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Cyclodextrin and Meglumine-Based Microemulsions as a Poorly Water-Soluble Drug Delivery System

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ABSTRACT

Cyclodextrins (CDs) and meglumine (MEG) are pharmaceutical excipients widely used to improve solubility of poorly water-soluble drugs. The purpose of this work was to study the effect of CDs or MEG on the internal microstructure of soya oil–based O/W microemulsions (MEs) and on the modulation of the solubility and release rate of Class II model hydrophobic drugs, sulfamerazine and indomethacin. The pseudoternary phase diagrams revealed that higher proportions of oil phase, as well as the presence of β-cyclodextrin (ßCD), methyl-ßCD, and MEG, favored the incorporation of the drugs. The conductivity studies, particle size, and zeta potential analysis showed that the O/W ME structure remained unaffected and that the ME presented reduced droplet sizes after the incorporation of the ligands. The drug-component interactions were assessed by proton nuclear magnetic resonance studies. The highest incorporations of sulfamerazine (35.6 mg/mL) and indomethacin (73.1 mg/mL) were obtained with the ME with $W = 5\%$, MEG and $W = 1.8\%$ ßCD in a phosphate buffer solution of pH 8, respectively. In addition, the ligands in ME significantly enhanced the released amount of the drugs, probably due to a solubilizing effect that facilitates the drug to penetrate the unstirred water layer adjacent to membranes.

Introduction

Microemulsions (MEs) are isotropic, optically clear nanostructured and thermodynamically stable multicomponent systems, composed of 2 immiscible liquids such as an aqueous component and an oily component, stabilized by an interfacial film of surfactants as emulsifying agents, frequently associated with a cosurfactant.[1-6] These systems often require high surfactant concentrations in order to provide very low interfacial tension. MEs have a powerful solubilization capacity for poorly water-soluble drugs,[4,7-10] and both hydrophilic and lipophilic drugs have shown a different release behavior from O/W and W/O MEs.[9-15] Recent studies carried out by our research group have demonstrated that soya oil–based MEs, containing sulfamerazine (SMR) and indomethacin (INM) (Figs. 1a and 1b), were able to solubilize high concentrations of the drugs (22.0 and 62.3 mg/mL, respectively) and to enhance the release rate of SMR due to its solubilizing property.[6]

Cyclodextrins (CD) (Fig. 1c) are cyclic torus-shaped molecules, consisting of 6, 7, or 8 d-(+)-glucopyranose units with a hydrophilic outer surface and a lipophilic central cavity and are among the most widely used hosts due to their ability to form complexes with drug molecules in aqueous solutions or in solid state by noncovalent interactions.[16,17] This phenomenon is successfully used to improve the biopharmaceutical properties of drugs.[18-20] In general, the inclusion complex of drugs exhibits a higher aqueous solubility[18,20-23] and a greater chemical stability than the pure drug.[6,24-29]

This approach can be applied in the context of the Biopharmaceutical Classification System[30-33] of drugs. Class II and IV drugs are poorly water soluble but permeable or poorly permeable, respectively, through the gut, meaning that the oral adsorption is limited by the solubility of the drug and the dissolution rate. Thus, the CD technology yields better results for such drugs belonging to Class II and IV because it leads to changes in the physical-chemical properties, making them behave as Class I drugs.[34]

N-acetyl glutamine, also known as meglumine (MEG) (Fig. 1d), is a polyhydroxy organic amine that has demonstrated to be a suitable counterion for salt formation with weakly acidic molecules. Such salt formation is a strategy to increase solubility,[6,20,29,35] drug release rate,[20,29,35-38] and stabilization.[38] In a previous study, we

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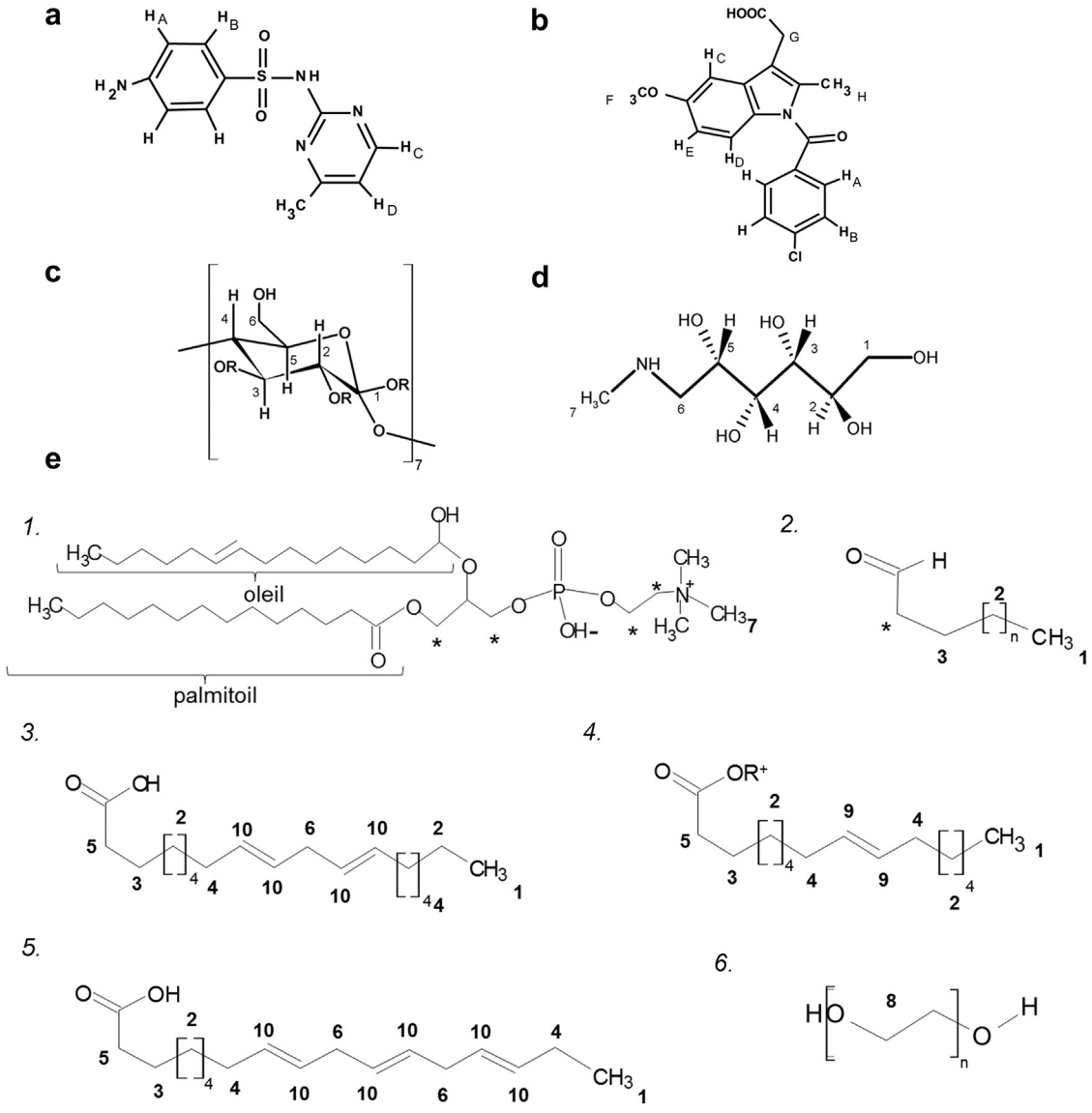


Figure 1. Chemical structure of (a) sulfamerazine; (b) indomethacin; (c) ßCD, MßCD, or HPßCD when R = H, -CH_3, or -$CH_2CH(OH)CH_3$, respectively; (d) MEG; (e) surfactants and the fatty acid components of the microemulsion: *1.* soy phosphatidylcholine (S); *2.* palmitic acid (O) or stearic acid (O) when n = 12 or 14, respectively; *3.* linoleic acid (O); *4.* oleic acid (O) or sodium oleate (S) when R = H or Na, respectively; *5.* linolenic acid (O); *6.* Eumulgin (S); *weak indistinguishable signals.

have shown that MEG promoted a significant increase in SMR solubility, which is a very slightly water-soluble sulfonamide (0.22 mg/mL).[20] In addition, the solubility improvement was increased by the formation of the drug:MEG:CD ternary complexes.[6,29]

The interaction of drugs with ME in combination with CDs or MEG could favorably modify the undesirable properties of pharmaceutical active substances and may enhance the individual benefit of the separated systems. Furthermore, the addition of the ligands as cosurfactants to the ME formulations may affect the stability and the structure of the system, allowing the reduction of the surfactant amount.[11,39,40]

From these concerns, the goal of the present research was to examine various formulation strategies of modified ME by adding ß-cyclodextrin (ßCD), methyl-ßCD (MßCD), hydroxypropyl-ßCD (HPßCD), or MEG to the aqueous phase of the systems, using SMR and INM as model hydrophobic drugs, which belong to Class II of Biopharmaceutical Classification System according to our previous findings.[6,20,41-43] Experimental approaches applied to these drugs could be extrapolated to other drugs with similar physicochemical properties. In order to assess the influence of the ligands on the ME microstructure, pseudoternary phase diagrams (PTPDs) and conductivity, particle size, and zeta potential determinations were conducted. Drug-component interaction studies were performed by ^{1}H NMR (nuclear magnetic resonance), and drug incorporation into ME and the *in vitro* release of the drugs from these systems in the presence of the ligands were also carried out.

Materials and Methods

Materials

The MEs used in this work were chosen according to previous studies.[6,44] Soy phosphatidylcholine (SPC) was purchased from Degussa Texturant Systems Deutschland GmbH & Co. (Hamburg, Germany); polyoxyethylenglycerol-40 hydrogenated Castor Oil (Eumulgin® HRE 40) (EU) (Sigma-Aldrich®); soy oil (Liza®) (O),

SMR, and INM were obtained from Parafarm®, Argentina (Fig. 1e). MEG was purchased from Sigma-Aldrich; ßCD ($M_W = 1135$), MßCD ($M_W = 1190$), and HPßCD ($M_W = 1325$) were kindly supplied by Ferromet® (agent in Argentina of Roquette®). 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 3 portions of 100 mL of acetone. All the other substances and solvents were of analytical reagent grade. The water used in these studies was obtained from a Millipore Milli-Q Water Purification System.

Methods

Pseudoternary Phase Diagram

The PTPDs of the systems were obtained, utilizing an SPC/EU/SO (35:35:30) surfactant mixture with an hydrophilic-lipophilic balance value of 12. The surfactant composition was chosen from 2 previous studies carried out by our research group, one of them about hydrophilic-lipophilic balance values for the surfactant system and the other on the same ME system without the addition of ligands.[6,44] Semisolid mixtures of oil/surfactants (O/S) (1.0 g), with weights ranging from 1:9 to 9:1 ratios, were titered with aqueous phase (W), under ultrasonic stirring using an Ultrasonic Liquid Processor, Heat System XL 2020 apparatus. The aqueous phases consisted of 1.8% ßCD, 12% MßCD, 2.5% MßCD, or 5% MEG in water, in relation to the maximum water solubility of each ligand. The whole study was carried out at room temperature. The transitions from semisolid mixture to opaque dispersion (emulsion) and from emulsion (EM) to optically clear ME or phase separation were sharp and reproducible with 0.1 mL of precision.

Conductivity (σ)

The conductivity (σ) of the systems was measured for 3 different concentrations of each ligand (0.005, 0.010, and 0.015 M) incorporated into the aqueous phase using a Digimed® DM-32 conductivity meter with a Digimed DMC-010M electrode. The conductivity meter was calibrated using a standard solution of 1413 μS/cm before testing. The measurements were carried out in triplicate at 25 ± 1°C.

Effect of the O/S Ratio on the Drug Incorporation Into ME With Ligands

A selected ME containing 2.5% SPC, 2.5% EU, 7.5% OS, and 7.5% O chosen from the ME region of the PTPD, with a fix 80% wt/wt of W, for obtaining an O/W ME with different aqueous phase contents (without ligand, 1.8% ßCD, 12% MßCD, 2.5% MßCD, or 5% MEG in water or in phosphate buffer solution of pH 8 [PBS 8]) was prepared to evaluate the influence of the system composition on the incorporated amount of SMR or INM. The ME was prepared by slowly adding the O phase amount to the semisolid mixture of SPC/EU/OS. Then, 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-μm filter, appropriately diluted with ethanol and analyzed at 230 or 270 nm for SMR or INM, respectively, using a Hewlett Packard 89090A UV-Visible spectrometer 1 cm path length cuvettes. The dissolved amount of drug was plotted against the O/S ratio. In order to elucidate the affinity of the drugs for the ligands in the systems, the apparent stability constants (K_C) of the drug:CD complexes were determined as a function of the ligand concentration ([CD]). Because the phase solubility diagrams of these complexes, reported in a previous work,[20] were of A_L type and assuming that the complexes were of 1:1 stoichiometry, the apparent stability (or formation) constants (K_C) were calculated using the slope from the linear regression analysis of the phase solubility isotherms by the following equation:

$$K_C = \text{slope}/S_0(1 - \text{slope}) \quad \text{(eq. 1)}$$

where S_0 is the water solubility of the pure drug.

For the ME without ligand, the K_C was also calculated to provide information about the affinity of the drugs for the surfactant/oil droplets. Both the CD and the droplets dispersed in the aqueous phase can incorporate the drugs in the internal hydrophobic compartment, which contribute to the reduction of the dielectric constant of the media, increasing the solubilization of lipophilic drugs.

Nuclear Magnetic Resonance Studies

^{1}H NMR studies were performed at 298 K in a Bruker® Avance II High Resolution Spectrometer equipped with a broad band inverse probe) and a variable temperature unit, using 5-mm sample tubes. Spectra of the unloaded and drug-loaded ME with different aqueous phase compositions (water, 1.8% ßCD, 12% MßCD, 2.5% HPßCD, or 5% MEG in water) were obtained by incorporating a 0.1-mL volume of D_2O into 0.5 mL of a (5/15/80) O:S:W (wt/wt/wt) ME. The spectra of the pure components were obtained by diluting appropriate amounts in D_2O for SPC, EU, and SO or in $DCCl_3$ for soy oil. All the studies were carried out at 400.16 MHz and the data were processed with the Bruker TOPSPIN 2.0 software. The residual solvent signal (4.80 ppm) was used as the internal reference. Induced changes in the ^{1}H NMR chemical shifts ($\Delta\delta$) for the drugs and ME components, originated from their interaction, were calculated according to the following equations:

$$\Delta\delta_{drug} = \delta_{D(ML)} - \delta_C \quad \text{(eq. 2)}$$

$$\Delta\delta_{drug} = \delta_{D(ML)} - \delta_{D(M)} \quad \text{(eq. 3)}$$

and

$$\Delta\delta_{ME} = \delta_{M(L)} - \delta_M \quad \text{(eq. 4)}$$

$$\Delta\delta_{ME} = \delta_{M(LD)} - \delta_{M(L)} \quad \text{(eq. 5)}$$

where $\delta_{D(ML)}$, δ_C, and $\delta_{D(M)}$ are the chemical shifts of the drug protons in the ME + ligand + drug system [D(ML)], in the corresponding complex with ßCD, MßCD, HPßCD, or MEG (C) or in the ligand-free ME [D(M)] and $\delta_{M(L)}$, δ_M, and $\delta_{M(LD)}$ are the chemical shifts of the ME signals incorporated in the ME + ligand system [M(L)], in the ligand and drug-free ME (M), or in the ME + ligand + drug system [M(LD)], respectively.

Droplet Size, Polydispersity, and Zeta Potential Measurement

The droplet size, polydispersity, and zeta potential of both unloaded and drug-loaded ME with different aqueous phase compositions (water, 1.8% ßCD, 12% MßCD, 2.5% HPßCD, or 5% MEG in water) were determined at 25°C, using a Beackman Coulter® Delsa™ Nano C Particle Analyzer. The intensity autocorrelation function was measured at a 165° angle, using a viscosity of 0.8878 Pas and a refractive index of 1.3328. The samples were appropriately diluted with water before their analysis.

In Vitro *Release of the Drugs From the Microemulsion*

The *in vitro* release of SMR and INM from the ME containing water or 1.8% ßCD, 12% MßCD, 2.5 % HPßCD, or 5% MEG in water as

the aqueous phase 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®). Cellulose acetate membrane (Sigma-Aldrich) with a pore size of 0.45 μm and an exposed area of 1.77 cm^2 was used. The drugs in an oral dose incorporated into the ME were loaded in the donor 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 267 nm for SMR or INM, respectively. Each experiment was performed at least 3 times and the results represent the experimental average. The initial concentration of the drug in PBS solution was held constant at 200 μg/mL.

Results and Discussion

Characterization Studies

Pseudoternary Phase Diagram

For ME, it is necessary to determine the phase diagram that describes the experimental conditions in which optically transparent systems can be obtained.[11] As shown in Figure 2, the PTPDs of the systems containing soy oil (O), SPC/EU/OS (S), with different aqueous phases (W) (1.8% ßCD, 12% MßCD, 2.5% HPßCD, or 5% MEG in water) were constructed to show the relationship between the composition and phase behavior of samples. The addition of the ligands in the aqueous phase expanded the region of the liquid isotropic phases from a minimum of 70% of W and a maximum of 16% of O to 60; 50; 50% of W and 20; 30; 35% of O with ßCD, MßCD, and MEG, respectively. This feature may be due to the presence of CD that acts as cosurfactants, leading to the formation of isotropic phases, which allows higher incorporation of W and O into the ME at the same S content. The presence of CD in the aqueous phase and the interfacial region can influence the optimal head group area of the surfactants by altering the aqueous solubility of the oil phase and drug compounds.[45] In contrast, a small reduction in the ME domain area was verified using HPßCD to a minimum of 75% of W and a maximum of 10% of O. MßCD and MEG were the most suitable cosurfactants when the ME was prepared within the testing range. The increment of the oil content incorporated in the ME provides a greater opportunity for the dispersion of poorly water-soluble drugs.[4]

Conductivity (σ)

Electrical conductivity is a structure-sensitive property. There are some studies that reported the strong correlation of this parameter with the internal microstructure of ME.[9,15,46,47] In our previous work, the electrical conductivity (σ) of ME with different O/S composition using water as aqueous phase was investigated. The conductivity values were within the range, from 4.0 to 21.3 mS/cm, respectively. It was observed that the systems exhibited a characteristic profile of percolative conductivity, which indicates that the amount of water in the formulations was above the critical fraction necessary to obtain stable O/W ME and that oil nanodroplets existed as a dispersed phase forming clusters in the bulk dispersion.[6] In this work, the electrical conductivity (σ) of the ME containing 2.5% SPC, 2.5% EU, 7.5% OS, and 7.5% O region of the PTPD with a fixed 80% wt/wt of W containing 3 different concentrations (0.005, 0.010, and 0.015 M) of each ligand (ßCD, MßCD, HPßCD, or MEG) incorporated in the aqueous phase was measured at 25 ± 1°C in order to characterize the inner structure of the systems (Fig. 3). The electric conductivity values of the formulations were within the tested range of ME_{1-5} (from 9.9 to 13.3 mS/cm), indicating oil-in-water dispersion type. In addition, the

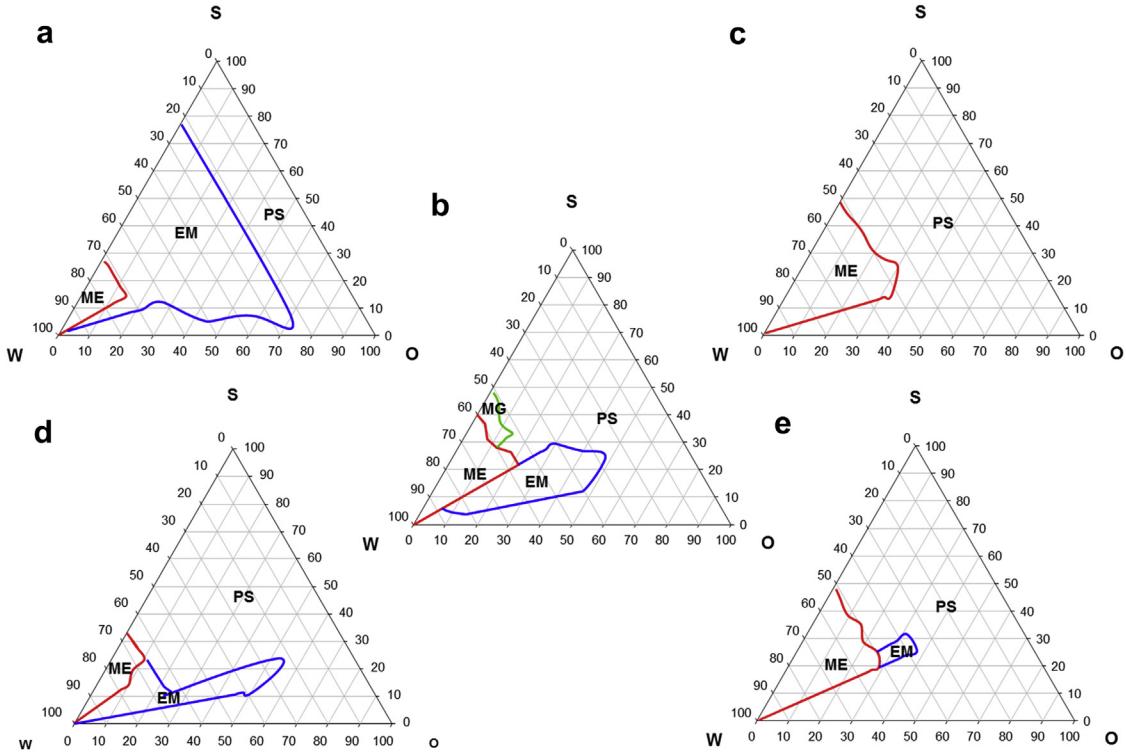
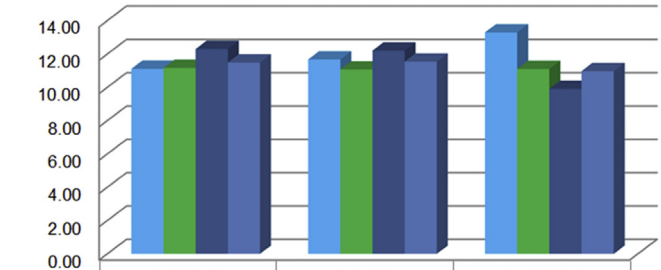


Figure 2. Pseudoternary phase diagram of ME containing (a) water; (b) 1.8% ßCD; (c) 12% MßCD; (d) 2.5% HPßCD; and (e) 5% MEG, in the aqueous phase. G, gel; SP, separation of phases.



	0.005 M	0.01 M	0.015 M
ßCD	11.108	11.673	13.292
MßCD	11.16	11.07	11.1
HPßCD	12.301	12.202	9,904
MEG	11,488	11,538	10,97

Figure 3. Variation of the electrical conductivity (σ) with the increment of the ligand concentration.

conductivity values slightly changed with the variation of the content of the ligands, which suggests that the ME microstructure remained unaffected with the increasing percentage of them.

Effect of the O/S Ratio on the Drug Dissolution in ME With Ligands

The solubilizing effect of the ME may contribute to the effect of the CDs and MEG associated with the systems. In order to evaluate the effect of the ligands on the solubilization of SMR and INM in ME, selected samples containing different O/S ratios and different aqueous phase contents (without ligand or with 1.8% ßCD, 12% MßCD, 2.5% MßCD, or 5% MEG in water or in PBS 8) were prepared and the O/S versus drug concentration was plotted, with the plots being presented in Figure 4. The solubilities of the drugs in the aqueous phase (S_w) of the ME, the maximum solubilities (S_{max}), and the apparent solubility increments achieved using the ME, with respect to the solubility of the drug in water or in PBS 8 (S_{max}/S_D) or in each drug-ligand system unassociated with the ME (S_{max}/S_w), as well as the stability constant (K_c) values, were determined and are presented in Table 1. As explained in our previous work on ligand-free ME,[6] a linear increase in the drug concentration was observed with the increase in the O/S ratio.

Even though the highest S_{max} was achieved with the ME with $W = 5\%$ MEG (35.6 mg/mL) for SMR and with $W = 1.8\%$ ßCD in PBS 8 (73.1 mg/mL) for INM, the highest values for K_c and the S_{max}/S_w ratios were obtained with W = water for both drugs, which indicates that the ligands improved the drug solubility by increasing the concentration solubilized in the aqueous phase, instead of being involved in the interaction of the drugs with the components of the ME system. In addition, the highest S_{max}/S_D ratios were achieved with $W = 5\%$ MEG in water for SMR and with W = water ME for INM, which supports the previous statement.

Drug-ME Component Interaction Studies

The intermolecular interactions between the drugs and the ME components were investigated by ^{1}H NMR analysis to shed some light on the location of SMR and INM molecules in ME systems. Spectra of the unloaded and drug-loaded ME with different aqueous phase compositions were obtained and the chemical shift displacements of SMR and INM incorporated in the ME:ligand:drug system ($\delta_{D(ML)}$) with respect to both the corresponding complex with each CD or MEG (δ_C) and the drug in the ligand-free ME ($\delta_{\mathbf{D(M)}}$) were determined. Also, the displacements of the ME component signals incorporated in the ME:ligand system ($\delta_{M(L)}$) were analyzed with respect to the ligand-free ME ($\delta_{\mathbf{M}}$) and the ME:ligand:drug system ($\delta_{M(\mathbf{LD})}$). These results are shown in Table 2. In the unloaded ME containing 1.8% ßCD, the downfield displacements were observed for all the ME signals. The most significant shifts corresponded to the side chain of EU and to the methylene group near the carboxylic acid moiety of OS, suggesting the presence of ßCD in the near proximity of surfactant molecules. In the SMR-loaded ME, containing 1.8% ßCD, upfield displacements with respect to ßCD:ME, SMR:ME, and SMR:ßCD were recorded for most of the ME and SMR signals, which may indicate that van der Waals or hydrophobic interactions between the drug, the CD, and the ME component occur. The higher displacements were observed for the SPC and EU signals, suggesting the interaction of SMR with the surfactants in the interface, which may be related to the lower dissolution of SMR in ME containing ßCD with respect to the ME without ligand. In the unloaded ME containing 12% MßCD, 2.5% HPßCD, and 5% MEG, the most significant upfield displacements were evidenced for the vinyl bond vicinal protons and the vinyl protons of the fatty acid, which may indicate the interaction of these ligands with OS, SPC, and the side chains of fatty acids. On the other hand, in the SMR-loaded ME containing 12% MßCD, a high upfield displacement for the vinyl bond vicinal protons and downfield displacements for the vinyl protons and for the methylene groups of the side chain of the fatty acid were observed. The SMR-loaded ME containing 2.5% HPßCD showed the most significant downfield displacements for the vinyl protons, for the

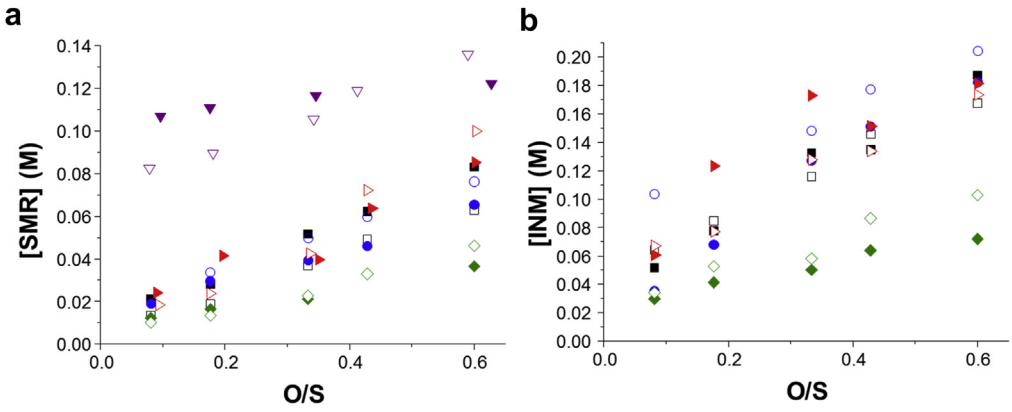


Figure 4. Incorporation curve of (a) SMR and (b) INM in microemulsions alone (■) or containing 1.8% ßCD (●), 12% MßCD (◆), 2.5% HPßCD (▶), or 5% MEG (▼) in water (filed symbol) or in PBS pH 8 (empty symbol) as the aqueous phase.

Table 1
Solubilities of the Drugs in the Aqueous Phase (S_w), Maximum Solubilities Achieved With the ME (S_{max}), Apparent Solubility Increment Achieved Using the ME (S_{max}/S_w or S_{max}/S_D), and Stability Constants (K_c)

Aqueous Phase (W)	Sulfamerazine					Indomethacin				
	S_W (mg/mL)	S_{max} (mg/mL)	S_{max}/S_w	S_{max}/S_D	K_C (M^{-1})	S_W (mg/mL)	S_{max} (mg/mL)	S_{max}/S_w	S_{max}/S_D	K_C (M^{-1})
Water	0.2213	21.9879	99.36		167.55	0.01824	67.01	3673.8		6771.83
PBS pH 8	1.6576	16.6277	10.03		17.72	1.4276	59.912	42.0		64.65
ßCD 1.8% in water	0.785	17.2991	22.04	78.17	31.37	0.0395	65.2573	1652.1	3577.70	3706.39
ßCD 1.8% in PBS pH 8	2.8678	20.1801	7.04	12.17	11.21	5.8606	73.1144	12.5	51.21	15.02
MßCD 1.8% in water	5.9205	9.62088	1.63	43.47	2.15	0.1065	25.768	242.0	1412.72	297.65
MßCD 1.8% in PBS pH 8	8.22	12.2428	1.49	7.39	2.48	14.6485	36.7737	2.51	25.76	3.67
HPßCD 2.5% in water	1.9	22.5290	11.86	101.80	17.71	0.028	3.1980	114.2136	175.33	3290.16
HPßCD 2.5% in PBS pH 8	0.69	26.3870	38.24	15.92	76.34	0.08	62.0631	775.7893	43.47	1198.334
MEG 5% in water	34.5344	32.3648	0.94	146.25						
MEG 5% in PBS pH 8	54.6461	35.9594	0.66	21.69						

methylene groups of the side chain of the fatty acid, for the signals corresponding to the methylene of the side chain of EU, and for the methylene group near the carboxylic acid moiety of OS, suggesting the presence of HPßCD in the near proximity of surfactant molecules. The SMR-loaded ME containing 5% MEG presented upfield displacements of the SPC, OS, and EU signals, which may be due to the shielding effect of the amine moiety of MEG. However, the SMR protons presented upfield displacements with respect to SMR:ME, suggesting the interaction with MEG. The SMR protons presented upfield displacements in all the ME, indicating the interaction with electron density donor moieties such as the side chains of fatty acids.

At the same time, in the INM-loaded ME containing 1.8% ßCD, upfield displacements were detected for most of the ME protons. The most significant shifts corresponded to the SPC, EU, and to the methylene groups of the side chain of fatty acid signals. Also, the INM protons presented upfield displacements, which are indicative of the interaction with the electron density donor moieties of the side chains of fatty acids. This may suggest the interaction of INM with both the oil phase and the surfactants, which is consistent with the higher solubilization of INM when the drug is incorporated in the ME containing ßCD. In the INM-loaded ME containing 12% MßCD, most of the ME protons presented downfield displacements, relative to those corresponding to the carboxylic acid vicinal protons, whereas the methylene side chain signals of the fatty acids were the most significantly changed. This evidence suggests that the dissolution of INM occurs mainly in the oil domain in this system. On the other hand, in the INM-loaded ME containing 2.5% HPßCD, the most significant chemical shifts were observed for the SPC and the methylene side chain signals of the fatty acids. In addition, for the ME containing MßCD and HPßCD, upfield displacements were recorded for H_A, H_B, and H_c, which have higher electron density, and for this reason they may interact with the electron donor moieties such as the side chains of fatty acids. However, downfield displacements were detected for H_D and H_E INM protons that present lower electron density and interact with electron acceptor moieties, such as the carboxylic acid of fatty acid or the amine of SPC moieties. These findings suggest the interaction of INM with both the surfactants and the oil phase in ME containing MßCD or HPßCD.

Droplet Size, Polydispersity, and Zeta Potential Measurement

The droplet size, polydispersity, and zeta potential for both unloaded and drug-loaded ME with different aqueous phase compositions (water, 1.8% ßCD, 12% MßCD, 2.5% HPßCD, or 5% MEG in water) were determined at 25°C. All the measurements showed a single peak, indicating a monomodal size distribution. The main results are summarized in Table 3. Reduced droplet size values (21-73 nm) were recorded in the presence of the ligands for the drug-loaded ME. This finding was probably due to the deposition of some drug and ligand molecules at the interface, which decreases the interfacial tension that increases the bend angle of the droplet, and thus reducing the droplet size. This is consistent with the results obtained by NMR that indicated the interaction of the ligands and drugs with the surfactants. As reported for the unloaded $ME_{1\text{-}5}$ in our previous work,[6] the negative surface potential value of the unloaded ME droplets was due to the presence of the SO in its ionized form at the oil/water interface (p*K*a = 6.2-7.3[48]). The zeta potential values for the ME containing ligands were closer to zero, which could be attributed to the interaction of the ligands with the SO and with the negatively charged drugs by electrostatic interaction with the amine moiety of MEG or by complexation with CDs.

In Vitro *Release of the Drugs From the ME*

Most drugs permeate through biological membranes by passive diffusion.

Adjacent to the membrane surface there is an unstirred water layer that may act as a diffusion barrier for rapid drug penetration.[49] It is well documented that CDs can, under certain conditions, enhance drug delivery through biological membranes.[11,49] On the other hand, ME has been shown to be efficient formulations for the transdermal and dermal delivery of, particularly, lipophilic compounds due to their solubilizing capacity and their components that may act as penetration enhancers.[9,12,13] The *in vitro* release and transport of SMR and INM from the ME containing water, 1.8% ßCD, 12% MßCD, 2.5% HPßCD, or 5% MEG in water as the aqueous phase were tested by evaluating the permeability across an artificial membrane (Fig. 5). As reported in our previous work,[6] a significant increase in the permeation was observed for SMR when it was incorporated in the *W* = water ME, achieving a 2-fold increase after 4 h, whereas the release profile of INM from *W* = water ME was similar to that of the control formulation, attaining about 70% of released drug and reaching a plateau at about 8 h. The presence of MEG or MßCD in the ME conferred the most significant increases in the release of drugs (95%-100%), reaching plateaus at 2 and 10 h for SMR and INM, respectively. This phenomenon may be due to the solubilizing effect of the ligands that promote the penetration of the drug through the water layer. In addition, an increased permeation was observed for both drugs employing ME with ßCD, attaining 90% for SMR and 75% for INM. Both drugs reached the plateau at 10 h. The results indicated that the combined effect of ME with CDs (mainly MßCD) or MEG may improve the diffusion of the lipophilic drugs though the unstirred water layer of biological membranes.

Table 2
Chemical Shift Displacements of (a) SMR and INM Incorporated in the ME:Ligand System ($\delta_{D(ML)}$) With Respect to the Corresponding Complex with ßCD, MßCD, HPßCD, or MEG (δ_C) and to the Drug in the Ligand-Free ME $\delta_{D(M)}$ and (b) ME Component Signals in the ME:Ligand System ($\delta_{M(L)}$) With Respect to the Ligand-Free ME (δ_M) and to the ME:Ligand:Drug System ($\delta_{M(LD)}$)

(a)	SMR:ßCD		SMR:MßCD		SMR:HPßCD		SMR:MEG	
	$\delta_{D(ML)}$-δ_C	$\delta_{D(ML)}$-$\delta_{D(M)}$	$\delta_{D(ML)}$-δ_C	$\delta_{D(ML)}$-$\delta_{D(M)}$	$\delta_{D(ML)}$-δ_C	$\delta_{D(ML)}$-$\delta_{D(M)}$	$\delta_{D(ML)}$-δ_C	$\delta_{D(ML)}$-$\delta_{D(M)}$
A	−0.2541	Ø	−0.1705	Ø	−0.1930	Ø	0.0065	Ø
B	−0.1331	−0.2543	−0.0115	−0.1765	−0.0789	−0.2426	0.0232	−0.1394
C	−0.1666	−0.1559	−0.1207	−0.1122	−0.0784	−0.1216	0.0091	−0.1491
D	−0.1698	0.075	0.1216	0.1768	−0.1396	0.0765	−0.0296	−0.2147
E	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
	INM:ßCD		INM:MßCD		INM:HPßCD			
	$\delta_{D(ML)}-\delta_C$	$\delta_{D(ML)}-\delta_{D(M)}$	$\delta_{D(ML)}-\delta_C$	$\delta_{D(ML)}-\delta_{D(M)}$	$\delta_{D(ML)}-\delta_C$	$\delta_{D(ML)}-\delta_{D(M)}$		
A	−0.0539	−0.0709	−0.0111	−0.061	−0.0571	−0.0015		
B	−0.1915	−0.0572	−0.1176	−0.0312	−0.2300	0.0319		
C	−0.4259	−0.0489	−0.3664	−0.043	−0.2846	−0.0281		
D	−0.3976	−0.0234	−0.2587	0.0884	−0.2978	0.0884		
E	−0.4081	−0.0313	−0.2962	0.0406	−0.2895	0.0644		
F	Ø	Ø	Ø	Ø	Ø	Ø		
G	Ø	Ø	Ø	Ø	Ø	Ø		
(b)	ME:ßCD		ME:MßCD		ME:HPßCD		ME:MEG	
	$\delta_{M(L)}$	$\delta_{M(L)}-\delta_M$	$\delta_{M(L)}$	$\delta_{M(L)}-\delta_M$	$\delta_{M(L)}$	$\delta_{M(L)}-\delta_M$	$\delta_{M(L)}$	$\delta_{M(L)}-\delta_M$
1	0.9616	0.0438	0.8913	−0.0265	0.9126	−0.0052	0.887	−0.0308
2	1.3645	0.0459	1.3001	−0.0185	1.3225	0.0039	1.2953	−0.0233
3	1.6439	0.0474	1.5748	−0.0217	1.5932	−0.0033	1.5697	−0.0268
4	2.1117	0.0433	2.0372	−0.0312	2.065	−0.0034	2.0419	−0.0265
5	2.3131	0.0573	2.2383	−0.0175	2.2584	0.0026	2.2333	−0.0225
6	2.8199	0.0211	2.7543	−0.0445	2.7611	−0.0377	2.7502	−0.0486
7	3.3267	0.0431	3.2569	−0.0267	3.2715	−0.0121	3.2543	−0.0293
8	3.7916	0.0556	3.7128	−0.0232	3.7279	−0.0081	3.707	−0.029
9	5.3799	0.0344	5.3143	−0.0312	5.3288	−0.0167	5.309	−0.0365
	SMR:ME:ßCD		SMR:ME:MßCD		SMR:ME:HPßCD		SMR:ME:MEG	
	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$
1	0.8751	−0.0865	0.9087	0.0174	0.9736	0.061	0.8709	−0.0161
2	1.2785	−0.086	1.30395	0.00385	1.3028	−0.0197	1.2778	−0.0175
3	1.5639	−0.08	1.5849	0.0101	1.5759	−0.0173	1.5633	−0.0064
4	2.0294	−0.0823	2.0615	0.0243	2.052	−0.013	2.0273	−0.0146
5	2.228	−0.0851	2.24365	0.00535	2.2343	−0.0241	2.2201	−0.0132
6	2.7431	−0.0768	2.3405	−0.4138	2.7527	−0.0084	2.74112	−0.00908
7	3.2334	−0.0933	3.2627	0.0058	3.264	−0.0075	3.2338	−0.0205
8	3.6995	−0.0921	3.72	0.0072	3.7122	−0.0157	3.6906	−0.0164
9	5.2938	−0.0861	5.3253	0.011	5.3162	−0.0126	5.2968	−0.0122
	INM:ME:ßCD		INM:ME:MßCD		INM:ME:HPßCD			
	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$	$\delta_{M(LD)}$	$\delta_{M(LD)}-\delta_{M(L)}$		
1	0.883	−0.0786	0.8996	0.0083	0.898	−0.0146		
2	1.2827	−0.0818	1.2962	−0.0039	1.3005	−0.022		
3	1.5708	−0.0731	1.5961	0.0213	1.5715	−0.0217		
4	2.0262	−0.0855	2.0508	0.0136	2.0282	−0.0368		
5	2.237	−0.0761	2.2636	0.0253	2.2449	−0.0135		
6	2.7512	−0.0687	2.7763	0.022	2.7481	−0.013		
7	3.1602	−0.1665	3.1729	−0.084	3.2436	−0.0279		
8	3.69905	−0.09255	3.7148	0.002	3.7158	−0.0121		
9	5.298	−0.0819	5.3397	0.0254	5.3182	−0.0106		

Ø, undistinguishable signal because of superposition.

Table 3
Particle Size, PDI, and Zeta Potential of Unloaded, SMR-Loaded, and INM-Loaded ME Containing ßCD, MßCD, HPßCD, or MEG

	O/S	Empty ME			SMR-Loaded ME			INM-Loaded ME		
		Particle Size (nm)	PDI	Zeta Potential (mV)	Particle Size (nm)	PDI	Zeta Potential (mV)	Particle Size (nm)	PDI	Zeta Potential (mV)
Plain ME	0.084	97 ± 6	0.279	−56.9 ± 8.7	106.6 ± 2.9	0.3	−57 ± 9	117 ± 1	0.304	−67.5 ± 1.43
ME + ßCD	0.178	103 ± 4	0.423	−40.7 ± 7.2	20.9 ± 0.9	0.3	−0.2 ± 0.04	73 ± 4	0.206	−0.24 ± 0.27
ME + MßCD	0.334	150 ± 2	0.38	−0.88 ± 0.1	41.7 ± 6	0.39	−10 ± 3	48 ± 6	0.569	0.34 ± 0.05
ME + HPßCD	0.428	183 ± 12	0.316	−60.8 ± 3.8	42.5 ± 6	0.26	−16 ± 0.3	24 ± 2	0.29	−2.59 ± 0.58
ME + MEG	0.62	96 ± 3	0.295	−0.6 ± 0.3	20.9 ± 0.9	0.3	−0.1 ± 0.05			

PDI, polydispersity index.

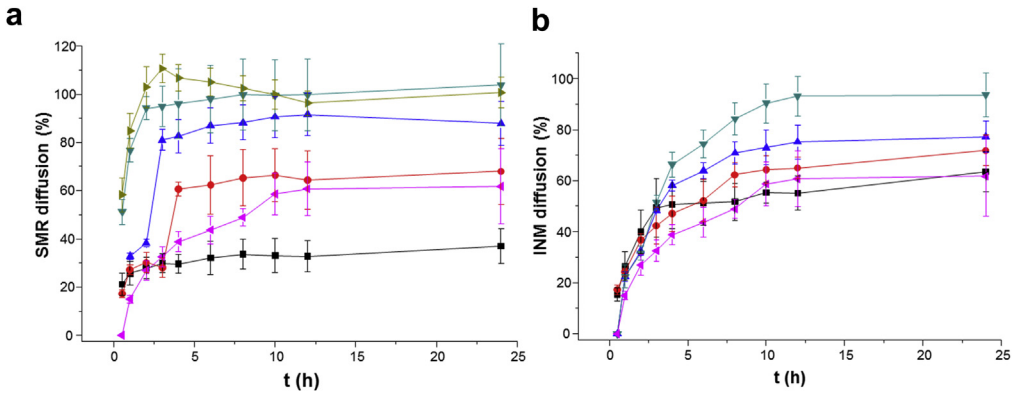


Figure 5. *In vitro* release profiles from a cellulose acetate membrane of (a) SMR and (b) INM alone (■), in microemulsions containing water (●), 1.8% ßCD (▲), 12% MßCD (▼), 2.5% HPßCD (◀), or 5% MEG (▶), as the aqueous phase.

Conclusions

In this study, we have demonstrated the advantages of the combination approach between the lipophilic drug complex, containing CD or MEG with ME. It was shown that the addition of ßCD, MßCD, or MEG to the aqueous phase expanded the isotropic region of the ME domain. The possibility of incorporating a higher oil content is a greater opportunity for the clear dispersion of lipophilic drugs such as SMR and INM. The conductivity studies indicated the obtainment of stable O/W ME and the existence of oil nanodroplets as a clear dispersed phase forming clusters in the bulk dispersion, which remains unaffected after the incorporation of the ligands.

High proportions of SMR and INM could be dissolved in most of the ME formulations, achieving the best value with the ME containing $W = 5\%$ MEG (35.6 mg/mL) and $W = 1.8\%$ ßCD in PBS 8 (73.1 mg/mL) for SMR and INM, respectively.

The NMR spectroscopy revealed that ßCD, MßCD, HPßCD, MEG, SMR, and INM interact with both the surfactants and the oil phase components. A reduction in the droplet size values (21-73 nm) was recorded in the presence of the ligands for the drug-loaded ME. A significant increase in the drug release from ME was achieved for SMR or INM when MEG or MßCD was used. These changes in the profiles may be due to a solubilizing effect of the ligands that facilitates the drug to penetrate the unstirred water layer.

The drug solubilization effect using O/W ME in combination with ßCD, MßCD, HPßCD, or MEG optimized in this study may improve the bioavailability of poorly water-soluble drugs, by using an easy technological strategy.

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