)	1.0% (w/w)
SDS (anionic)	268 ± 0.5	268 ± 0.5	268 ± 0
CTAC (cationic)	268 ± 0	268 ± 0	268 ± 0
CPB (amphoteric)	268 ± 0	268 ± 0.5	233 ± 24.5
NaCl (Na^+)	251 ± 24.5	268 ± 0.5	268 ± 0
CaCl$_2$ (Ca^{+2})	268 ± 0	250 ± 25.0	233 ± 24.5
AlCl$_3$ (Al^{+3})	268 ± 0	268 ± 0	250 ± 25.0
NaHCO$_3$ (CO_3^-)	268 ± 1.2	268 ± 0.5	268 ± 0
Na$_2$CO$_3$ (CO_3^{-2})	268 ± 0	268 ± 0	268 ± 0.5

Results of the influence on droplet size value in nm (mean of three values obtained and their standard deviation) of the samples containing surfactants and salts as additives, 48 hours after their addition.

the same occurred for the analyzed nano-emulsions. Trivalent cations had the most significant effects on zeta potential of the nano-emulsion, while no significant difference was observed between monovalent and divalent cations (variance data are not shown); we might suggest that these results would be influenced by concentrations employed in percentage (w/w).

The results showed that the addition of some electrolytes could significantly change the zeta potential and consequently the droplets' surface charge density at the pH value investigated, while others could not. In spite of this, all samples analyzed did not demonstrate any sign of macroscopic instability (flocculation, creaming, coalescence, separation, etc). For that reason, it can be assumed that electrolytes, that could not be adsorbed specifically on the surface of the droplets, for example mono- and divalent ions, did not change zeta potential significantly. Thus, a decrease in double layer thickness was not enough to decrease system stability. This adsorption

TABLE 2
Zeta potential of the samples (mV) containing electrolytes (surfactants/salts)

	0.1% (w/w)	0.5% (w/w)	1.0% (w/w)
SDS (anionic)	-55.2 ± 7.4	-66.8 ± 5.0	-73.0 ± 1.7
CTAC (cationic)	-26.0 ± 2.3	4.9 ± 5.7	12.9 ± 2.14
CPB (amphoteric)	-45.0 ± 5.4	-45.1 ± 1.8	-47.8 ± 3.4
NaCl (Na^+)	-47.3 ± 0.4	-38.7 ± 2.4	-36.7 ± 1.9
CaCl$_2$ (Ca^{+2})	-46.3 ± 6.6	-46.1 ± 0.2	-42.2 ± 4.5
AlCl$_3$ (Al^{+3})	-42.5 ± 0.9	-39.6 ± 0.8	-28.2 ± 9.1
NaHCO$_3$ (CO_3^-)	-53.6 ± 2.6	-63.9 ± 3.7	-62.0 ± 1.2
Na$_2$CO$_3$ (CO_3^{-2})	-62.8 ± 0.6	-64.5 ± 8.1	-50.2 ± 1.7

Results of the influence on zeta potential value in mV (mean of three values obtained and their standard deviation) of the samples containing surfactants and salts as additives, 48 hours after their addition.

probably happened with samples containing CTAC and AlCl$_3$. We might still suggest that this fact could be related to the pH of the studied samples. Depending on the pH, the corresponding concentration of H$^+$/OH$^-$ ions on the O/W interface might change the droplet charge. At lower pH, such as observed for CTAC and AlCl$_3$, the absolute value of the negative electric charge of oil droplets decreases as pH value decreases, and the corresponding concentration of H$^+$ ions on the O/W interface will gradually weaken and even neutralize the net negative charge. At higher pH, such as for NaHCO$_3$ and NaCO$_3$, selective adsorption of OH$^-$ ions on the O/W interface results in the development of stronger negative charges on the oil droplets. Compared to Cl$^-$ or Na$^+$ ions, H$^+$ or OH$^-$ ions are much more easily adsorbed in the region near the O/W interface and thus affect the net electric charge on the dispersed droplets in each case.[23] Therefore, when pH values of the emulsions were close to neutral (see Table 1), the adsorbed H$^+$ or OH$^-$ on the interface (nonionic surfactant of the polyethoxylated type) did not permit enough bonding between droplet interface and additives in this study.[20,24,32]

The change in the zeta potential of emulsion droplets would be influenced by different ways depending on the additive amount in the film formed on the oil/water interface.[20] In this study, no significance difference was observed among the three different concentrations used (variance data are not shown). This may suggest that these results have been influenced by the concentrations of additives used, thus a narrow concentration range where a possible unstability would take place could not be found. It is probable that the concentrations used may have been above this range.[11]

Furthermore, another important topic reported in the literature that was demonstrated by our results is that the zeta potential value lost its importance in emulsion stability analysis when high electrolyte levels are present.[11] Moreover, it is important to notice that if the emulsion remains stable even after the addition of high-additives contents, certainly the stability is not due to electric double layer repulsion, and other stabilizing mechanisms must be considered. It may be concluded that electrostatic repulsion is not the only factor preventing coalescence, and the role of hydrogen bonding should be kept in mind since it leads to a steric impediment close to the droplet surfaces.[13]

A decrease in the absolute value of zeta potential should usually be correlated to a higher tendency to flocculate. Generally, lower zeta potential (in module) might not stabilize O/W emulsions, therefore these values indicate an inadequate electrostatic repulsion between droplet surfaces.[11,19,20] Phenomena such as flocculation and coalescence would become possible, therefore these processes can increase droplet sizes. Nevertheless, after droplet size measurements (Table 3) it could be observed that the additives did not demonstrate significant influence on this parameter, even for the emulsions containing CTAC and AlCl$_3$, which showed significant

decrease in zeta potential. The decrease of zeta potential is not always related to the increase in droplet size, especially when steric mechanisms are possibly predominant (variance data are not shown). Consequently, the stability of the emulsions in the absence of electrostatic mechanisms (in other words, after the zeta potential values have been suppressed in samples containing CTAC and AlCl$_3$) is possible evidence that the steric component is the main constituent of the electrosteric stability of these canola oil/water nano-emulsions.

CONCLUSION

The results suggest that additives influenced the interfacial structure of the droplet emulsions, without affecting macroscopic stability. The zeta potential obtained before and after the tests suggest that emulsion stability did not exclusively depend on electrostatic stabilization; in this case the steric component should be the main mechanism of a possible electrosteric stabilization of the nano-emulsions in question. As a result, these nano-emulsion systems obtained from a pair of nonionic surfactants and by emulsion inversion phase method could be applied the development of pharmaceutical and/or cosmetic dosages where high levels of hydrolyzable active are required. Furthermore, it was concluded that zeta potential and droplet size analyses have efficiently assessed nano-emulsion stability and demonstrated usefulness to characterize these colloidal systems during manufacture and quality control of pharmaceutical and/or cosmetic usage.

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