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Solubility and release modulation effect of sulfamerazine ternary complexes with cyclodextrins and meglumine

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ABSTRACT

This study investigated the effect on solubility and release of ternary complexes of sulfamerazine (SMR) with β-(βCD), methyl-(MβCD) and hydroxypropyl-β-cyclodextrin (HPβCD) using meglumine (MEG) as the ternary component. The combination of MEG with MβCD resulted the best approach, with an increased effect (29-fold) of the aqueous solubility of SMR. The mode of inclusion was supported by 2D NMR, which indicated that real ternary complexes were formed between SMR, MEG and MβCD or HPβCD. Solid state analysis was performed using Fourier-transform infrared spectroscopy (FT IR), differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD), which demonstrated that different interactions occurred among SMR, MEG and MβCD or HPβCD in the ternary lyophilized systems. The ternary complexes with βCD and MβCD produced an additional retention effect on the release of SMR compared to the corresponding binary complexes, implying that they were clearly superior in terms of solubility and release modulation.

1. Introduction

Cyclodextrins (CDs) (Fig. 1a) are cyclic torus-shaped molecules, consisting of 6–8 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 bind organic molecules in aqueous solutions and in the solid state by non-covalent interactions [1,2]. The inclusion complex normally exhibits a higher aqueous solubility [3–6], and a greater chemical stability than the pure drug [7–11], and also affects the release rate of drugs in physiological fluids [12–14].

However, the high molecular weight, cost, production capability and possible parenteral toxicity have hindered the use of CDs in pharmaceutical formulations, leading their concentrations being minimized whenever [15,16]. The total solubility of a drug in the presence of CDs can be highly enhanced by pH adjustment or the use of an appropriate third component, including the addition of polymers to the complexation media [15,17], drug ionization and salt formation [7,18,19] or the addition of organic salts [16].

N-acetyl glucamine, also known as meglumine (MEG) (Fig. 1b), is a polyhydroxy organic amine that has been demonstrated to raise solubility [20,21], drug release rate [21–24] and stabilization [24] of weakly acidic molecules. In a previous work developed in our laboratory, meglumine showed a significant solubilization enhancement of sulfamerazine (SMR) (Fig. 1c), which is a very slightly water-soluble (0.22 mg/ml) [21] sulfonamide, compared with the free drug and with the SMR-CD complexes, and was demonstrated to be responsible for a solubility improvement via multiple factors rather than just providing a favorable pH. Moreover, it was demonstrated that the complexation with βCD, MβCD, HPβCD and MEG resulted in a decrease in the release rate of the drug through cellulose acetate membrane, thereby enabling sustained drug delivery systems. [21] Other formulation strategies have been applied to SMR, such as the use of co-solvent mixtures [25], nanocrystals [26], amorphous solid dispersions containing polymers [27], pectin films [28], chitosan membranes [29,30] and phase transformation [31,32], with Rajendiran et al. also describing the binary complexes of this drug with βCD and αCD [33]. In

Abbreviations: SMR, sulfamerazine; MEG, meglumine; K_c, apparent stability constant; PSS, phase solubility studies; S_0, intrinsic solubility of the drug; δ_S, δ_T, δ_B, δ_M, chemical shifts of SMR alone, in the ternary and binary complexes with CD or MEG, respectively; FDBS, freeze-dried binary system; FDTS, freeze-dried ternary system; TPM, ternary physical mixtures; S_{max}, maximum solubility; $K_{c\,TER}$, K_c value of the ternary system; $K_{c\,BIN}$, K_c value of the binary system; BPM, binary physical mixture.

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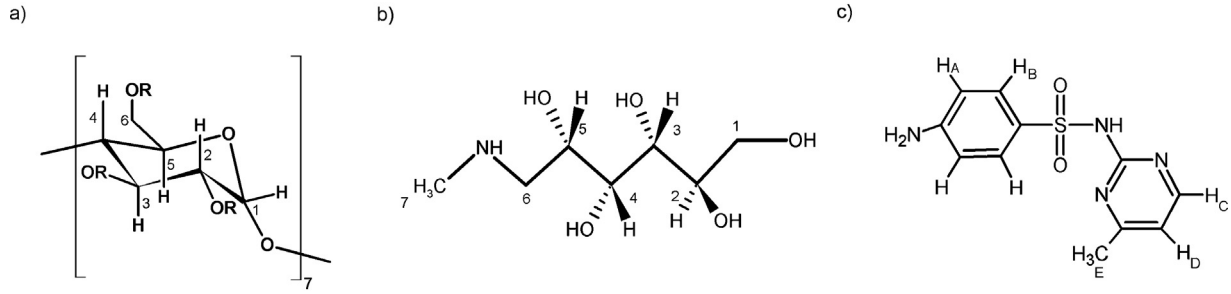

Fig. 1. Chemical structure of: (a) βCD, MβCD or HPβCD with R = H; —CH_3 or —$CH_2CH(OH)CH_3$, respectively; (b) MEG and (c) sulfamerazine.

addition, it is important to note that the preparation of multicomponent inclusion complexes has been previously described in a patent of Chiesi et al. (1998). By using a drug bearing an acidic group, a CD and a basic compound, they found that the simultaneous salt formation with the suitable basic counter-ions and complexation with CDs dramatically increased the aqueous solubility of the drug [34]. In another study, Redenti et al. showed that complexation and simultaneous salt formation resulted in a higher solubility of a drug in comparison with simple binary complexes [35], thereby permitting the production of oral formulations with an increased dissolution rate and bioavailability of the complexed drug over a wider pH range [36]. Recently, in our laboratory, the solubility and dissolution profile of sulfisoxazole was improved by the development of a ternary system with HPβCD and triethanolamine (TEA) as the co-solubilizing agent [37]. Furthermore, the formation of an inclusion multicomponent complex of acetazolamide with HPβCD and TEA significantly enhanced the water solubility and the dissolution rate of the drug, resulting in a powerful strategy for ocular formulations, since this was able to reduce the intraocular pressure in rabbits [38].

Following on from these studies, we now aimed to examine various formulation approaches, namely ternary cyclodextrin complexes made with three different CDs, with varying abilities to improve the solubility of poorly soluble SMR and using meglumine as the ternary component to intensify the solubilization by cyclodextrins. Also, the ability of the ternary complexes to deliver SMR and to sustain its release was examined.

2. Materials and methods

2.1. Materials

Sulfamerazine was obtained from Parafarm®, Argentina. Meglumine was purchased from Sigma–Aldrich®, USA, with βCD, MβCD KLEPTOSE® CRYSMEB (DS = 0.5) and HPβCD (DS = 0.45–0.95) being kindly supplied by Ferromet® (agent in Argentina of Roquette®). All the other materials and solvents were of analytical reagent grade, and the water used in these studies was generated by a Millipore Milli-Q Water Purification System.

2.2. Phase solubility studies (PSS)

Solubility diagrams were obtained according to the Higuchi and Connors method [39], and each experiment was performed in triplicate. Excess amounts of SMR, to create saturation conditions, were added to aqueous or buffered solutions containing a 3 mM concentration of MEG and βCD (0–16 mM), MβCD (0–100 mM) or HPβCD (0–18 mM), in relation to their aqueous solubilities. The suspensions formed were sonicated in an ultrasonic bath for 15 min every 12 h to favor solubilization before being placed in a 25.0 ± 0.1 °C constant temperature bath [HAAKE DC10 thermostat (Haake®, Paramus, NJ, USA)] for 72 h. After equilibrium was reached, the suspensions were filtered through a 0.45 μm membrane filter (Millipore®, USA) and analyzed in a *Shimadzu® UV-160* spectrophotometer. The equilibrium pH of each solution was measured (*Hanna® HI 255* pH-meter) and the apparent stability constants (K_c) of the SMR:CD:MEG complexes were determined as a function of the CD concentration (CD) added [12]. From A_L-type isotherms or from the linear portion of the A_N or A_P phase solubility diagrams, the apparent stability (or formation) constants K_c were calculated assuming a 1:1 drug–CD stoichiometry and taking into account the value of the slope using the following equation:

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of the pure drug.

2.3. Nuclear magnetic resonance (NMR) studies

Heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) 2D NMR experiments were performed at 298 K in a Bruker® Advance II High Resolution Spectrometer equipped with a broad band inverse (BBI) probe and a variable temperature unit (VTU) using 5 mm sample tubes, with spectra being obtained at 400.16 MHz. Equimolar ratio complexes (1:1:1) and pure substance solutions were prepared in D_2O by incorporating an excess amount of the drug with a fixed concentration of each ligand, which were then sonicated for 1 h to favor the maximum SMR solubilization in order to obtain a measurable concentration of the drug and to form complexes. All suspensions were filtered before their analysis and the NMR 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 SMR originating from their interactions with the ligands were calculated according to the following equations:

$$\Delta\delta = \delta_T - \delta_S; \quad \Delta\delta = \delta_T - \delta_B \quad \text{and} \quad \Delta\delta = \delta_T - \delta_M$$

where δ_S, δ_T, δ_B and δ_M are the chemical shifts of SMR alone, in the ternary and binary complexes with CD or MEG, respectively.

2.4. Solid sample preparations

Freeze-dried binary (FDBS) and ternary (FDTS) systems were obtained by the freeze-drying technique using a Labconco® Freeze Dry 4.5 apparatus. Equimolar ratio systems (1:1:1) and pure substance aqueous solutions were prepared by incorporating an excess amount of the drug with a fixed concentration of the ligands, which were sonicated for 1 h. The suspensions were filtered and then frozen at −40 °C until their complete solidification had been achieved for the freeze-drying procedure. In contrast, ternary physical mixtures (TPM) were prepared using the same molar ratios, by simple mixing in an agate mortar.

Table 1
SMR solubilities, apparent stability constants (K_c) and solubility increments (SI).

		Isotherm	R^2	S_{max} (mg/ml)	S_{max}/S_0	S_{max}/S_{MEG}	$K_{c\ TER}$ (M^{-1})	$K_{c\ BIN}$ (M^{-1})	pH
SMR:βCD: MEG	Water	A_p	0.9809	0.97 ± 0.05	4 ± 1	2.5 ± 0.3	35 ± 14	198 ± 22	7.43
	pH 2.0	A_N	0.9635	1.30 ± 0.08	4 ± 1	2.4 ± 0.3	200 ± 18	207 ± 45	1.72
	pH 8.0	A_L	0.9431	2.36 ± 0.05	1.5 ± 0.2	1.15 ± 0.09	15 ± 4	31 ± 8	8.03
SMR:MβCD: MEG	Water	A_L	0.8295	6.28 ± 0.06	29 ± 2	3.5 ± 0.3	29 ± 3	73 ± 21	7.85
	pH 2.0	A_L	0.9945	2.8 ± 0.5	9 ± 1	3.6 ± 0.6	42 ± 2	118 ± 29	1.81
	pH 8.0	A_N	0.9190	11 ± 1	7 ± 1	5.0 ± 0.8	88 ± 9	59 ± 13	7.95
SMR:HPβCD: MEG	Water	A_L	0.9964	4.4 ± 0.4	20 ± 5	1.6 ± 0.3	76 ± 9	144 ± 26	7.92
	pH 2.0	A_L	0.9732	1.23 ± 0.01	3.9 ± 0.7	1.70 ± 0.09	58 ± 4	65 ± 17	1.85
	pH 8.0	A_L	0.8706	2.5 ± 0.1	1.5 ± 0.3	1.1 ± 0.1	7 ± 3	20 ± 4	8.00

S_{max}: maximum solubility; S_0: intrinsic solubility of the drug (0.22 mg/ml in H_2O; 0.32 mg/ml at pH 2.0 and 1.6 mg/ml at pH 8.0); S_{MEG}: solubility of SMR in a 0.003 M solution of MEG; TER: ternary complex; BIN: corresponding binary complex with each CD.

2.5. Fourier-transform infrared spectroscopy (FT IR)

The FT IR spectra of pure substances, physical mixtures and binary and ternary systems were recorded as potassium bromide discs using a Nicolet 5 SXC FT IR spectrometer, and the signal changes of the corresponding functional groups were compared in order to analyze the mode of interaction between them.

2.6. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The thermal behavior of the drug and complexes was studied by heating the samples in pierced aluminum-crimped pans (pinhole) under nitrogen gas flow, over the temperature range of 25–350 °C, at a 10 °C min^{-1} heating rate. The samples containing MEG were analyzed only in the temperature range from 25 to 200 °C, due to premature thermal degradation of MEG. The DSC and TG curves were obtained on DSC TA 2920 and on TG TA 2920 equipments, respectively. Data were obtained and processed using the TA Instruments Universal Analysis 2000 software.

2.7. X-ray diffractometry (XRD)

The X-ray diffraction analyses were performed for pure substances, physical mixtures, binary and ternary systems using the Rikugu® model Dmax 2500PC X-ray diffractometer with a 2θ range between 4° and 50°, utilizing Cu Kα radiation (λ = 1.5406 Å) at scanning rate of 0.05°/min. The XRD patterns were recorded under ambient temperature conditions. The diffraction patterns of pure SMR and MEG correspond to the lyophilized plain components.

2.8. In vitro-release studies

2.8.1. Release experiments

Release experiments of SMR and complexes were carried out 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 membranes with a pore size of 0.45 μm and exposed area of 1.77 cm^2 were used (Sigma–Aldrich®, USA), with aqueous formulations (0.3 ml) with pure SMR and ternary complexes in the determined stoichiometric relation and in an oral dose quantity being loaded into the donor compartment. A 10 mM phosphate buffer solution (PBS) of pH 7.4 was used as the diffusion medium in the donor and receptor cells, and samples (2.0 ml) were withdrawn from the receiver compartments at fixed intervals and replaced automatically with an equal volume of previously warmed PBS. The SMR concentration was measured spectrophotometrically at 240 nm, with the initial concentration of the drug alone or in the complexes in PBS solution being maintained at 200 μg/ml.

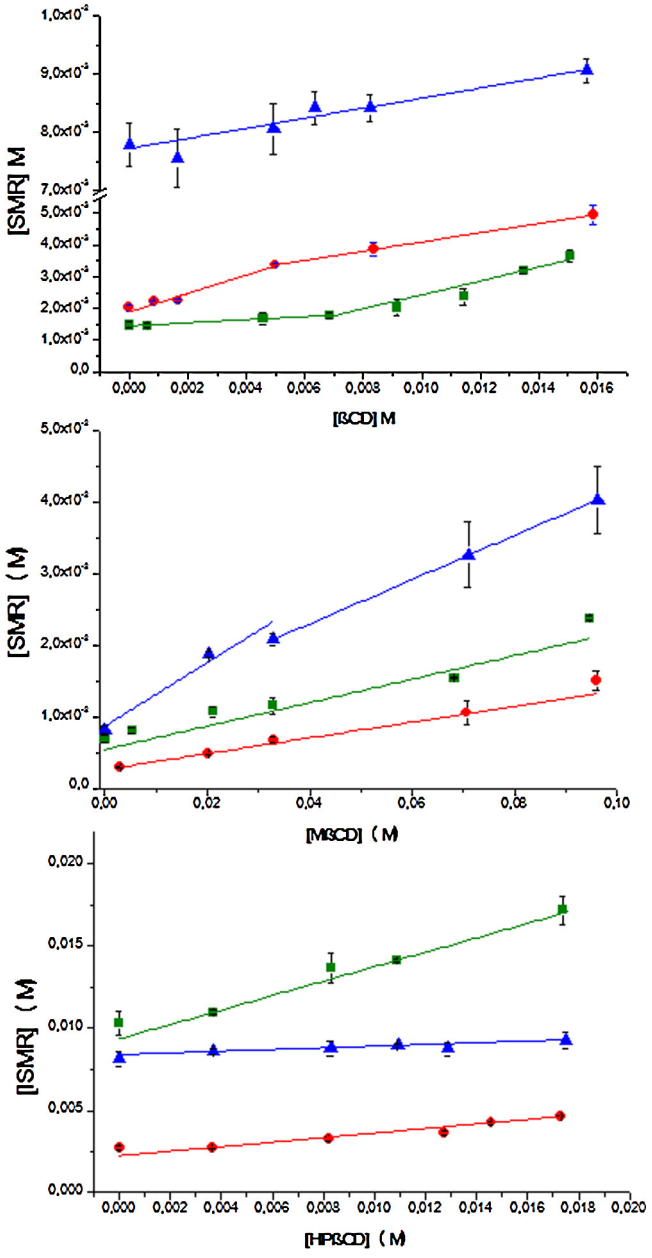

Fig. 2. Phase solubility diagrams for sulfamerazine ternary complex with MEG and the βCD, MβCD or HPβCD at 25 °C in: water (■); pH 2.00 (●) and pH 8.00 (▲) solution.

Table 2
Chemical shifts of sulfamerazine alone (δ_S), in the binary complex with MEG (δ_M) or with the corresponding CD (δ_B) and in the ternary complex (δ_T).

	SMR:βCD:MEG							SMR:MβCD:MEG					SMR:HPβCD:MEG				
	δ_S	δ_M	δ_B	δ_T	$\delta_T-\delta_S$	$\delta_T-\delta_B$	$\delta_T-\delta_M$	δ_B	δ_T	$\delta_T-\delta_S$	$\delta_T-\delta_B$	$\delta_T-\delta_M$	δ_B	δ_T	$\delta_T-\delta_S$	$\delta_T-\delta_B$	$\delta_T-\delta_M$
H_A	8.1764	8.0290	8.3176	8.0858	−0.0906	−0.2318	0.0568	8.3406	8.1989	0.0225	−0.1417	0.1699	8.2656	8.1574	−0.0190	−0.1082	0.1284
H_B	7.7369	7.6342	7.7968	7.6916	−0.0453	−0.1052	0.0574	7.7530	7.7306	−0.0063	−0.0224	0.0964	7.7543	7.7247	−0.0122	−0.0296	0.0905
H_C	6.8670	6.7889	6.9471	6.8119	−0.0551	−0.1352	0.0230	6.9449	6.7982	−0.0688	−0.1467	0.0093	6.8932	6.8404	−0.0266	−0.0528	0.0515
H_D	6.8091	6.6471	6.8322	6.6844	−0.1247	−0.1478	0.0373	6.6426	6.7543	−0.0548	0.1117	0.1072	6.8034	6.7758	−0.0333	−0.0276	0.1287
H_E	2.3901	2.2701	2.4765	2.3214	−0.0687	−0.1551	0.0513	2.4522	2.3873	−0.0028	−0.0649	0.1172	2.4323	2.3704	−0.0197	−0.0619	0.1003

Table 3
Chemical shifts of each CD alone (δ_{CD}), in the binary (δ_B) and the ternary complexes (δ_T).

	SMR:βCD:MEG					SMR:MβCD:MEG					SMR:HPβCD:MEG				
	$\delta_{\beta CD}$	δ_B	δ_T	$\delta_T-\delta_{\beta CD}$	$\delta_T-\delta_B$	$\delta_{M\beta CD}$	δ_B	δ_T	$\delta_T-\delta_{M\beta CD}$	$\delta_T-\delta_B$	$\delta_{HP\beta CD}$	δ_B	δ_T	$\delta_T-\delta_{HP\beta CD}$	$\delta_T-\delta_B$
H_1	5.0678	5.1184	5.0976	0.0298	−0.0208	5.2507	5.2447	5.2808	0.0301	0.0361	5.2517	5.2372	5.2565	0.0048	0.0193
H_1[a]	–	–	–	–	–	5.0638	5.0581	5.0981	0.0343	0.0400	5.0978	5.0886	5.1054	0.0076	0.0168
H_2	3.6470	3.4301	3.6758	0.0288	0.2457	Overlapped with H_4			–	–	3.6489	3.6302	3.6900	0.0411	0.0598
H_2[a]	–	–	–	–	–	3.3993	3.3993	3.4222	0.0229	0.0229	–	–	–	–	–
H_3	3.9654	3.9952	3.9872	0.0218	−0.0080	4.0081	3.9952	4.0231	0.0150	0.0279	3.9839	4.0458	4.8735	0.8896	0.8278
H_3[a]	–	–	–	–	–	3.9541	3.9541	3.9683	0.0142	0.0142	–	–	–	–	–
H_4	3.5835	3.6322	3.611	0.0272	−0.0215	Overlapped with H_2			–	–	3.5214	3.5256	3.5348	0.0134	0.0092
H_5	3.8505	3.8709	3.8800	0.0295	0.0091	3.6698	3.7985	3.7990	0.1292	0.0005	3.7454	3.8476	[b]	–	–
H_6	3.8774	3.9216	3.9024	0.0250	−0.0192	3.8624	3.8877	3.8973	0.0349	0.0096	3.8947	3.8932	3.9009	0.0062	0.0077
OCH_3	–	–	–	–	–	3.5518	3.5788	3.6024	0.0506	0.0236	–	–	–	–	–

[a] Methylated position.
[b] Undistinguishable.

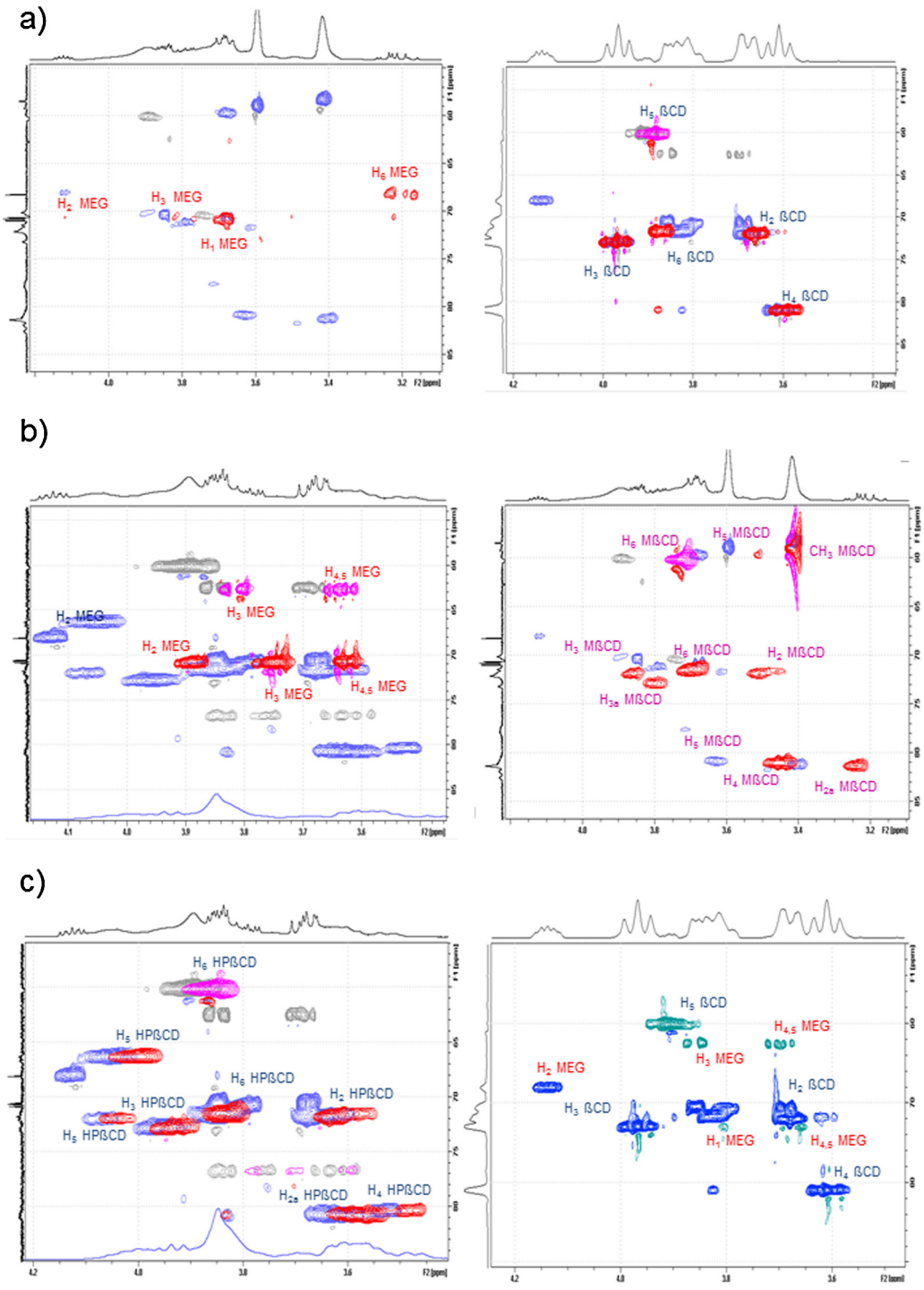

Fig. 3. Partial contour plots of HSQC 2D NMR spectra of: (a) SMR:βCD:MEG; (b) SMR:MβCD:MEG and (c) SMR:HPβCD:MEG complexes, overlay with the corresponding CD and MEG binary complex (left and right, respectively).

Each experiment was performed at least three times and the results were the averages. The data are presented as the mean ± standard deviation. The significant differences of the induced changes in release due to the incorporation in the ternary complexes compared with the pure drug and with the SMR:CD and SMR:MEG complexes were assessed with one-way analysis of variance (ANOVA). Results were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Phase solubility studies (PSS)

In order to evaluate the formation of ternary complexes between SMR, MEG and the three CDs (βCD, MβCD or HPβCD), and to analyze the effect of these on the aqueous solubility of the drug, phase solubility diagrams of SMR in water, and in solutions of pH 2.0 and

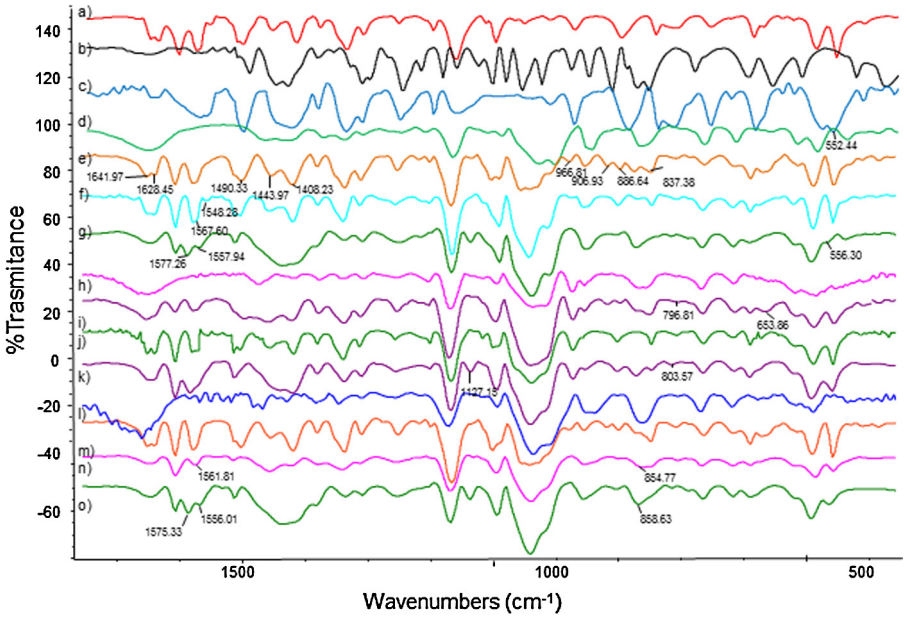

Fig. 4. FT-IR spectra of: (a) pure MEG; (b) pure SMR; (c) binary freeze-dried systems (BFDS) with MEG; (d) pure βCD; (e) ternary physical mixtures (TPM) with βCD; (f) BFDS with βCD; ternary freeze-dried systems (TFDS) with βCD; (h) pure MβCD; (i) TPM with MβCD; (j) BFDS with MβCD; (k) TFDS with MβCD; (l) pure HPβCD; (m) TPM with HPβCD; n) BFDS with HPβCD; (o) TFDS with HPβCD.

pH 8.0 were obtained for each CD with the addition of a fixed concentration of MEG (3 mM) as the ternary component, as shown in Fig. 2. In general, most of the isotherms could be classified as A_L-type diagrams according to Higuchi and Connors [39], showing that SMR solubility increased linearly as a function of CD concentration and that soluble complexes were formed without the occurrence of precipitation in the range of CD concentrations used. These results were in agreement with those previously reported by our research group for the SMR:CD and SMR:MEG binary complexes [40,21] with the exception being the isotherms of SMR:βCD:MEG and SMR:MβCD:MEG in solutions of pH 2.0 and pH 8.0, respectively, which were of A_N-type, suggesting the self-association of the CD molecules or an alteration in the effective nature of the solvent in the presence of large concentrations of ligand, thus affecting the apparent degree of complexation [39,41]. In contrast, the diagram of SMR:βCD:MEG in water was of A_P-type, suggesting an increase of the host–guest ratio at high concentrations of this CD.

Taking into account the results reported for the corresponding binary complexes [21], where the phase solubility diagrams with the three CD were classified as A_L-type, it should be stated that, in some cases, the introduction of MEG modifies the way of complexation of the drug with the CD, with slope values of less than one in all the diagrams, indicating the formation of drug-CD complexes with a 1:1 stoichiometry in solution. In addition to the apparent stability constants (K_c), calculated by the Higuchi and Connors equation, the maximum solubilities (S_{max}) achieved, the solubility increments and the stability constants (K_c) for all the systems are presented in Table 1. From these values, a different interaction between the drug and the three CDs in the presence of MEG could be deduced. The highest stability constant was determined for the SMR:βCD:MEG complex at pH 2.0, whose value was statistically equal to the one of the binary SMR:βCD system, indicating that the interaction with βCD was produced more efficiently when the weak sulfonamide acid group of the drug existed in its unionized form, suggesting that the MEG did not affect the incorporation of SMR inside the cavity. The K_c values of the other ternary systems ($K_{c\,TER}$) were lower than those of the corresponding binary ones ($K_{c\,BIN}$), except for the ternary complex with MβCD at pH 8.0, whose $K_{c\,TER}$ value was slightly higher than $K_{c\,BIN}$. In addition, it is important to comment that in a previous study it was determined that the solubility of SMR diminished at pH 2.0 with increasing concentrations of MEG, a fact that was attributed to the electrostatic repulsion of MEG and SMR, since both presented their amino groups protonated at this pH value [21]. However, this behavior was not observed for the ternary systems at pH 2.0 for the MEG concentration used in these studies. Related to this, by analyzing the S_{max} values obtained for each system it could be concluded that, despite in all of them an improvement being observed for the ternary systems with MβCD and HPβCD in water, the rise in the S_{max} values were in fact higher, with increases of 29- and 20-folds, respectively, with respect to the solubility in the absence of ligands.

According to these above results, we can conclude that an improvement in the solubility occurred due to the co-presence of CD and MEG, since the S_{max} values achieved with the ternary systems were higher than those obtained with the binary complexes. Finally, the behavior of the three CDs suggests that their chemical structures influenced the different modes of interaction with SMR in the presence of MEG.

3.2. Nuclear magnetic resonance (NMR) studies

For studying the modes of interaction between SMR, MEG and each of the three CD in the ternary complexes, the chemical shifts of protons in the pure components, the binary and ternary complexes were compared. Since the ^{1}H NMR spectra of CD and MEG interfered with each other, which was also reflected in ROESY, heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC) 2D NMR experiments were performed in order to elucidate the signals with a higher resolution. The chemical shifts were extracted from the HSQC spectra (Fig. 3) and are listed in Tables 2 and 3. In the ternary complexes, all SMR protons presented upfield displacements in comparison with the pure drug and the corresponding binary complexes with each CD, suggesting a predominating shielding phenomenon of the amine moiety of MEG in the proximity of the sulfonamide group of SMR, which was also observed previously in the SMR:MEG binary system [21]. In contrast, all SMR protons presented downfield displacements in comparison with the SMR:MEG binary system, probably

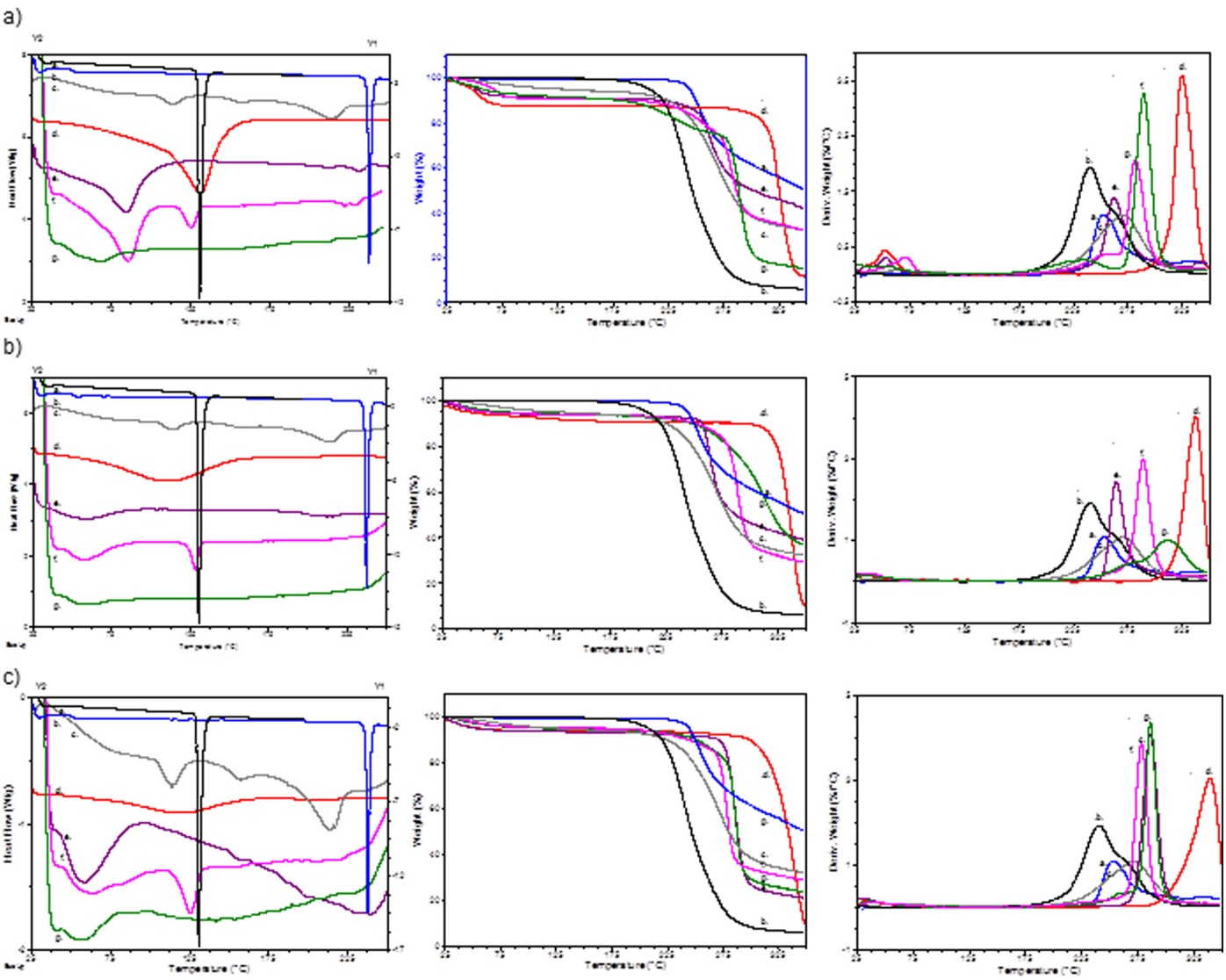

Fig. 5. DSC, TG and DTG curves of: (a) SMR:βCD:MEG; (b) SMR:MβCD:MEG and (c) SMR:HPβCD:MEG complexes. **a**. Pure SMR: blue; **b**. Pure MEG: black; **c**. Binary freeze-dried systems (BFDS) with MEG: gray; **d**. Pure CD: red; **e**. BFDS with the CD: purple; **f**. Ternary physical mixtures (TPM): pink; **g**. Ternary freeze-dried systems (TFDS): green; Scales: Y1 for SMR and MEG; Y2 for the remaining curves. (For interpretation of the references to color in this legend, the reader is referred to the web version of the article.)

due to Van Der Waals interactions between SMR and the hydrophobic inner side of CD, as observed for the binary complexes [21].

The CD protons presented downfield displacements in comparison with the corresponding pure form of the ligands, with the most significant shifts being observed in H_5 and H_3 for MβCD and HPβCD ternary systems, respectively. The βCD protons presented upfield displacements in the SMR:βCD:MEG system with respect to the binary SMR:βCD complex, probably due to the shielding effect of the MEG amino moiety, which may indicate the interaction of βCD with uncomplexed MEG. In contrast, the MβCD and HPβCD protons presented downfield displacements in comparison with the binary systems, suggesting the interaction of the SMR:MEG pair with the internal hydrophobic cavity of the CD by Van Der Waals forces. These findings may explain the high increase in solubility observed in the phase solubility studies carried out in water, when SMR was combined with MβCD or HPβCD in the presence of MEG, indicating the formation of real ternary complexes.

3.3. Fourier-transform infrared spectroscopy (FT IR)

FT IR spectroscopy was used to assess the interaction between SMR and MEG with βCD, MβCD or HPβCD in the solid state, since upon complexation of the guest, shifts or changes in the absorption spectrum occur [17]. The FT IR spectra of pure substances, binary freeze-dried systems (FDBS), ternary physical mixtures (TPM) and ternary freeze-dried systems (FDTS), are shown in Fig. 4. The characteristic bands of SMR presented changes in the FDTS compared with the TPM and the FDBS, as the aromatic ring vibration signals (1642 and 1630 cm^{-1}) were shifted to higher or lower frequencies in the FDTS, with βCD and HPβCD (1557; 1577 and 1556 cm^{-1}) or disappeared, in comparison with the corresponding FDBS (1548; 1567 and 1575; 1562 cm^{-1}). In particular, the pyrimidine ring vibration bands (1444 and 1407 cm^{-1}) were modified to a single band (1423 cm^{-1}) or disappeared in the FDTS, as with βCD, with respect to the TPM and the FDBS (1490; 1444; 1408 and 907 cm^{-1}), which may have been related to the different interactions of βCD with SMR in the presence of MEG, as observed in the NMR studies. Moreover, the S=O stretching band of SMR (1092 cm^{-1}), which was exhibited at 1127 cm^{-1} in the FDBS and the TPM, was absent in the FDTS with MβCD, and the S—O stretching bands in the TPM and FDBS with βCD and MβCD (967; 837; 887 and 797 cm^{-1}, respectively) were shifted (797–804 cm^{-1}) or disappeared. In addition, many other signals of the FDBS and the TPM spectra corresponding to the fingerprint region of SMR were shifted in the FDTS with βCD

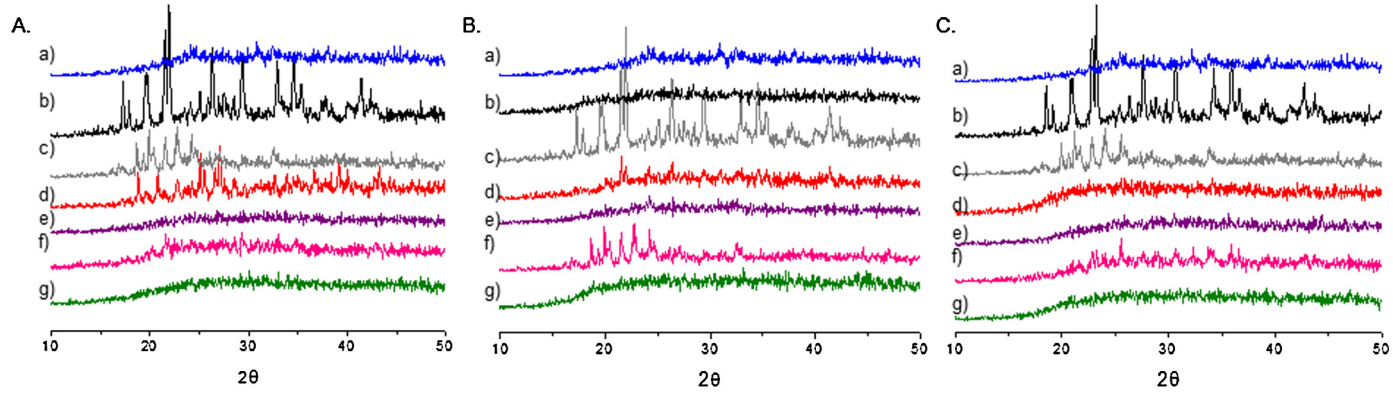

Fig. 6. DRX patterns of: (A) SMR:βCD:MEG; (B) SMR:MβCD:MEG and (C) SMR:HPβCD:MEG complexes. (a) Pure lyophilized SMR; (b) pure lyophilized MEG; (c) binary freeze-dried systems (BFDS) with MEG; (d) pure CD; (e) BFDS with the CD; (f) ternary physical mixtures (TPM); and (g) ternary freeze-dried systems (TFDS).

(556–552 cm^{-1}) or HPβCD (855–859 cm^{-1}) or disappeared with MβCD (654 cm^{-1}). These results confirm that a different interaction occurred between SMR, MEG and each of the CDs when the ternary systems were prepared by means of lyophilization, indicating the formation of new compounds that differed from the corresponding binary complexes, as previously shown by the NMR studies and the solubility enhancements.

3.4. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

In a recent work developed in our laboratory, the formation of true inclusion complexes of SMR with βCD, MβCD and HPβCD and a binary system with MEG were successfully described by DSC and TGA [21]. The SMR thermal curve was typical of a crystalline substance and was characterized by a sharp endothermic peak (at 237.51 °C), assigned to its melting point, which decomposed above 270 °C. Moreover, CDs lose water at temperatures in the range from 25 to 150 °C and decompose above 250 °C. On the other hand, the MEG DSC curve presented a sharp endothermic peak with no loss of weight, corresponding to its melting point and decomposing above 200 °C [21]. In the present work, the thermal behavior of the SMR:MEG:CD ternary systems was monitored by using the same procedure in order to compare the patterns obtained with the pure substances and the FDBS, and to be able to assess the formation of the ternary complexes which are illustrated in Fig. 5. In the curves of the TPMs with the three CDs, the endothermic peaks corresponding to the SMR and MEG melting points were absent or slightly shifted to lower temperatures. In addition, the dehydration events of βCD, MβCD and HPβCD were also shifted to lower temperatures (85.39, 63.09 and 63.09 °C, respectively) in comparison with the corresponding pure ligands. These events may indicate that weak molecular interactions occurred between the components at high temperatures, but was not evidence of the formation of true inclusion compounds. In contrast, in the DSC curves of the FDTS, the melting peaks of SMR and MEG were absent. Also, the dehydration event of HPβCD in the FDTS was shifted to a lower temperature (56.05 °C) than the one of the corresponding TPM (63.09 °C). Furthermore, the decomposition of the FDTS with βCD and MβCD occurred at higher temperatures (289.28 and 312.19 °C) than the corresponding FDBS (262.34 and 264.25 °C) and TPM (281.84 and 288.85 °C), probably demonstrating the formation of a new entity with a higher thermal stability. These results suggest that a different interaction occurred between SMR and CD in the presence of MEG, when the ternary systems were prepared by means of lyophilization, and support the previous findings from the NMR studies, which indicated the formation of true inclusion complexes.

3.5. X-ray diffractometry (XRD)

The powder X-ray patterns of pure lyophilized substances, binary freeze-dried systems (FDBS), ternary physical mixtures (TPM) of the lyophilized components and ternary freeze-dried systems (FDTS) are shown in Fig. 6. The molecular state of the binary complexes of SMR with βCD, MβCD, HPβCD and MEG was previously described by our research group [21] using powder XRD, where the presence of each species as an isolated solid was confirmed in the binary physical mixture (BPM) and a drug amorphization was instead induced when the CD solid complexes were obtained by the freeze-drying technique, as deduced by the

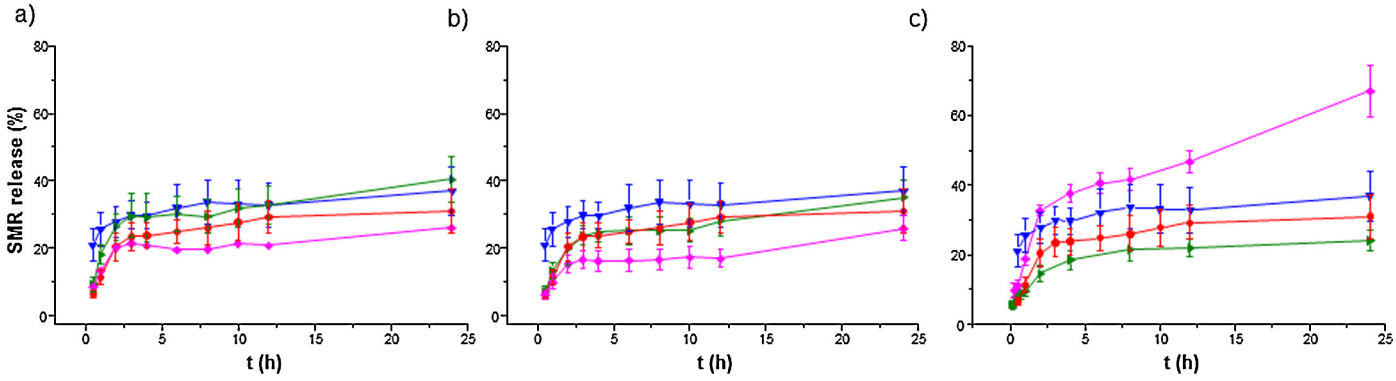

Fig. 7. Permeation profiles of: SMR (▼); SMR:MEG complex (►); SMR:CD complex (●); SMR:CD:MEG complex (♦); (a) βCD; (b) MβCD; (c) HPβCD (each value represents the mean ± S.D. of $n \geq 3$).

superposition of the pure substance patterns or by the presence of large diffraction bands in which it was no longer possible to distinguish the characteristic peaks of the drug. From these bases, the molecular state of SMR:MEG:CD ternary complexes was also investigated using the same procedure with the diffractograms of the TPMs being found to be approximately the superposition of the patterns of the raw materials, in agreement with that observed in the BPM. On the other hand, the FDTS showed diffused diffraction patterns with a decreased crystalline character with respect to the FDBS, where the crystalline diffraction peaks corresponding to isolated MEG, which were detectable in SMR:MEG FDS, were no longer visible. This may indicate that the combination of each CD and MEG presented a higher efficacy toward drug amorphization than the binary complexes, when the systems were obtained by means of lyophilization.

3.6. Release rate studies

The ability of the ternary complexes to deliver SMR and to sustain its release was examined by determining the diffusion of the drug through cellulose acetate membranes at 37 °C for 24 h. The statistical analysis of the results ($p < 0.05$) shown in Fig. 7 indicated the modification of the in vitro release of the drug as was observed with the binary complexes [21], and also an additional retention effect produced by the presence of MEG in the ternary complexes with βCD and MβCD, thus allowing a more sustained release than that of the corresponding binary ones. In contrast, the SMR:MEG:HPβCD complex showed a significantly higher diffusion compared with the SMR:HPβCD formulation, thereby, providing a significant improvement ($p < 0.05$). Different explanations have been reported for the release modulation effect of CD complexation. For example, Constantin et al. established that compounds that form strong inclusion complexes with CDs display high values of retention time, while chemicals that form weak inclusion complexes display low values, suggesting that the release rate of drugs is mainly controlled by the dissociation of the guest molecule from CDs [42]. Meanwhile, Loftsson et al. postulated that the rates for formation and dissociation of drug/CD complexes are very close to diffusion-controlled limits, with complexes being continually formed and broken down. Consequently, the presence of water-soluble drug/CD complexes at the hydrated membrane surface frequently increases the availability of dissolved drug molecules, especially of lipophilic drugs with poor aqueous solubilities [16]. In addition, Matsuda et al. indicated that the enhancing effects of hydrophilic CDs on drug release from the complex might be explained by the increase in the thermodynamic activity of drugs in the vehicle. In contrast, hydrophobic CDs may modulate the release of drugs from the system [43]. Therefore, the different behaviors observed for the ternary complex with HPβCD may be explained not only because of the lower complexation effectiveness ($K_{c\,TER} = 7 \pm 3\ M^{-1}$) of HPβCD with SMR when a buffer solution of pH 8.0 was used (as revealed by the phase solubility studies) compared with the βCD ($15 \pm 4\ M^{-1}$) and MβCD ($88 \pm 9\ M^{-1}$) ternary complexes, (since in both a retention effect was observed), but also by the hydrophilic properties of HPβCD that increased the thermodynamic activity of the drug in the system and consequently produced the transport of the drug through the membrane.

4. Conclusions

A significant increasing effect on the solubilizing ability of MβCD and HPβCD occurred in the presence of MEG. Using NMR studies, the formation of genuine ternary complexes were demonstrated when SMR and MEG were combined with the three CDs, while by IR-spectroscopy, DSC, TG and XRD it was shown that different interactions occurred between them when the ternary systems were prepared by means of lyophilization. Moreover, a higher efficacy toward drug amorphization was found for the lyophilized ternary complexes than the binary ones. Also, the ternary complex with HPβCD provided significant enhancement effects on the release of SMR, compared to the corresponding binary systems, as with the ternary complexes with βCD and MβCD producing additional retention effects. The ternary systems were clearly superior over binary complexes in terms of solubility and release modulation.

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