Improvement of water solubility of sulfamethizole through its complexation with β- and hydroxypropyl-β-cyclodextrin Characterization of the interaction in solution and in solid state

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Abstract

The aim of this study was to increase the solubility of sulfamethizole in water by complexing it with β-cyclodextrin (BCD) and hydroxypropyl-β-cyclodextrin (HPBCD). The interaction of sulfamethizole with the cyclodextrins was evaluated by the solubility, ^{1}H NMR spectrometry and molecular modelling. The stability constants calculated from the phase solubility method increase in order HPBCD<BCD. From the NMR studies could be concluded that the sulfamethizole:cyclodextrin mole ratio was 1:1 (mol/mol) in the BCD complex and 2:3 (mol/mol) in the HPBCD complex. In both cases the sulfamethizole moiety included in the cyclodextrin was the thiadiazole group. MM2 calculations, either in vacuum or in the presence of a solvent, support this structure. Solid inclusion complexes of sulfamethizole with BCD and HPBCD were obtained by freeze drying 1:1 (mol/mol) solutions in aqueous ammonium hydroxide. Host–guest interactions were studied in the solid state by powder X-ray diffractometry and differential scanning calorimetry. The dissolution rates of sulfamethizole increased by the complexation with BCD or HPBCD. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Sulfamethizole; β-Cyclodextrin; Hydroxypropyl-β-cyclodextrin; NMR; Molecular modelling; Dissolution

1. Introduction

Sulfamethizole is a short-lived sulphonamide used in the treatment of urinary tract infections alone or in combination with other antibiotics. Its main target pathogen is *Escherichia coli*. It acts by taking the place of a folic acid precursor required for bacterial reproduction. The feasibility of sustained release sulfamethizole formulations with more convenient administration schedules is complicated by the low solubility of the drug in water (1 part in 2000 parts of water) (BIAM, 1999; Martindale, 1999).

In this work, we investigated whether this difficulty could be overcome by inclusion-complexation of sulfamethizole with cyclodextrin, a technique that is becoming increasingly popular for improving the solubility of poorly soluble drugs (Stella and Rajewski, 1997; Veiga et al., 1998; Lutka, 2000), for increasing bioavailability (Soliman et al., 1997; Miyake et al., 1999; Siefert et al., 1999) or stability (Lu et al., 2000), or for reducing side effects (Otero-Espinar et al., 1991). Specifically, the interaction between sulfamethizole and β- and hydroxypropil-β-cyclodextrin have been studied in solution and in solid state. We prepared and characterized solid inclusion complexes of sulfamethizole with β-cyclodextrin (BCD) and hydroxypropyl-β-cyclodextrin (HPBCD) by freeze-drying, comparing in particular their drug dissolution characteristics with those of the uncomplexed drug.

2. Materials and methods

2.1. Materials

Sulfamethizole (4-amino-*N*-[5-methyl-1,3,4-thiadiazol-2-yl]-benzenesulphonamide) was supplied by Sigma Chemical Co. (St. Louis, MO, USA,). β-Cyclodextrin

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(BCD) was donated by Roquette-Laisa (Barcelona, Spain), and hydroxypropyl-β-cyclodextrin (HPBCD; degree of substitution 2.7) by Janssen Pharmaceutica N.V. (Beerse, Belgium).

2.2. Solubility studies

Solubility diagrams were obtained according to Higuchi and Connors (1965). Excess amounts of sulfamethizole were added to water containing various concentrations of cyclodextrins and the suspensions were shaken at 37°C. After equilibrium was reached (about 7 days), drug concentration was analyzed spectroscopcally (Shimadzu UV-240-Graphicord) at 264 nm ($E_{1\%,1\ cm}$=577.31). The apparent stability constant of the complex, K, assuming that a 1:1 complex is initially formed, was calculated from the initial straight-line portion of the solubility diagrams. All samples were prepared in duplicate.

2.3. ^{1}H NMR studies

^{1}H NMR spectra in 0.2 N solutions of NaOD in deuterated water were obtained at 25°C with a Bruker WM 300 apparatus operating at 300 MHz, using as internal reference the peak at δ=4.6 ppm due to DHO and H_2O impurities. To determine the stoicheiometry of the complexes by the continuous variation method (Job, 1928), 9.7×10^{-3} M solutions of BCD and sulfamethizole or 8.5×10^{-3} M solutions of HPBCD and sulfamethizole in the deuterated NaOD solution, were mixed in various proportions while keeping the total concentration fixed, and the changes in the most sensitive NMR signal were noted.

2.4. Molecular modelling

Using fixed geometries for β-cyclodextrin and sulfamethizole taken from the Cambridge Structural Database, the optimal geometries of BCD–sulfamethizole complexes in vacuo and in the presence of water molecules were calculated using MM2 force field as implemented in HyperChem (Release 3.0 for Windows). The presence of water was simulated by placing the solute molecules in a 16×12×16 Å water box with a minimum distance of 2.0 Å between solute and solvent molecules, which means that 35 water molecules were included in the model.

2.5. Preparation of inclusion complexes

Equal mole amounts of sulfamethizole and cyclodextrin were dissolved in 5% (w/v) aqueous ammonium hydroxide, the solutions were frozen by immersion in liquid nitrogen, and the frozen solutions were lyophilized in a Labconco Lyph-lock 6 apparatus.

2.6. Preparation of physical mixtures

Sulfamethizole and cyclodextrin were passed through 0.5-mm meshes and equal mole amounts were mixed in a Turbula T2C mixer for 10 min.

2.7. Powder X-ray diffractometry

Diffractograms of the solid inclusion complexes were obtained with a Philips PW 1710 BASED diffractometer using Cu K$_\alpha$ radiation and 2θ scans at a scan rate of 2°/min.

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed with a Shimadzu DSC-50 apparatus equipped with a TA-5 WS/PC thermal analyzer. Thermograms were run between 50 and 250°C at a scan rate of 10°C/min.

2.9. Dissolution studies

The dissolution behaviour of the complexes at 37°C were compared with that of pure sulfamethizole and of physical sulfamethizole–cyclodextrin mixtures using transparent gelatin capsules containing quantities of formulation equivalent to 50 mg of sulfamethizole. Tests were carried out in a USP23 Method II apparatus (Turu-Grau) using 900 ml of distilled water and a stirring speed of 50 r.p.m.; the capsules were enclosed in stainless steel baskets to prevent floating. All experiment were made in triplicate. At pre-specified times, 5-ml samples were extracted and filtered, and the concentration of sulfamethizole was determined spectrophotometrically at 264 nm. The resulting dissolution curves were characterized by the corresponding 60-min dissolution efficiency (Khan, 1975). The statistical significance of differences among formulations was estimated by one-way analysis of variance and the Student–Newman–Keuls test.

3. Results and discussion

3.1. Solubility studies

Fig. 1 shows the phase solubility diagrams of sulfamethizole with the cyclodextrins used, BCD and HPBCD, at 37°C. The solubility of the drug increased linearly as a function of the CD concentration, a feature of the A_L-type complex, showing that water-soluble complexes exit in solution. These results show, in the experimental conditions used, the formation of a 1:1 (mol/mol) inclusion complex with both cyclodextrins because the slope of the diagrams is lower than one (Higuchi and Connors, 1965). The apparent 1:1 stability constant (K), calculated from the initial straight-line part of the solubility curves
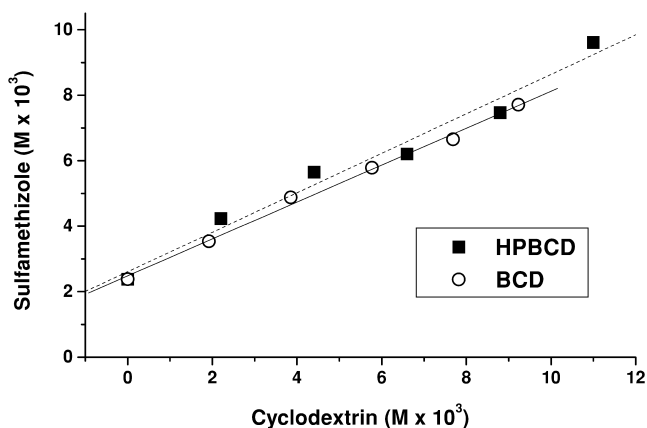
Fig. 1. Phase solubility diagrams for sulfamethizole with increasing concentrations of BCD and HPBCD.

were 651.85 M^{-1} for BCD and 563.95 M^{-1} for HPBCD, which indicates a similar interaction between the drug and both cyclodextrin derivatives in the conditions used in the study.

3.2. ^1H NMR studies

The inclusion of a guest molecule in a cyclodextrin ring shifts the NMR signals of included guest protons dow-

nfield and those of affected host protons upfield, thereby throwing light on the interactions, position and orientation of the guest molecule (Ueda and Nagai, 1981; Cabral Marques et al., 1990; Djedaïni et al., 1990; Torres-Labandeira et al., 1993). The spectra run in this work have no signals that are not present in the components, showing that under the working conditions complexation–decomplexation is a dynamic process with the same time scale as NMR (Djedaïni et al., 1990).

An increase of the concentration of cyclodextrin caused downfield shifts in the methyl proton signals and upfield shifts in those of the benzene protons adjacent to the sulphonamide group, but hardly affected those of the benzene protons adjacent to the amino group (Fig. 2). The deshielding of the methyl group is interpreted as a consequence of its inclusion in the cyclodextrin, and the shielding of the protons adjacent to the sulphonamide group as due to the formation of hydrogen bonds with the hydroxyl groups at the edge of the cyclodextrin cavity (C6–OH). It is concluded that the part of the sulfamethizole molecule that is included in the cyclodextrin is the thiadiazole moiety.

Increasing BCD concentration caused upfield shifts in the NMR signals of the cyclodextrin protons inside the cavity (H3 and H5/H6), especially for H5/H6 (Fig. 3 and Table 1). This suggests that the molecule of sulfamethizole
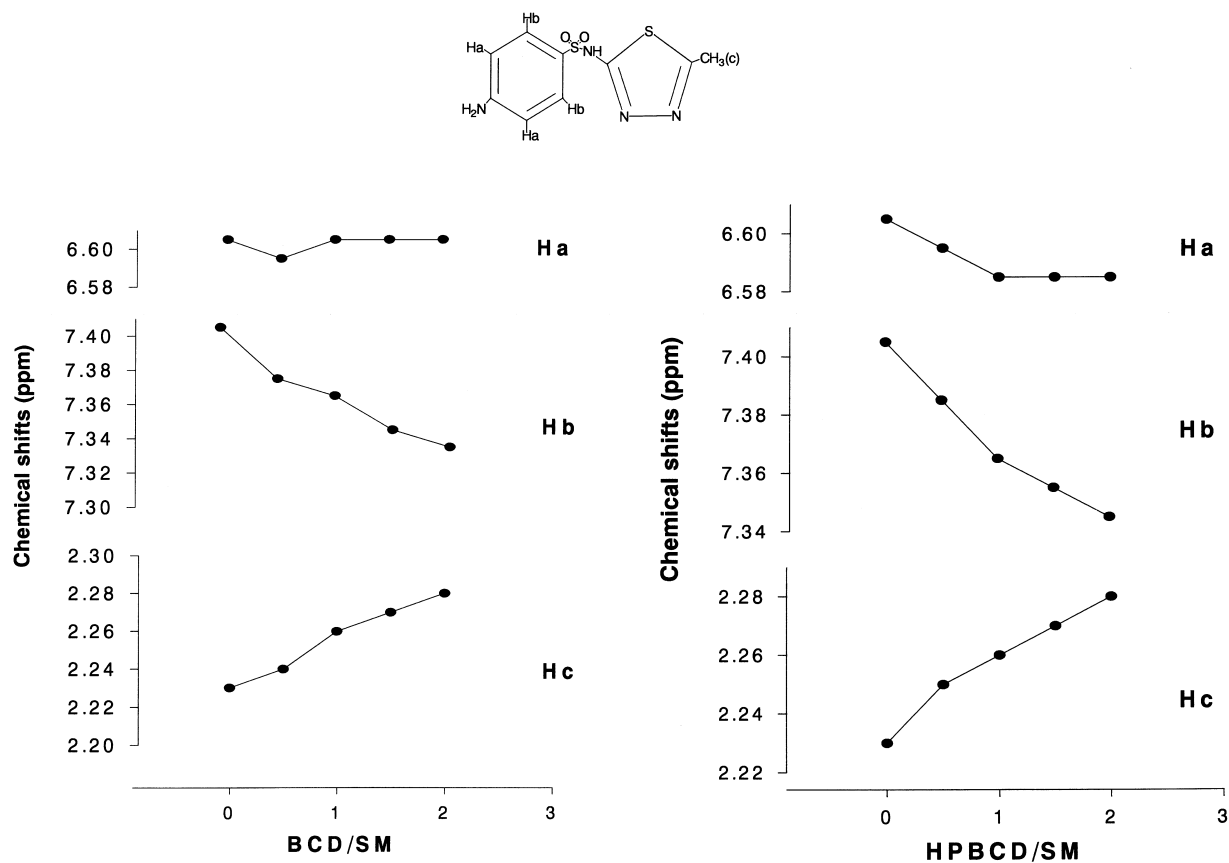

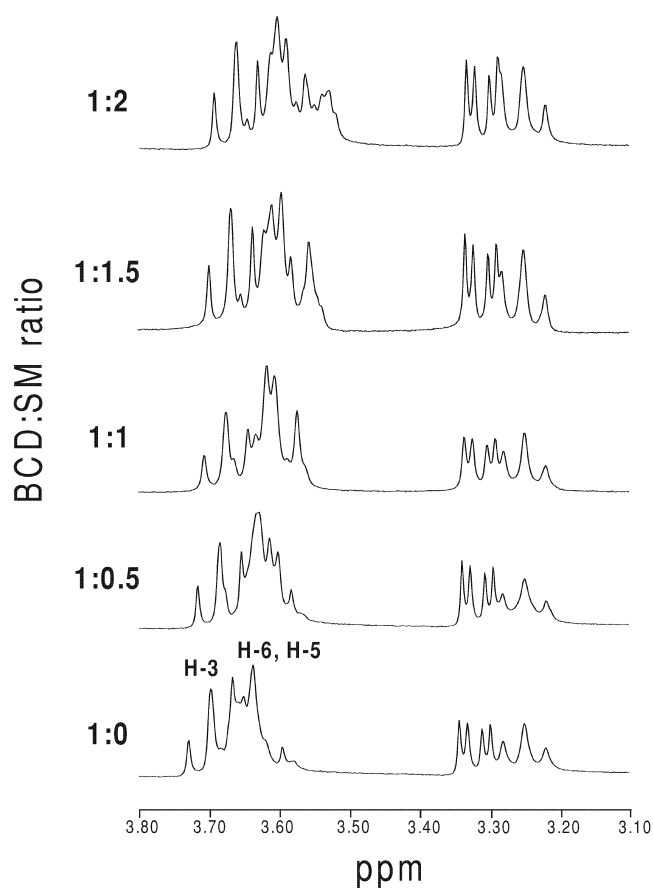Fig. 2. Chemical shifts of sulfamethizole protons plotted against cyclodextrin:sulfamethizole mole ratio.

Fig. 3. Partial ^{1}H NMR spectra of mixtures of sulfamethizole and BCD in different mole ratios.

Fig. 4. Job plots of Hc protons for mixtures of sulfamethizole with BCD or HPBCD. r=Mole fraction of sulfamethizole.

enters the narrow end of the cyclodextrin. No such analysis was possible for HPBCD because it is a mixture of different structures.

To determine the stoicheiometry of the complexes by Job's continuous variation method (Job, 1928), equimolar cyclodextrin and sulfamethizole solutions were mixed in various proportions while keeping the total molar concentration fixed, and changes in the chemical shift of the sulfamethizole H_c (methyl) protons were recorded. For BCD, the resulting plot of [sulfamethizole]$\times\Delta\delta(H_c)$ against r, the mole fraction of sulfamethizole, (Fig. 4) shows a maximal peak at r=0.5, indicating the formation

of 1:1 complexes; the corresponding plot for HPBCD peaked at r=0.4, indicating 2:3 sulfamethizole–HPBCD complexes. A 2:3 stoicheiometry has previously been reported for inclusion complexes of various steroids with γ-cyclodextrin (Uekama et al., 1982).

3.3. Molecular modelling

Molecular modelling has became an interesting tool to know the host–guest interactions in cyclodextrin technology (Amato et al., 1992a,b; Ganza González et al., 1994; Díaz et al., 1998; Lipkowitz et al., 1998). In this work, molecular modelling was performed to throw further light on the structure of the BCD complexes (no such study is possible for the HPBCD complexes because HPBCD is a mixture of different structures). Table 2 lists the energies of the conformers detected (the conformer labels indicate the sulfamethizole group first entering the cyclodextrin cavity and which of the cyclodextrin orifices it enters). Regardless of the presence of water, in the conformer of lowest energy (Fig. 5) the thiadiazole group was included in the cyclodextrin by entering its narrow end, in agreement with the conclusions of the NMR study. This result contrasts with our previous findings for the salbutamol–BCD complex where the presence of water molecules changes the orientation of the host molecule compared to in vacuo calculations (Estrada et al., 2000).

Table 1
Changes in the chemical shifts of BCD protons in the presence of increasing quantities of sulfamethizole

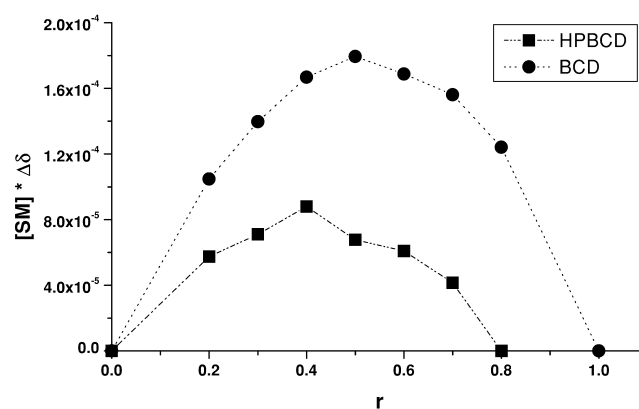| BCD:SM	Changes ($\Delta\delta$) in chemical shifts (ppm)	
	H3	H6, H5
1:0.5	0.0125	0.0221
1:1.0	0.0220	0.0366
1:1.5	0.0395	0.0461
1:2.0	0.0462	0.0738

Table 2
Energies, in the presence and absence of water, of the four possible conformations of sulfamethizole–BCD complexes, as calculated by MM2

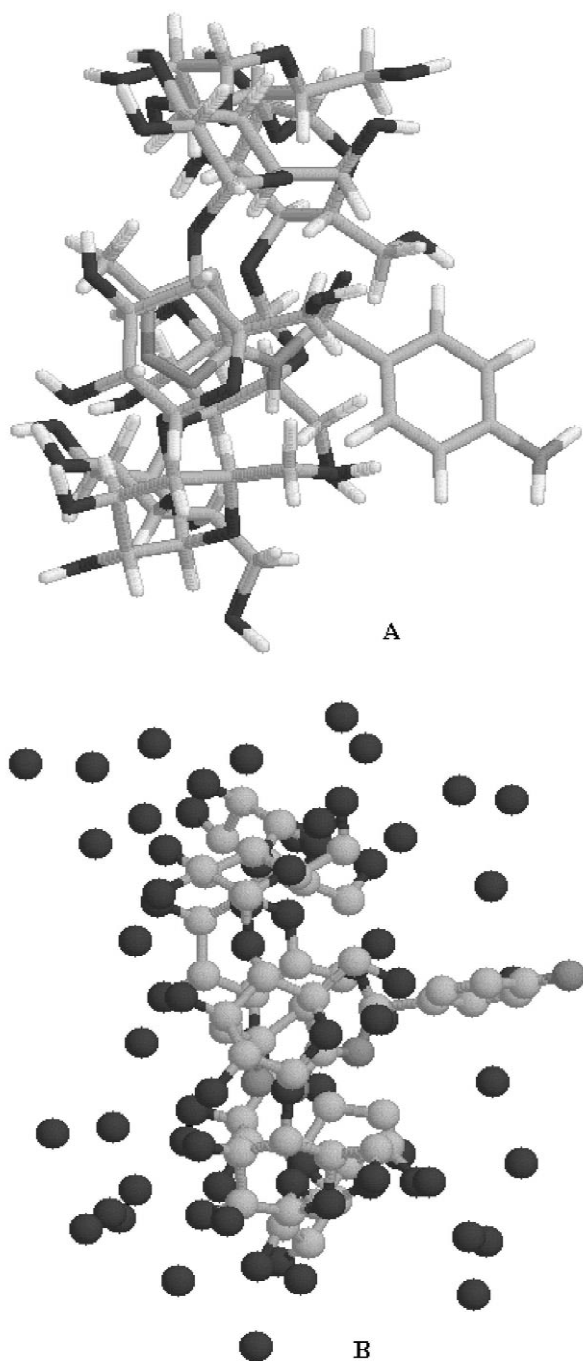| Conformer	MM2 (kcal/mol)	MM2 with water (kcal/mol)
Methyl in broad end	102.469	59.333
Methyl in narrow end	101.057	47.645
Amino in broad end	102.187	64.719
Amino in narrow end	103.656	67.882

Fig. 5. Least energy structures of SM-BCD in vacuo (A) and in the presence of water (B).

3.4. Preparation and characterization of the solid inclusion complexes

Freeze-drying is a common pharmaceutical technique that has proved to be useful for preparing cyclodextrin inclusion complexes of other drugs (Kurozumi et al., 1975). Solid inclusion complexes were prepared in a 1:1 (mol/mol) drug:cyclodextrin ratio as found in the phase solubility diagrams for both CD derivatives. A good

solubilization of sulfamethizole occurs upon the addition of ammonium hydroxide. This additive is used in the preparation of crystalline cyclodextrin complexes. In this work, the endothermic DSC peak at 210°C which is characteristic of sulfamethizole is present in the thermograms of the physical sulfamethizole–cyclodextrin mixtures but not in those of the inclusion complexes (Fig. 6), which confirms the formation of true inclusion complexes. Similarly to HPBCD, the X-ray diffractograms of the complexes consisted fundamentally of a single very broad band, whereas those of physical mixtures of the same compositions corresponded to superimposition of the diffractograms of the individual components (Fig. 7).

3.5. Dissolution of sulfamethizole

Fig. 8 shows the dissolution profiles of sulfamethizole and of its 1:1 physical mixtures as well as its inclusion complexes with BCD and HPBCD, together with the corresponding mean values of the 60-min dissolution efficiency (DE_{60}) (Khan, 1975). The analysis of variance showed that there are significant differences among the formulations ($F_{4,10}=35$, $\alpha<0.05$), which in order of increasing DE_{60} were ranked by the Student–Newman–Keuls test as follows: SM=SM+BCD<SM+HPBCD< SM–BCD complex=SM–HPBCD complex where SM indicates sulfamethizole and the plus sign a physical mixture. The solubility of sulfamethizole was therefore significantly improved by complexation with the cyclo-
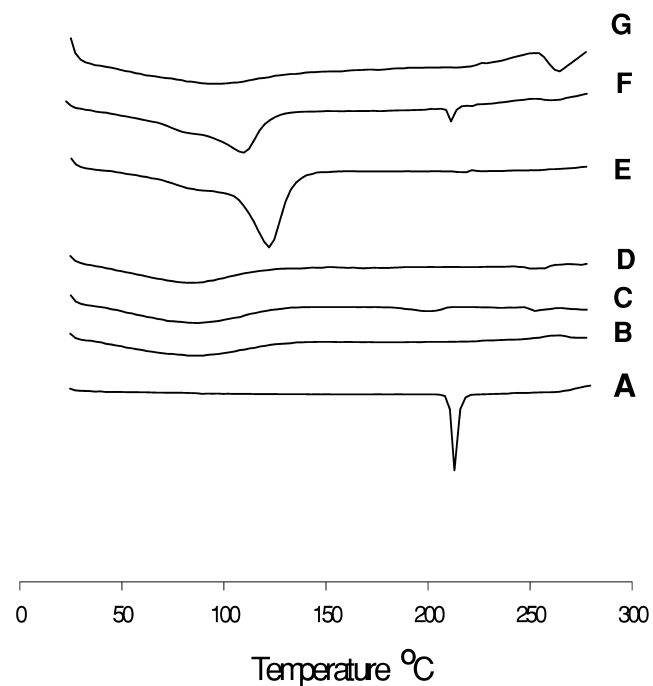

Fig. 6. DSC thermograms of (A) sulfamethizole (SM), (B) HPBCD, (C) SM+HPBCD (physical mixture), (D) lyophilized SM–HPBCD, (E) BCD, (F) SM+BCD (physical mixture), and (G) lyophilized SM–BCD.
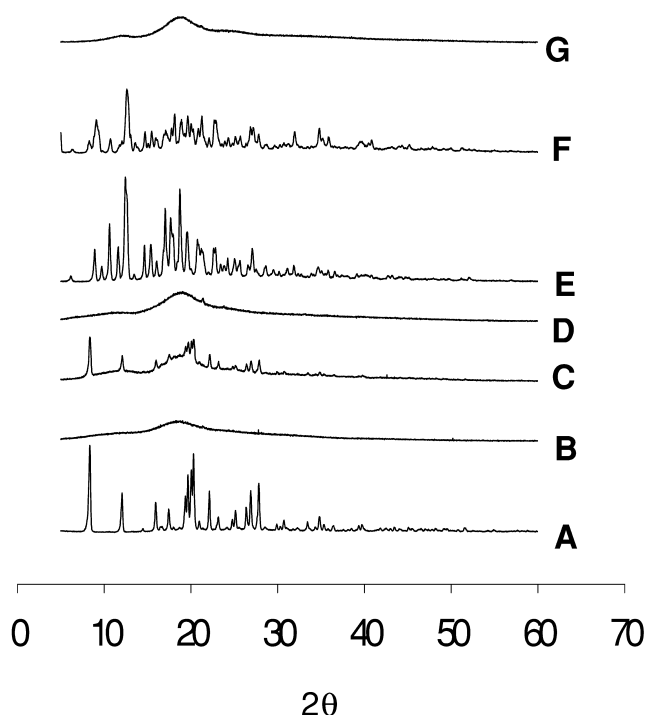
Fig. 7. X-ray diffractograms of (A) sulfamethizole (SM), (B) HPBCD, (C) SM+HPBCD (physical mixture), (D) lyophilized SM–HPBCD, (E) BCD, (F) SM+BCD (physical mixture), and (G) lyophilized SM–BCD.

dextrins, which did not differ significantly from each other in this respect.

In conclusion, lyophilization of solutions of sulfamethizole with β-cyclodextrin and hydroxypropyl-β-cyclodextrin in aqueous ammonium hydroxide solution produces solid-state sulfamethizole–cyclodextrin inclusion complexes, as shown by powder X-ray diffractometry and differential scanning calorimetry. NMR studies show their stoichiometry to be 1:1 for the sulfamethizole–BCD complex and 2:3 for the sulfamethizole–HPBCD complex,

and that in both cases it is the sulfamethizole thiadiazole moiety that is included in the cyclodextrin. Complexation significantly facilitates the dissolution of the drug, without there being any significant differences between the two cyclodextrins in this respect.

Acknowledgements

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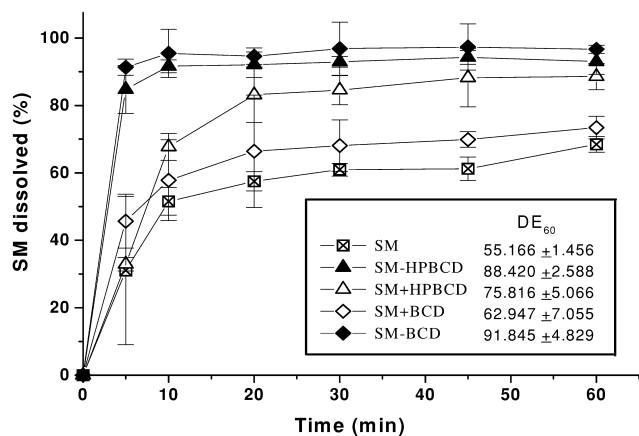

Fig. 8. Dissolution profiles of sulfamethizole (SM) and of its 1:1 physical mixtures (SM+BCD, SM+HPBCD) and its inclusion complexes (SM–BCD, SM–HPBCD) with BCD and HPBCD, together with the corresponding mean values of the 60-min dissolution efficiency (DE_{60}).

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