

Development and Characterization of a Biocompatible Soybean Oil-Based Microemulsion for the Delivery of Poorly Water-Soluble Drugs

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ABSTRACT: The aim of this work was the development and characterization of a biocompatible microemulsion (ME) containing soybean oil (O), phosphatidylcholine/sodium oleate/Eumulgin[®]HRE40 as the surfactant mixture (S) and water or buffer solution as the aqueous phase (W), for oral delivery of the poorly water-soluble drugs sulfamerazine (SMR) and indomethacin (INM). A wide range of combinations to obtain clear oil-in-water (o/w) ME was observed from pseudo-ternary phase diagrams, which was greater after the incorporation of both drugs, suggesting that they acted as stabilizers. Drug partition studies indicated a lower affinity of the drugs for the oil domain when they were ionized and with increased temperature, explained by the fact that both drugs were introduced inside the oil domain, determined by nuclear magnetic resonance. High concentrations of SMR and INM were able to be incorporated (22.0 and 62.3 mg/mL, respectively). The ME obtained presented an average droplet size of 100 nm and a negative surface charge. A significant increase in the release of SMR was observed with the ME with the highest percentage of O, because of the solubilizing properties of the ME. Also, a small retention effect was observed for INM, which may be explained by the differences in the partitioning properties of the drugs. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci* 104:3535–3543, 2015

Keywords: microemulsions; solubility; sulfamerazine; indomethacin; surfactants; sulfonamides; NMR spectroscopy; drug release

INTRODUCTION

Microemulsions (ME) are isotropic, optically clear nanostructured and thermodynamically stable dispersions, composed of two nonmiscible liquids such as an aqueous phase and an oily phase, stabilized by an interfacial film of surfactants often associated with a cosurfactant.^{1–5} The formation of water-in-oil (w/o) or oil-in-water (o/w) ME is dependent on the properties of the surfactant mixture, the oil–surfactant ratio and the temperature.⁶ It has also been reported that the ME structure plays an important role in drug release.^{7–10}

Over the last decade, a large number of studies on the ME systems available for pharmaceutical application has been published.^{4,5,7,11–15} These systems involve the use of biocompatible components with the ability to improve the solubility of sparingly soluble drugs.^{8,9,16–18} Soy phosphatidylcholine (SPC) is a widely used biocompatible surfactant of natural origin from a component of cell membranes and is a known pharmaceutical ingredient that can be used for all administration routes including i.v.^{1,19,20} In addition, monounsaturated fatty acids, such as oleic acid, have received increasing attention as penetration enhancers.¹⁰ A typical analysis of pure soybean oil indicates that the main composition is palmitic acid (9%–13%), stearic acid (3%–6%), linoleic acid (50%–57%), oleic acid (17%–26%), and linolenic acid (5%–10%). Interest in using nonionic surfactants associated with a cosurfactant is growing in ME composition, mainly because of the high stability, low toxicity,

and biodegradability of these compounds. Polyoxyethylenglycerol (PEG)-40 hydrogenated castor oil is a nonionic surfactant for pharmaceutical use^{21,22} that provides adequate conditions to stabilize w/o ME when used in surfactant mixtures.^{12,23,24}

The effect of ME on drug delivery has been well described in the literature and reveals a favorable influence on the modification of the bioavailability of many drugs, such as the antimicrobial activity of glycerol monolaurate by using o/w ME;⁵ tween-based MEs has been developed for the delivery of a fixed-dose combination of three first-line antitubercular drugs;¹¹ and other MEs have been produced for the solubilization of many drugs such as azithromycin,²⁵ chloramphenicol,⁴ and several water-soluble peptides of different molecular structures, sizes, and charges in w/o MEs containing long- or medium-chain triglycerides.⁶ In addition, several recent studies have suggested that MEs have the potential to increase transdermal drug delivery of both hydrophilic and lipophilic drugs, such as naproxen,²⁶ testosterone,¹⁰ curcumin,² T4,²⁷ ketoprofen, lidocaine, and caffeine.²⁸

The main purpose of this work was the development of a biocompatible ME for the oral delivery of sparingly water-soluble acid/ionic drugs, using sulfamerazine (SMR) and indomethacin (INM) as model drugs. Experimental approaches applied to these drugs can also provide information for other poorly water-soluble drugs with similar physicochemical properties. The obtained systems were characterized using pseudo-ternary phase diagrams (PTPD), polarizing light microscopy, ¹H nuclear magnetic resonance (NMR) spectroscopy, conductivity, and particle size and zeta potential measurements. In addition, the incorporation of the drugs related to the system composition and the *in vitro* release from selected ME were also analyzed.

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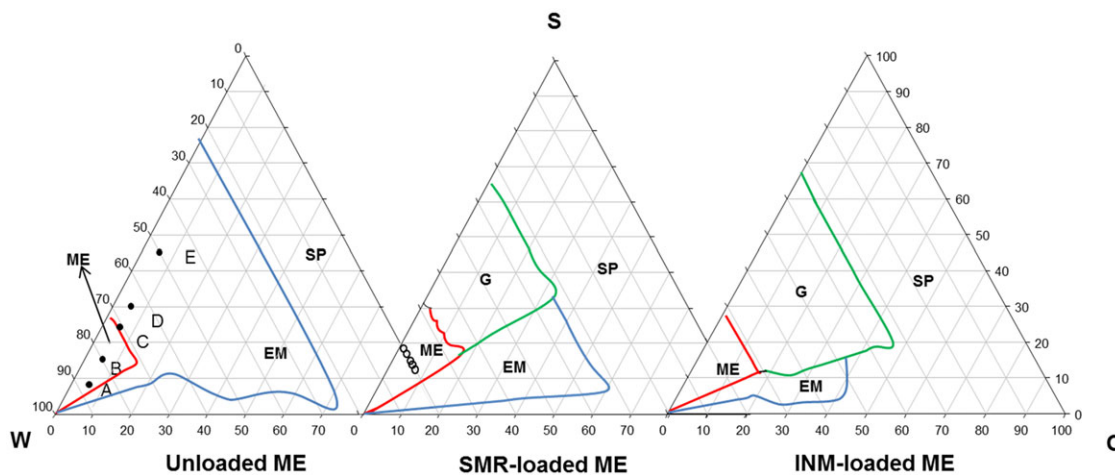


Figure 1. Pseudo-ternary phase diagram of: unloaded, SMR-loaded, and INM-loaded ME. (a–e) Selected formulations for polarized light microscopy study. Selected MEs for incorporation studies. ME, microemulsion; EM, emulsion; G, gel; SP, separation of phases.

MATERIALS AND METHODS

Materials

The MEs used in this work were chosen according to a previous study.¹² SPC was purchased from Degussa Texturants Systems Deutschland GmbH and Company (Hamburg, Germany); (PEG)-40 hydrogenated castor oil (Eumulgin® HRE 40) (EU) (CAS number 61788-85-0) was purchased from Pharma Special (São Paulo, Brazil); soybean oil (Liza®) (O), SMR, and INM were obtained from Parafarm® (Buenos Aires, Argentina); sodium oleate (SO) was obtained from the stoichiometric reaction of oleic acid with 1 M NaOH solution for 30 min. The precipitate was filtered and washed with three portions of 100 mL of acetone. All the other materials and solvents were of analytical grade or better. Purified water was obtained from a Millipore Milli-Q Water Purification System.

Methods

Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were obtained from previous studies of our research group, utilizing hydrophilic–lipophilic balance (HLB) values for the same surfactant system used in this study.¹² The surfactant system containing an SPC–EU–SO (35:35:30) mixture with an HLB value of 12 was used to define the phase diagrams. Semisolid mixtures of oil–surfactant (O–S) (1.0 g) with weights ranging from 1:9 to 9:1 ratios were titrated with aqueous phase under ultrasonic stirring using a Ultrasonic Liquid Processor, Heat System XL 2020 apparatus. The whole study was carried out at room temperature. The transitions from semisolid mixture to opaque dispersion (emulsion), and from emulsion to optically clear ME or phase separation (PS), were sharp and reproducible with 0.1 mL of precision. For the drug-loaded ME, 0.021 or 0.0071 g, the SMR or INM were added, respectively, to the S–O mixture before titration with the aqueous phase, and the procedure was followed as described above for drug-free ME.

Polarizing Light Microscopy

Drug-loaded and drug-unloaded ME, with constant 5% content of oil phase selected from the PTPD, were analyzed by

polarized light microscopy (Jenamed 2; Carl Zeiss®, Jena, Thuringia, Germany). to differentiate MEs (nonbirefringent) from liquid crystalline structures (birefringent). The O–S–W percent composition of samples analyzed were: A = 5:8:87; B = 5:15:80; C = 5:24:71; D = 5:71:65; and E = 5:65:50 (see Fig. 1 for phase diagram illustration). A digital camera (Nikon CoolPix 990, Tokyo, Japan) was attached to the microscope for capturing the images.

Conductivity (σ)

The conductivity (σ) was measured for both unloaded and drug-loaded ME as a function of the O–S ratio or drug content using a Digimed® DM-32 conductivity meter with a Digimed® DMC 010 M electrode with a cell. The conductivity meter was calibrated using a standard solution of 1413 $\mu\text{S}/\text{cm}$ before testing. The selected composition for all studies are presented in Table 1. All measurements were carried out in triplicate at $25 \pm 1^\circ\text{C}$.

Determination of Partition Coefficients

In order to estimate the partition behavior of the drugs in ME systems, studies between the used aqueous phases and soybean oil were performed. The drug partition coefficients were calculated according to the concentrations of SMR or INM remaining in the phases after separation of 1:1 water–oil systems.

To carry this out, 3 mL of water, 10 mM potassium phosphate buffer solution (PBS) of pH 7.4 or 8 and soybean oil, were added to 5 or 10 mg of SMR or INM, respectively. These mixtures were maintained at 25 or $37 \pm 1^\circ\text{C}$ with constant agitation of 200 rpm for 72 h using a Shaker Ferca® (Santa Fe, Argentina), before being centrifuged for 30 min at 700 g for PS using a Rolco® (Buenos Aires, Argentina) centrifugator. The concentration of the substrates was calculated by measuring the absorbance using a Cary 60, Agilent Technologies® (Santa Clara, CA, USA) UV–Visible spectrometer.

The oil/water partition coefficients were calculated using the following equation:

$$\log P = C_{\text{oil}}/C_{\text{water}}$$

where C_{oil} and C_{water} are the concentrations of drug in oil and in water, respectively.

Table 1. Percentage Composition (w/w), Particle Size, Polydispersity Index (PDI), and Zeta Potential of Unloaded, SMR-Loaded, and INM-Loaded ME (See Fig. 1 for Phase Diagram Illustration)

Composition	Formulas (%)					Unloaded ME			SMR-Loaded ME			INM-Loaded ME			
	FCS	EU	OS	O	W	O/S	Average Size (nm)	PDI	Zeta Potential (mV)	Average Size (nm)	PDI	Zeta Potential (mV)	Average Size (nm)	PDI	Zeta Potential (mV)
ME ₁	8.5	8.5	1.5	1.5	80	0.0796	145 ± 1	0.247	-66.1 ± 0.4	85.4 ± 0.6	0.241	-61 ± 2	105 ± 2	0.299	-73.2 ± 0.3
ME ₂	7.0	7.0	3.0	3.0	80	0.1765	150 ± 1	0.180	-52 ± 13	115 ± 7	0.296	-51 ± 1	136 ± 7	0.250	-54 ± 1
ME ₃	5.0	5.0	5.0	5.0	80	0.3333	177 ± 4	0.271	-68 ± 3	138 ± 2	0.311	-59 ± 5	160 ± 2	0.271	-72 ± 3
ME ₄	4.0	4.0	6.0	6.0	80	0.4286	152.6 ± 0.4	0.294	-56.0 ± 0.5	118 ± 4	0.290	-67 ± 3	132 ± 3	0.317	-58 ± 5
ME ₅	2.5	2.5	7.5	7.5	80	0.6000	101 ± 6	0.279	-57 ± 9	107 ± 3	0.300	-61 ± 2	117 ± 1	0.304	-68 ± 1

Effect of the O–S Ratio on the Drug Incorporation into ME

Selected samples containing different O–S ratios, chosen from the ME region of the PTPD, with a fix 80% (w/w) of W (for obtaining biocompatible o/w ME), were prepared to evaluate the influence of the system composition on the incorporated amount of SMR or INM (Table 2). MEs were prepared by slowly adding the O phase amount to the semisolid mixture of SPC/EU/OS, and the corresponding volume of W was added with gentle stirring to enable the dissolution of the surfactant. The dispersion was then sonicated using an Ultrasonic Liquid Processor, Heat System XL 2020 apparatus for a 10-min period, with pulses of 59 s every 20 s. Excess amounts of SMR or INM were dissolved directly in the liquid ME, and the dispersions were sonicated again for a 15-min period. The suspensions were filtered through a 0.45- μ m filter, appropriately diluted with ethanol and analyzed at 230 or 270 nm for SMR or INM, using a 89090A, Hewlett Packard® (Palo Alto, CA, USA) UV–Visible spectrometer and 1 cm path length cuvettes. The dissolved amount of drug was plotted against the O–S ratio.

NMR Studies

¹H NMR studies were performed at 298 K on a Advance II High Resolution Bruker® (Billerica, MA, USA) spectrometer equipped with a broad band inverse probe and a variable temperature unit using 5 mm sample tubes. The spectra of the unloaded and loaded ME were obtained by incorporating a 0.1-mL volume of D₂O to 0.5 mL of a (5:15:80) O–S–W (w/w/w) SMR-free and drug-loaded ME. The spectra of the pure components were obtained by diluting appropriate amounts in D₂O for SPC, EU, and O or in DCCl₃ for soy oil. All the studies were carried out at 400.16 MHz and the data were processed with the TOPSPIN 2.0 software, Bruker® (Billerica, MA, USA). The residual solvent signal (4.80 ppm) was used as the internal reference. Induced changes in the ¹H NMR chemical shifts ($\Delta\delta$) for the drugs and ME components, originating from their interaction, were calculated according to the following equations:

$$\Delta\delta = \delta_{\text{loadedME}} - \delta_{\text{unloadedME}}$$

and

$$\Delta\delta = \delta_{\text{druginME}} - \delta_{\text{drug}}$$

Droplet Size, Polydispersity, and Zeta Potential Measurement

The droplet size, polydispersity, and zeta potential of the ME were determined at 25°C using a Delsa™ Nano C Particle Analyzer, Beckman Coulter® (Pasadena, CA, USA). The intensity autocorrelation function was measured at a 165° angle using a viscosity of 0.8878 Pa.s and a refractive index of 1.3328 for the bulk medium. The samples were appropriately diluted with water before analysis.

In Vitro Drug Release from the ME

The *in vitro* release of SMR and INM from ME₅ was determined using a MicroettePlus® Vertical diffusion Franz cell apparatus with automatic sampling at 37 ± 2°C and a 300-rpm stirring rate (Hanson Research Corporation®, Chatsworth, CA, USA). Cellulose acetate membrane with a pore size of 0.45 μ m and an exposed area of 1.77 cm² was used (Sigma–Aldrich®, Buenos Aires, Argentina). The pure drugs in an oral dose in PBS solution or incorporated into the ME were loaded in the donor

Table 2. Drug Partition Coefficients Between Different Aqueous Phases and Soy Oil Performed at 25°C and 37°C, Percentages of Ionized and Unionized Drugs at Different pH Calculated Using the Henderson–Hasselbalch Equation and Intrinsic Solubilities of the Drugs (S_0), Maximum Solubilities (S_{max}), and Apparent Solubility Increase Achieved Using the ME

Aqueous phase (W)	pH	Sulfamerazine						Indomethacin									
		Partition Coefficients			pKa ₁ = 2.6	pKa ₂ = 6.9		Partition Coefficients			pKa = 4.5	Apparent Solubility Increase					
		at 25°C	at 37°C	[B]		[BH ⁺]	[AH]	[A ⁻]	at 25°C	at 37°C		[AH]	[A ⁻]	S_{max} (mg/mL)	S_0 (mg/mL)	Apparent Solubility Increase	
Water	5.5	8 ± 1	3.7 ± 0.2	94.78	5.22	80.22	19.78	0.2213	219.879	99.38	809 ± 152	398 ± 94	26.89	73.11	0.01824	623.168	34.164.912
PBS 7.4	7.4	2.5 ± 0.3	2.7 ± 0.8	99.18	0.82	37.75	62.25	/	/	/	7.5 ± 0.3	2.8 ± 0.3	5.22	94.78	/	/	/
PBS 8	8	0.26 ± 0.09	0.14 ± 0.03	99.55	0.45	24.97	75.03	16.576	166.277	100.312	3.0 ± 0.3	0.26 ± 0.08	2.93	97.07	14.276	5.991.334	419.679

compartment. A 0.01-M, pH 7.4, PBS solution was used as the diffusion medium in the donor and receptor cells. Samples (2.0 mL) were withdrawn from the receiver compartments at fixed intervals and replaced automatically with an equal volume of previously warmed PBS. Drug concentration was spectrophotometrically measured at 240 or 225 nm for SMR or INM, respectively. Each experiment was performed at least three times and the results represent the experimental average. The initial concentration of the drug in the PBS solution was maintained at 200 µg/mL.

RESULTS AND DISCUSSION

Characterization Studies

Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams describe the experimental conditions in which components may be combined to obtain different organized systems.⁹ The results for the systems containing soybean oil (O) as the oil phase, SPC/EU/OS (S) as the surfactant mixture and water (W) as aqueous phase are presented in Figure 1, in which it is possible to observe a wide range of combinations among the formulation components to obtain clear o/w ME, in which large volumes of W and O can be added to maintain the thermodynamic stability of system. In the PTPD for drug-unloaded systems, the transition from separated phases to normal opaque emulsions, and from emulsion to translucent ME, was clearly observed. It could be seen that depending on the O content, up to 22% of W is needed to obtain emulsions systems. Clear and translucent liquid ME prevailed in a region above 72% of W and below 14% of O content.

For both the SMR and INM drug-loaded systems, a similar region of o/w ME was obtained and also a higher viscosity and a translucent system area designated as gel (G) were verified. The PTPD showed that the translucent and isotropic ME regions were greater when compared with drug-free systems, containing a higher O ratio of 19%, which suggests that the drug acted as a stabilizer for ME. It was also observed that depending on the O and S proportions, less than 20% of water was necessary to obtain G or emulsions systems.

Polarizing Light Microscopy

In order to evaluate the refringence behavior of the systems, selected samples with 5% fix O (Figs. 1a–1e) were analyzed by polarizing light microscopy with the microphotographs being shown in Figure 2 for unloaded, SMR-loaded, and INM-loaded ME.

It was verified that all samples showed a nonbirefringent behavior, as dark fields were observed in the microphotographs. The results indicated that the transparency of the unloaded systems was not modified, and in the SMR-loaded systems only with the highest S proportion (formulation a-E and b-E) was it suggested that modification of the structure occurred, which may have induced the formation of more organized systems. For the INM-loaded systems, the transparency was independent of the S proportion, therefore making it possible to affirm that the structural organization of the systems remained unaffected.

The results confirmed that formulations A and B were systems organized as oil nanodroplets dispersed in the continuous aqueous phase (ME) and the formulations C, D, and E

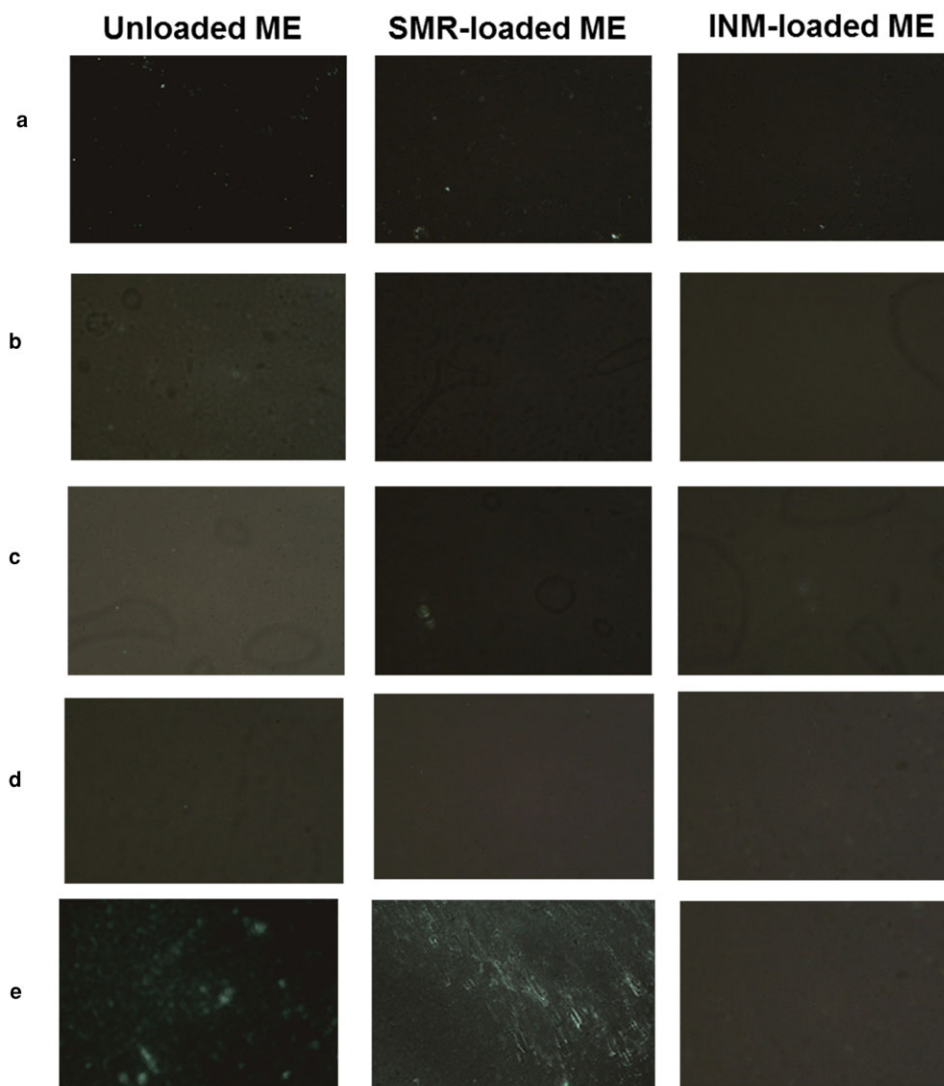


Figure 2. Polarizing light microscopy images of selected samples (a–e) from the PTPD for unloaded, SMR-loaded, and INM-loaded systems.

were emulsions or G, for unloaded and drug-loaded systems, respectively.

Conductivity (σ)

The electrical conductivity (σ) was measured as a function of the composition of the MEs, as a strong correlation has been previously demonstrated between them.^{10,11,29,30} Formulations were analyzed with different O–S ratios and along the line in Figures 1a–1e, which represented a wide range of stable MEs containing increasing amounts of water. In Figure 3, it can be observed that the plot of σ versus water content exhibits the profile characteristic of percolative conductivity, where this parameter decreases with increasing water content and oil phase proportion. This phenomenon suggests that the amount of water in the formulations is above the critical fraction, and is adequate for obtaining o/w ME. In addition, the dependence of the drug amount on the conductivity was also evaluated, with no significant changes in conductivity being recorded, indicating that the internal microstructural organization of the ME continues in the o/w ME regime, even in the presence of these drugs.

Determination of Partition Coefficients of the Drugs

The drug partition coefficients between the oil phase and the aqueous phase used in the studies were estimated at 25°C and 37°C, and the results are presented in Table 2. For both SMR and INM, smaller $C_{o/w}$ values were obtained when the pH was higher, indicating lower affinity of the drugs for the oily phase. This profile may be related to the increase in the ionized drug fraction present, according to the values calculated from the Henderson-Hasselbalch equation (Table 2). The $C_{o/w}$ values were higher at 25°C, probably because of the higher solubility of the drugs in the aqueous phases at 37°C, indicating a higher affinity for the hydrophilic phases at increased temperature. On the basis of the partition and solubility data, it can be concluded that INM presents a higher affinity toward the lipophilic component than SMR.

Study of the Effect of the O–S Ratio on the Incorporation of the Drugs into the ME

In order to evaluate the effect of the O–S ratio on the solubilization of drugs in the ME, incorporation studies were performed

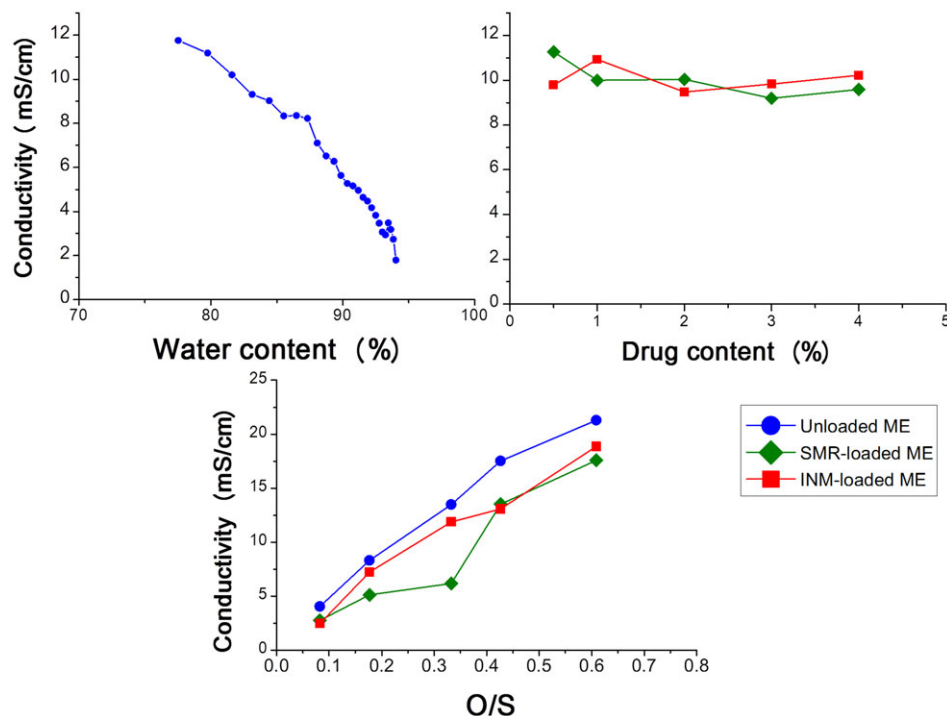


Figure 3. Variation in the electric conductivity (σ) as a function of: water content (%) (along line a–c shown in the pseudo-ternary diagram shown in Fig. 1); the O–S ratio; the drug content (%); for unloaded, SMR-loaded, and INM-loaded MEs.

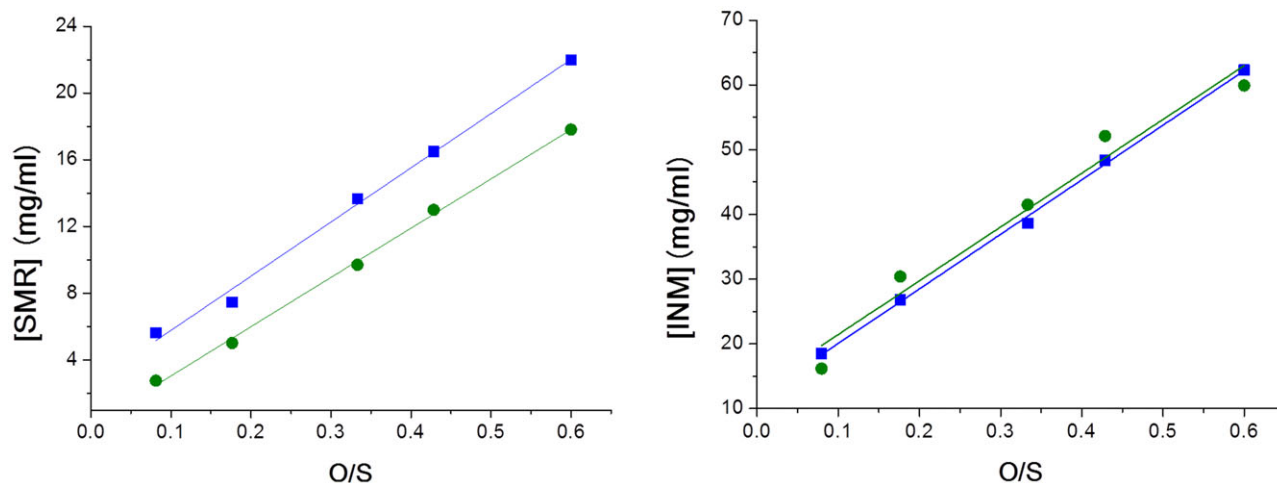


Figure 4. Incorporation curve of (a) SMR and (b) INM in O–W MEs containing water (■) or PBS 8 (●) in the aqueous phase.

using water and PBS 8 as the aqueous phase, with the plots being presented in Figure 4. Also, a PBS solution at pH 2 was tested as the aqueous phase, as it was not possible to obtain ME systems because of SO prevailing in the neutral form at this media ($pK_a = 6.2\text{--}7.3^{31}$), thus decreasing the polarity of the carboxylate group and reducing the tensioactive activity. When water was used as the aqueous phase, with a fix 80% proportion, a linear increase in the drug solubilization was verified in relation with the O–S ratio. The maximum concentration of SMR dissolved was 22.0 mg/mL for SMR and 62.3 mg/mL for INM. In contrast, the maximum concentration of incorporated drugs was achieved at 16.6 and 59.9 mg/mL for SMR and INM, respectively, when PBS 8 was used as the aqueous phase. In

addition, the solubility rises were higher when water was used as the aqueous phase (Table 2), which might have occurred because both active ingredients are ionized at pH 8, thus hindering the partitioning of the drugs into the oil droplet domain and suggesting that the drug solubilization into ME was mainly because of the hydrophobic effect on the incorporation of the drugs.

Drug–Component Interaction Studies

^1H NMR studies were performed to determine the possible location of each drug in the colloidal dispersion. On the basis of the chemical shifts of the pure components, the signals of the

Table 3. Structures of Surfactants, the Fatty Acid Components of the Microemulsion, and the Drugs and chemical shifts of (a) unloaded and drug-loaded ME and (b) SMR and INM in D₂O and in ME

a.

b.

c.

d.

e.

f.

(a)	Signal	$\delta_{\text{unloaded ME}}$	$\delta_{\text{SMR-loaded ME}}$	$\delta_{\text{SMR-loaded ME}} - \delta_{\text{unloaded ME}}$	$\delta_{\text{INM-loaded ME}}$	$\delta_{\text{INM-loaded ME}} - \delta_{\text{unloaded ME}}$
	H ₁	0.9178	1.0297	0.1119	0.8923	-0.0255
	H ₂	1.3186	1.4416	0.123	1.282	-0.0366
	H ₃	1.5965	1.7139	0.1174	∅	–
	H ₄	2.0684	2.179	0.1106	2.0307	-0.0377
	H ₅	2.2558	2.3716	0.1158	2.2256	-0.0302
	H ₆	2.7988	2.9097	0.1109	2.7589	-0.0399
	H ₇	3.2836	3.3947	0.1111	3.1897	-0.0939
	H ₈	3.736	3.8531	0.1171	3.7025	-0.0335
	H ₉	5.3455	5.4674	0.1219	5.3932	0.0477

g.

h.

(b)	Signal	δ_{SMR}	$\delta_{\text{SMR in ME}}$	$\delta_{\text{SMR}} - \delta_{\text{SMR in ME}}$	δ_{INM}	$\delta_{\text{INM in ME}}$	$\delta_{\text{INM}} - \delta_{\text{INM in ME}}$
	H _A	8.1764	∅	–	7.7464	7.7499	0.0035
	H _B	7.7369	7.918	0.1811	7.6335	7.4916	-0.1419
	H _C	6.867	6.9364	0.0694	7.1147	7.141	0.0263
	H _D	6.8091	6.5874	-0.2217	7.1006	6.7812	-0.3194
	H _E	2.3921	∅	–	6.7834	6.569	-0.2144
	H _F	–	–	–	3.902	∅	–
	H _G	–	–	–	3.61	∅	–
	H _H	–	–	–	2.2563	∅	–

a, soy phosphatidyl choline (S); b, palmitic acid (O) or stearic acid (O) when $n = 12, 14$, respectively; c, linoleic acid (O); d, oleic acid (O) or sodium oleate (S) when $R = H$ or Na , respectively; e, linolenic acid (O); f, Eumulgin R (S); g, sulfamerazine; h, indomethacin; ∅, undistinguishable signal because of superposition. *, weak undistinguishable signals.

systems were assigned. The chemical shifts of the protons of the unloaded and drug-loaded ME, as well as those of SMR and INM, are presented in Table 3. Downfield displacements were observed for the signals corresponding to the vicinal protons of the sulfonamide group of SMR and also for the ones corresponding to the side chains of fatty acids. These observations may indicate that van der Waals or hydrophobic interactions occurred, suggesting that SMR was located in the oil domain of the ME, which is consistent with the increase in the quantity of SMR solubilized in function of the O–S ratio increments. For the INM–ME formulation, upfield displacements of the aromatic protons H_B, H_D, and H_E of INM were observed, which could have been because of their proximity to the amine group of SPC, as the protons of the methyl groups attached to the nitrogen atom moved upfield and suggests that INM

interacts with SPC by electrostatic attractions. Also, the vinyl protons of the fatty acid presented downfield displacements, whereas the hydrophobic alkyne protons showed upfield shifts, which may indicate the presence of hydrophobic or Van Der Waals interactions of INM with these protons. These results indicate that INM was incorporated into the oil domain of the ME, which is consistent with the higher solubilization of this drug with increasing O–S ratio reported in section *Study of the Effect of the O–S Ratio on the Incorporation of the Drugs into the ME*.

Droplet Size and Zeta Potential Measurement

The droplet size and the zeta potential of the systems were measured, with values obtained in the range 101–177 nm and (–66) to (–52) mV, respectively, for unloaded ME and a single peak

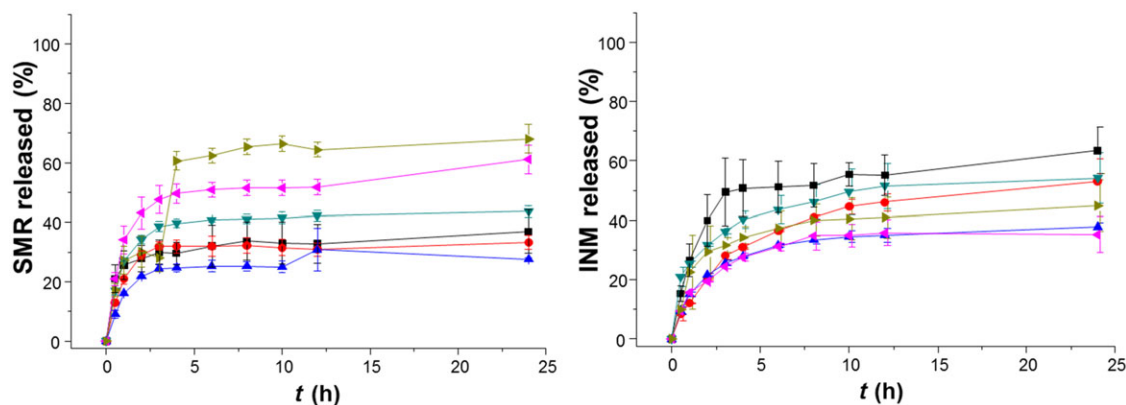


Figure 5. *In vitro* release profiles from a cellulose acetate membrane of (a) SMR and (b) INM from PBS pH 7,4 (■); ME₁ (●); ME₂ (▲); ME₃ (▼); ME₄ (◆); or ME₅ (▷) (each value represents the average \pm SD of $n \geq 3$).

in size distribution (Table 1). As it was observed for ME₁₋₃, the droplets grew and their average diameter enhanced because of the increasing oil content in the internal phase. Also, the decreasing amount of EU, which is a nonionic surfactant that stabilizes the internal phase of the ME by reducing the interfacial tension and thereby decreasing the size, caused the growth of the droplets size. The negative surface potential value of the unloaded ME droplets was because of the presence of SO in its ionized form at the oil–water interface ($pK_a = 6.2\text{--}7.3$ ³¹). Reduced droplet size values were recorded in the presence of SMR or INM, probably because of the deposition of some drug molecules at the interface affecting the mobility of the surfactant and thus reducing the droplet size. On the contrary, the addition of SMR and INM did not significantly change the negative surface potential value of the unloaded ME droplets, which may be indicating that most of the drug remained inside the oily internal phase of the ME, as both active molecules would be expected to be ionized in this medium (at $pH 9.8 \pm 0.9$), which is in agreement with the results of the NMR and the incorporation studies.

In Vitro Release of the Drugs from ME

The effect of ME₅ on the release and transport of the drugs was tested, by evaluating the diffusion across an artificial membrane, and the results are presented in Figure 5. Significant increases in the diffusion of SMR incorporated in the ME 3, 4, and 5 were observed, with a twofold increase obtained after 4 h with ME₅. These three ME presented the highest oil phase proportions, indicating that the higher SMR release have been because of the solubilizing properties of the ME, as previously reported by Padula et al.²⁷ The release patterns of INM from the different ME were similar, with a small retention effect being observed compared with the INM control formulation. These results can be explained by means of the differences in partitioning, which are related to the lipophilicity of the drugs (section *Determination of Partition Coefficients of the Drugs.*), with INM being highly hydrophobic presenting a greater affinity for the oil phase, and encountering maximum hindrance in release. The retention ability of the INM by the internal oil phase was also evidenced by the higher increase in the incorporation of INM into ME (section *Drug–Component Interaction Studies*).

CONCLUSIONS

The results presented in this paper have demonstrated that ME systems can be obtained from a simple procedure using highly biocompatible components. The PTPDs for both drug-loaded and drug-unloaded ME systems with a broad isotropic region were obtained, supported by polarized light microscopy. Conductivity determinations indicated that stable o/w ME may be obtained for a wide range of the components, and suggested that the internal microstructure is represented by oil droplet dispersion forming clusters that remained unaffected because of drug incorporation. The NMR spectroscopy determinations showed that both the SMR and INM drugs were incorporated into the oil droplet domain of the ME. The ME obtained presented an average droplet size of 100 nm and a negative surface charge. The formulations were able to incorporate high concentrations of both drugs and to enhance the release rate of SMR, thus highlighting the potential of the new systems mainly as carriers for the prolonged and controlled delivery of hydrophobic drugs. Our results also offer information on other poorly water-soluble drugs with similar physicochemical properties.

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