

# *Binding of Sulfamethazine to $\beta$ -cyclodextrin and Methyl- $\beta$ -cyclodextrin*

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**AAPS PharmSciTech**

An Official Journal of the American Association of Pharmaceutical Scientists

e-ISSN 1530-9932

AAPS PharmSciTech

DOI 10.1208/s12249-013-9958-9



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## Research Article

Binding of Sulfamethazine to  $\beta$ -cyclodextrin and Methyl- $\beta$ -cyclodextrinAriana Zoppi,<sup>1</sup> Alicia Delrivo,<sup>1</sup> Virginia Aiassa,<sup>1</sup> and Marcela Raquel Longhi<sup>1,2</sup>

Received 5 December 2012; accepted 14 March 2013

**Abstract.**  $\beta$ -cyclodextrin ( $\beta$ CD) and methyl- $\beta$ -cyclodextrin (M $\beta$ CD) complexes with sulfamethazine (SMT) were prepared and characterized by different experimental techniques, and the effects of  $\beta$ CD and M $\beta$ CD on drug solubility were assessed via phase-solubility analysis. The phase-solubility diagram for the drug showed an increase in water solubility, with the following affinity constants calculated:  $40.4 \pm 0.4$  (pH 2.0) and  $29.4 \pm 0.4$  (pH 8.0)  $M^{-1}$  with  $\beta$ CD and  $56 \pm 1$  (water),  $39 \pm 3$  (pH 2.0) and  $39 \pm 5$  (pH 8.0)  $M^{-1}$  with M $\beta$ CD. According to  $^1H$  NMR and 2D NMR spectroscopy, the complexation mode involved the aromatic ring of SMT included in the M $\beta$ CD cavity. The complexes obtained in solid state by freeze drying were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy, and thermal analysis. The amorphous complexes obtained in this study may be useful in the preparation of pharmaceutical dosage forms of SMT.

**KEYWORDS:** complexation; cyclodextrin; infrared spectroscopy; thermal analysis.

## INTRODUCTION

The efficacy of drug therapy administered by an extravascular route is influenced by oral drug absorption, which in turn depends on physicochemical, physiological, and dosage form variables (1). SMT ( $pK_1=7.4$  and  $pK_2=2.6$ ; Fig. 1a) (2,3), a short-acting sulfonamide, is frequently administered as a “triple sulfa drug” formulation with sulfadiazine (SDZ) and sulfamerazine (SMR). Sulfonamides are synthetic agents classified as antibacterial compounds, which exerts their bacteriostatic activity by competition with *p*-aminobenzoic acid that is involved in the enzymatic synthesis of dihydrofolic acid. These synthetic compounds play an important role as effective chemotherapeutics of bacterial and protozoal diseases and also exhibit growth-promoting properties in veterinary medicine. These drugs still exhibit a wide interest in veterinary medicine because of their broad-spectrum of activity and low cost (4–9).

However, sulfonamides present unfavorable physicochemical properties such as low water solubility, which is a major concern for its therapeutic use and still needs to be investigated. Their therapeutic effects can be influenced by the composition of the formulation, for example cyclodextrins (CDs; Fig. 1b) are useful functional excipients employed in the pharmaceutical industry as complexing agents in order to increase the aqueous solubility of poorly soluble drugs and to improve their bioavailability (10–13).

In previous studies, we evaluated the ability of CDs to increase the solubility of different sulfonamides, including

SMT, SDZ, and SMR (14–19). The affinity and three-dimensional structure of these complexes were determined by experimental and molecular modeling techniques. However, a detailed description of SMT/CD complex, justifying their inclusion in pharmaceutical formulations of triple sulfa drugs, remains uninvestigated. Therefore, the main purpose of this study was to further investigate the behavior of this complex in aqueous solution and solid state, taking into account the effects of pH and complexation with the  $\beta$ CD derivative M $\beta$ CD. In this way, the influence of the ionization state of the drug and the substitution of the native CD in the solubility of the drug was assessed. It is also important to note that Sourbaji *et al.* (20) has shown that the preparation of SMT/ $\beta$ CD complex, in solid state, applying the kneading method was unsuccessful on the basis of DSC studies. Therefore, it is necessary to study a different preparation method such as freeze-drying method for this system to obtain a real inclusion complex in solid state.

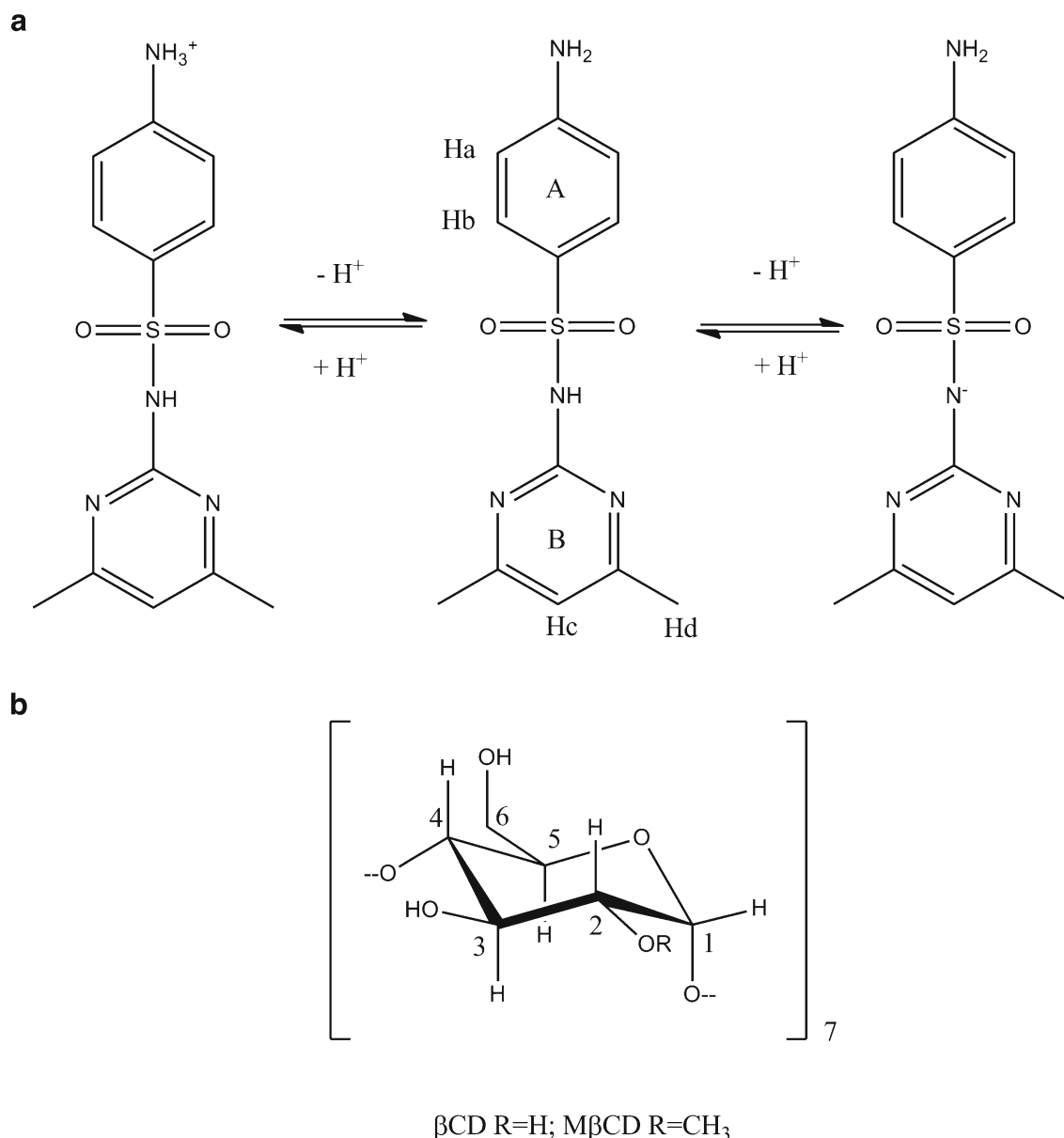
## MATERIALS AND METHODS

## Materials

Sulfamethazine sodium was procured from Parafarm (Argentina), and the SMT base was obtained by neutralization with aqueous HCl solution and subsequent recrystallization from ethanol, m.p. 198°C. The D<sub>2</sub>O 99.9 at.% D used in NMR studies was purchased from Sigma, while  $\beta$ CD (MW=1,135) and M $\beta$ CD (MW=1,190) were kindly supplied by Roquette (France). All other materials and solvents were of analytical reagent grade. A Milli-Q Water Purification System (Millipore, Billerica, MA) generated the water used in these studies. In addition, the H<sub>2</sub>KPO<sub>4</sub>/HNa<sub>2</sub>PO<sub>4</sub> at pH 2.0 and H<sub>2</sub>KPO<sub>4</sub> at

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**Fig. 1.** Chemical structure and proton atom numbering scheme of: **a** SMT (in the protonated, neutral, and anionic forms) and **b**  $\beta\text{CD}$  and  $\text{M}\beta\text{CD}$

pH 8.0 buffers were used (pH-meter HI 255, HANNA Instrument, Romania), with their ionic strengths being adjusted to 0.5 by addition of NaCl and KCl, respectively.

### Phase Solubility Studies

The effects of  $\beta\text{CD}$  and  $\text{M}\beta\text{CD}$  on the solubility of SMT were studied as follows: an excess of SMT was added to aqueous buffered solutions of pH 2.0, pH 8.0, or water containing different amounts of  $\beta\text{CD}$  (0–13.2 mM) or  $\text{M}\beta\text{CD}$  (0–105 mM for water and 0–84 mM for pH 2.0 and 8.0). The resulting suspensions were sonicated in an ultrasonic bath for 1 h and placed in a 25.0°C ( $\pm 0.1$ ) thermostated water bath (Circulators HAAKE F3-K, Germany) for 72 h. After equilibrium was reached, the suspensions were filtered through a 0.45- $\mu\text{m}$  membrane filter (Millipore) and analyzed by UV–Vis spectrophotometry. Each experiment was repeated at least three times and the results

reported were the mean values. The affinity constant ( $K_C$ ) values for the corresponding SMT/CD complex were calculated from the slope of the phase-solubility diagrams (PSD) and intrinsic solubility ( $S_0$ ) according to Eq. (1):

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

The quantitative determinations of SMT were performed spectrophotometrically (Shimadzu UV-Mini 1240 spectrophotometer) at 262 nm. The stability of the drug was determined in water at 25°C with no drug degradation being found after 72 h of incubation.

### NMR Studies

All experiments were performed on a Bruker Avance II High Resolution Spectrometer equipped with a broad band

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inverse probe and a variable temperature unit (VTU). The spectra were measured at 298 K. The complex was prepared in D<sub>2</sub>O and the concentration was 1 mM.

### <sup>1</sup>H NMR

These spectra were obtained at 400.16 MHz, with the chemical shift of the residual solvent at 4.8 ppm used as an internal reference. Induced changes in the <sup>1</sup>H chemical shifts for SMT,  $\beta$ CD, and M $\beta$ CD ( $\Delta\delta$ ) originated due to their complexation were calculated using Eq. 2:

$$\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}} \quad (2)$$

2D ROESY—the geometry of the inclusion complex was studied by two-dimensional rotating frame Overhauser experiments (2D ROESY). The pulse sequence was: roesygpph19; 2D ROESY with cw spinlock for mixing; phase sensitive and water suppression using a 3-9-19 pulse sequence with gradients; p15 (f1 channel), pulse for ROESY spinlock (200,000 $\mu$ s);  $d1/2$  s,  $d19$  (delay for binomial water suppression),  $d19=(1/(2*d))$ ,  $d$ =distance of next null (in hertz) use gradient ratio: gp 1/gp 2 (30:30); for  $z$ -only gradients, gpz1, 30% and gpz2, 30% use gradient files; gpnam1, SINE.100 and gpnam2, SINE.100. Before Fourier transformation, the matrix was zero filled to 4,096 ( $F_2$ ) by 2,048 ( $F_1$ ), and Gaussian apodization functions were applied in both dimensions.

### Preparation of Complexes in Solid State

Solutions of SMT with  $\beta$ CD or M $\beta$ CD (1:1 molar ratio) were prepared in water. The resulting solutions were placed in an ultrasonic bath for 1 h, before being incubated at 25.0°C ( $\pm 0.1$ ) in a thermostatic water bath (Circulators HAAKE F3-K, Germany) for 48 h. Then, the solutions were frozen at  $-40^\circ\text{C}$  and freeze drying was started (Freeze Dri 4.5 Labconco Corp., Kansas City, MI).

### Fourier Transform Infrared Spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy (FT-IR) spectra of SMT and its complexes with  $\beta$ CD and M $\beta$ CD were measured as potassium bromide disks on a Nicolet 5 SXC FT-IR Spectrometer. The FT-IR spectra of complexes were compared with those of the corresponding 1:1 molar ratio physical mixture and also with pure SMT,  $\beta$ CD, and M $\beta$ CD. All

spectra were obtained and processed using EZ OMNIC E.S.P v.5.1 software.

### Scanning Electron Microscopy Studies

Microscopic morphological structures of the raw materials and the binary systems were investigated and photographed using a scanning electron microscope LEO Model EVO 40XVP. The samples were fixed on a brass stub using double-sided aluminium tape and then made electrically conductive by employing gold coating in a vacuum by a sputter coater PELCO Model 3. The magnification selected was sufficient for appreciating in detail the general morphology of the samples under study.

### Differential Scanning Calorimetry and Thermogravimetric Analysis

Differential scanning calorimetry (DSC) measurements of the pure materials and binary systems were carried out using a DSC TA 2920 (TA Instruments, Inc., New Castle, DE). The thermal behavior was studied by heating 1–3 mg of samples in a sealed aluminum pan, from 25°C to 350°C at a rate of 10°C/min, under nitrogen gas flow, using a sealed empty pan as the reference. Indium (99.98%, mp 156.65°C; Aldrich, Milwaukee, WI) was used as standard for calibrating the temperature.

Thermogravimetric analysis (TGA) curves of the different samples were recorded on a TGA 2950 (TA Instruments, Inc.), using the same conditions as in the DSC studies. The TG temperature axes were calibrated with the Curie point of Ni (353°C). In both cases, data were obtained and processed using TA Instruments Universal Analysis 2000 software.

## RESULTS AND DISCUSSION

### Phase Solubility Studies

As discussed above, SMT is an ampholyte that can exist in the protonated, neutral, or anionic forms depending on the pH of the aqueous solutions. Consequently, in order to verify the pH effect on SMT interaction with the CD cavity, the PSD were determined in water and in phosphate buffer solutions (pH 2.0 and 8.0). For all pH values, the increase in solubility occurred as a linear function of CD concentration, corresponding to the A<sub>L</sub>-type profile defined by Higuchi &

**Table I.** Data from the Phase-Solubility Curves of SMT with  $\beta$ CD and M $\beta$ CD at Different pH Values

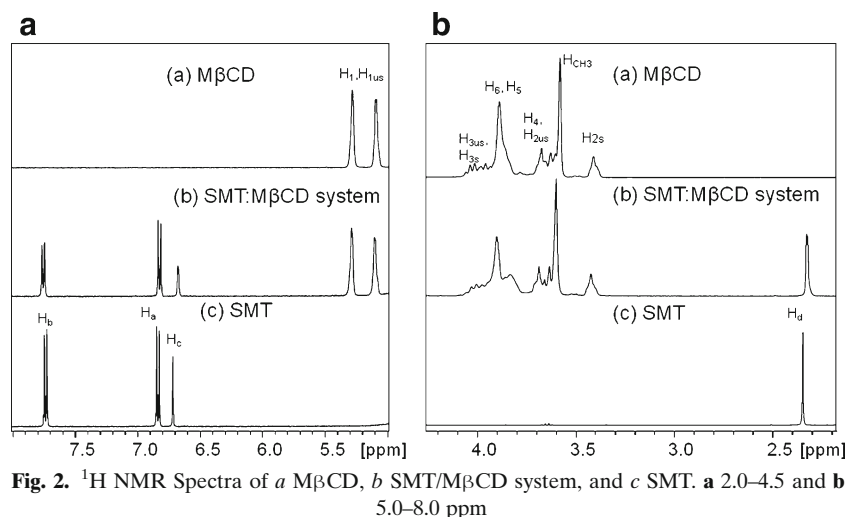
Solvent	CD	$K_C$ (M <sup>-1</sup> )	Final pH	$S_0$ (mg/ml)	$S_{\text{max}}$ (mg/ml)	ES
Water <sup>a</sup>	$\beta$ CD	101 $\pm$ 4	6.6	0.42 $\pm$ 0.02	0.93 $\pm$ 0.03	2
Buf. pH 2		40.4 $\pm$ 0.4	2.1	1.1 $\pm$ 0.2	1.63 $\pm$ 0.02 <sup>b</sup>	1.5
Buf. pH 8		29.4 $\pm$ 0.4	8.4	1.93 $\pm$ 0.06	2.6 $\pm$ 0.1 <sup>b</sup>	1.3
Water	M $\beta$ CD	56 $\pm$ 1	6.4	0.42 $\pm$ 0.02	7.7 $\pm$ 0.1 <sup>c</sup>	18
Buf. pH 2		39 $\pm$ 3	2.2	1.1 $\pm$ 0.2	4.4 $\pm$ 0.1 <sup>d</sup>	4
Buf. pH 8		39 $\pm$ 5	8.4	1.93 $\pm$ 0.06	3.5 $\pm$ 0.5 <sup>d</sup>	2

<sup>a</sup> Zoppi *et al.* (15)

<sup>b</sup> SMT solubility in  $\beta$ CD (13.2 mM) solutions

<sup>c</sup> SMT solubility in M $\beta$ CD (105 mM) solutions

<sup>d</sup> SMT solubility in M $\beta$ CD (85 mM) solutions



**Fig. 2.**  $^1\text{H}$  NMR Spectra of *a* M $\beta$ CD, *b* SMT/M $\beta$ CD system, and *c* SMT. **a** 2.0–4.5 and **b** 5.0–8.0 ppm

Connors (21) where slope values less than unity indicate the occurrence of soluble complexes of 1:1 mol/mol stoichiometries. The corresponding  $K_C$ ,  $S_0$ , maximum solubility ( $S_{\max}$ ) and efficiency of solubility ( $S_{\max}/S_0$ ) values were calculated from each PSD and are presented in Table I.

In water, the affinity constant of the M $\beta$ CD complex was lower than that of  $\beta$ CD. This lower affinity may be due to the absence of a hydrogen bond interaction between the guest molecule and the substituted OH moiety in the M $\beta$ CD, or alternatively to a steric hindrance effected by the methyl groups in the substituted CD which in turn impedes the inclusion of the guest molecule. Although SMT/M $\beta$ CD systems presented low  $K_C$  values compared with those containing  $\beta$ CD (except at pH 8.0), the overall increase in the solubility of the drug was higher because it was possible to add larger amounts of M $\beta$ CD due to its higher aqueous solubility compared with  $\beta$ CD. On analyzing the effects of pH, it can be observed that the values of  $K_C$  for the complexes with both CDs were lower when the drug was ionized, which is not favorable for complex formation. Finally, in the case of the complexes with  $\beta$ CD, the  $S_{\max}$  was higher in aqueous buffered solutions, because of the combined effect of the complex formation and the existence of the drug as an ionized species. However, for the complexes with M $\beta$ CD, the higher value of  $S_{\max}$  was obtained in water because it was possible to add larger amounts of M $\beta$ CD than in buffer solution.

## NMR Studies

### $^1\text{H}$ NMR Experiments

Figure 2 shows the SMT and M $\beta$ CD spectra obtained before and after complexation, from which the corresponding

**Table II.** Chemical Shifts for the Protons of SMT in the Free and Complex Forms

Proton	SMT (ppm)	SMT/M $\beta$ CD (ppm)	$\Delta\delta$ (ppm)
H <sub>a</sub>	6.8380	6.8263	-0.0117
H <sub>b</sub>	7.7374	7.7575	0.0201
H <sub>c</sub>	6.7181	6.6789	-0.0392
H <sub>d</sub>	2.3474	2.3272	-0.0202

chemical shifts ( $\delta$ ) and chemical shift displacements ( $\Delta\delta$ ) were determined (Tables II and III). The protons lying in the interior of the M $\beta$ CD hydrophobic cavity ( $H_3$  and  $H_5$ ) exhibited displacements in their  $\delta$  values in the presence of the guest molecule, suggesting the formation of an inclusion complex. Both protons showed a marked shielding effect, indicative of the inclusion of an electron rich moiety. In addition, all drug protons suffered marked displacements, with the protons of the methyl group showing broader peaks in the spectrum of the complex than in the free drug (Fig. 2). This behavior was probably associated with a decrease in the mobility of a moiety of this drug, caused by the complex formation with M $\beta$ CD.

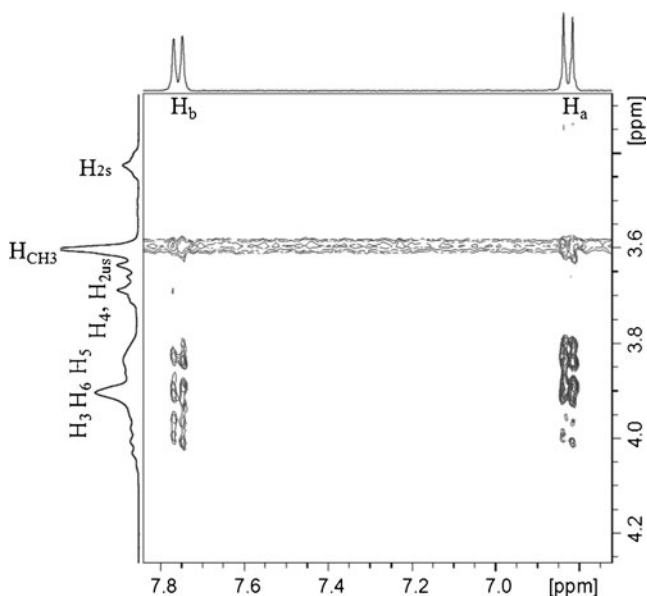
### 2D ROESY Experiments

This experiment was carried out to further investigate the inclusion of SMT into the M $\beta$ CD cavity and to determine the mode of interaction. Figure 3 shows partial contour plots of 2D ROESY spectra for the SMT/M $\beta$ CD complex. Intermolecular cross-peaks can be seen between H<sub>a</sub> and H<sub>b</sub> protons of guest molecules and H<sub>5</sub> and H<sub>6</sub> protons of M $\beta$ CD, again revealing the formation of an inclusion complex and suggesting that the ring A of SMT was inserted into the M $\beta$ CD hydrophobic cavity. These studies show that the inclusion mode of the complex with M $\beta$ CD was equivalent to the complex obtained with  $\beta$ CD, as was previously reported (15).

**Table III.** Chemical Shifts for the Protons of M $\beta$ CD in the Free and Complex Forms

Proton	SMT (ppm)	SMT/M $\beta$ CD (ppm)	$\Delta\delta$ (ppm)
H <sub>1s</sub>	5.2815	5.2874	0.0059
H <sub>1us</sub>	5.0880	5.1049	0.0169
H <sub>3us</sub>	4.0369	4.0054	-0.0315
H <sub>3s</sub>	3.9591	4.0054	0.0463
H <sub>4</sub> -H <sub>2us</sub>	Overlapped		
H <sub>6</sub>	3.8898	3.9026	0.0128
H <sub>5</sub>	3.7849	3.8325	0.0476
H <sub>4</sub>	3.6280	3.6593	0.0313
H <sub>CH3</sub>	3.5806	3.6002	0.0196
H <sub>2s</sub>	3.4111	3.4239	0.0128

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**Fig. 3.** Partial contour plot of the 2D ROESY spectrum of the SMT/ $M\beta$ CD system

## Fourier Transform Infrared Spectroscopy

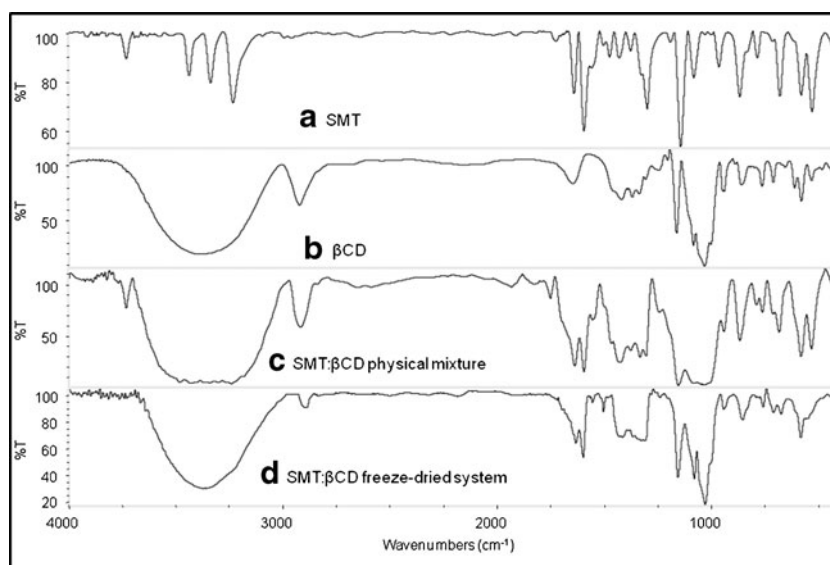
It has been vastly reported that the variation of the shape, shift, and intensity of the FT-IR absorption bands of the guest or host molecule can provide enough information for the occurrence of complexation (22). The infrared spectra of the binary systems taken in the region of 4,000–400  $\text{cm}^{-1}$  were compared with that of the free drug (Figs. 4 and 5). In the SMT spectrum, the bands that appear between 3,500 and 3,400  $\text{cm}^{-1}$  are due to asym ( $\text{NH}_2$ ) and sym ( $\text{NH}_2$ ) vibrations of the amino ( $\text{NH}_2$ ) group of the drug. The band for the sulfonamidic ( $\text{N-H}$ ) group is observed around 3,125  $\text{cm}^{-1}$ . On the other hand, the spectral region from 4,000 to 3,000  $\text{cm}^{-1}$  is

difficult to analyze for  $\beta$ CD and its complexes due to primary and secondary  $-\text{OH}$  groups of  $\beta$ CD and water molecules of crystallization (23). For this reason only the SMT bands between 1,640 and 900  $\text{cm}^{-1}$  were used to depict the inclusion complex disposition. Spectra of all binary systems did not show new peaks indicating that no chemical bonds were created in the formed compounds. The spectra for the physical mixtures (Figs. 4c and 5c) consisted of an overlay of the pure compounds spectra. On the other hand, the FT-IR spectra of the complexes prepared by the freeze-drying method (Figs. 4d and 5d) showed appreciable shifts and reduction in intensity of the characteristic SMT bands, evidencing the presence of more or less intense solid-state interactions between the components. The scissoring vibrations for the amino ( $-\text{NH}_2$ ) groups that appear at 1,640  $\text{cm}^{-1}$  in the free drug spectra was shifted to a lower frequency (1,630  $\text{cm}^{-1}$ ) in the complexes prepared by freeze-drying method. Furthermore, a reduction of the intensities of the bands at 1,330  $\text{cm}^{-1}$  (assigned to  $\text{SO}_2$  asymmetric stretching) and at 967  $\text{cm}^{-1}$  (assigned to  $\text{S-N}$  stretching) was observed for these systems, probably owing to a restriction of the vibration related to the complexation process. These changes in the characteristic bands of pure drug confirm the existence of the inclusion complexes as new compounds with different spectroscopic bands. It should be noted that these results are in agreement with the inclusion mode previously reported in the 2D ROESY experiments.

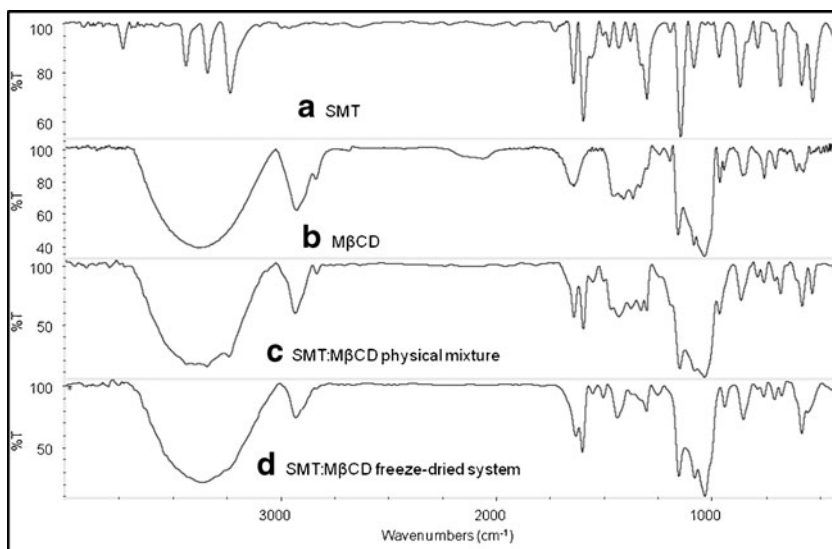
## Scanning Electron Microscopy Studies

Scanning electron microscopy (SEM) is used to study the microscopic aspects of the raw materials and their corresponding treated samples. Although this study is inadequate to affirm inclusion complexation, it helps to assess the existence of a single component in the preparations obtained (24).

Figure 6 shows the SEM images of SMT,  $\beta$ CD,  $M\beta$ CD alone, and the binary systems prepared by physical mixture and freeze-drying methods. It can be seen that SMT is present



**Fig. 4.** IR spectra of a) SMT, b)  $\beta$ CD, c) SMT/ $\beta$ CD physical mixture system, and d) SMT/ $\beta$ CD freeze-drying system

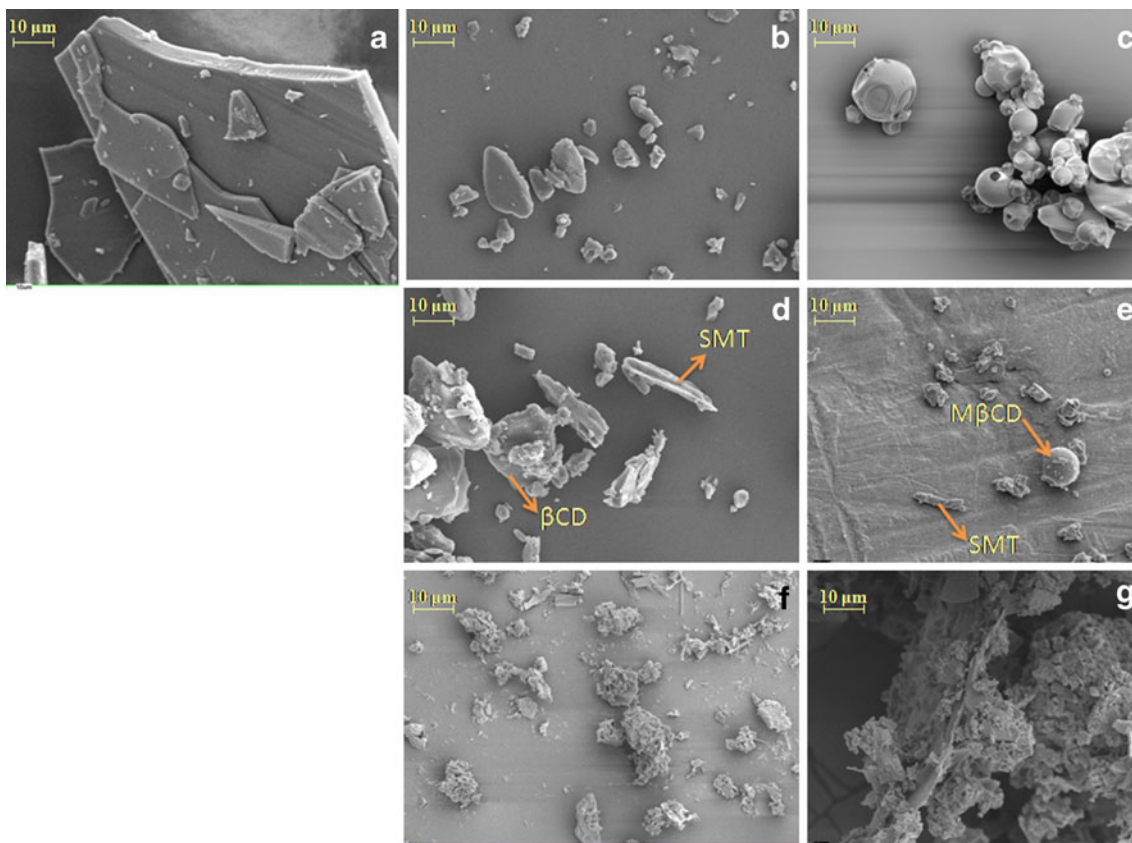


**Fig. 5.** IR spectra of *a* SMT, *b* MβCD, *c* SMT/MβCD physical mixture system, and *d* SMT/MβCD freeze-drying system

as crystals of irregular shapes and sizes, while βCD exhibits a parallelogram shape and MβCD is composed of spherical particles with amorphous character.

SEM images of the physical mixtures revealed the characteristic SMT crystals, which were mixed with CDs particles, thus confirming the presence of crystalline drug, where residual CDs particles were easily identified.

Finally the morphology of the particles of SMT/βCD and SMT/MβCD system prepared by freeze-drying presents significant changes in their shape and aspect, which is indicative of the presence of a new solid phase. These finding may be simply a consequence of a crystalline habitus change in the systems or may support the evidence of the presence of a new solid complex.



**Fig. 6.** Scanning electron microphotographs of *a* SMT, *b* βCD, *c* MβCD, *d* SMT/βCD physical mixture system, *e* SMT/MβCD physical mixture system, *f* SMT/βCD freeze-drying system, and *g* SMT/MβCD freeze-drying system



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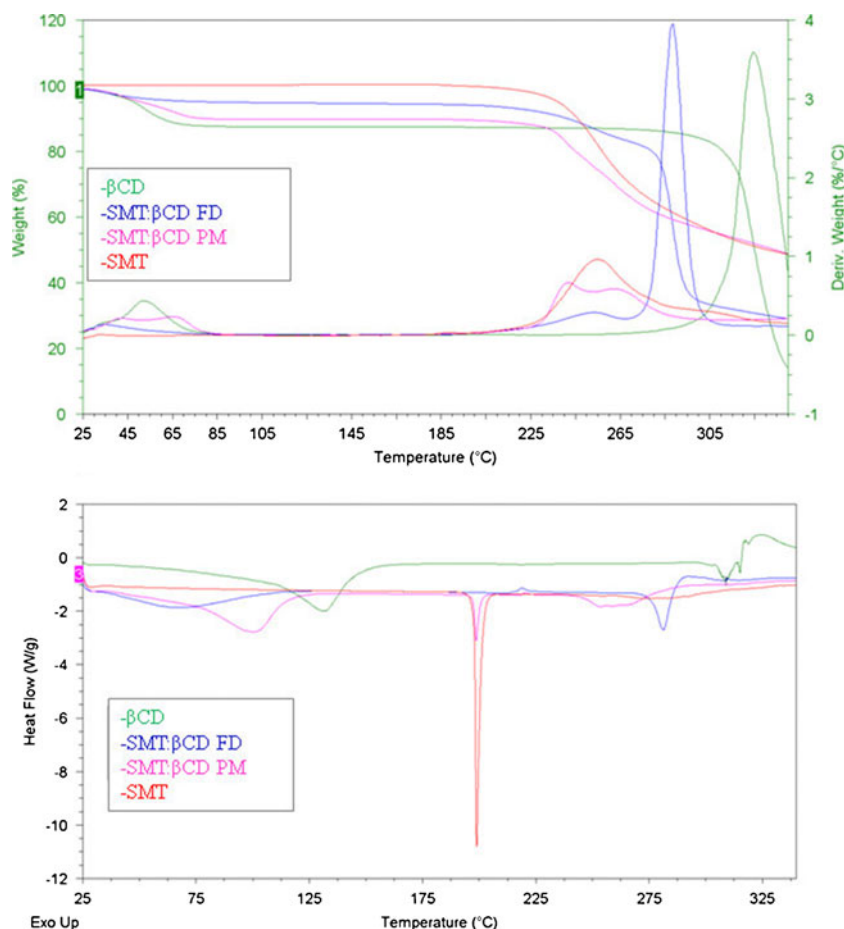


Fig. 7. DSC and TG curves of  $\beta$ CD (green), SMT (red), SMT/ $\beta$ CD physical mixture system (SMT/ $\beta$ CD PM; pink), and SMT/ $\beta$ CD freeze-drying system (SMT/ $\beta$ CD FD; blue)

### Differential Scanning Calorimetry and Thermogravimetric Analysis

Several thermoanalytical techniques are often used for studying complexation, being TGA and DSC as the most commonly used techniques (13). The DSC and TG curves of pure components and binary systems with  $\beta$ CD and M $\beta$ CD are shown in Figs. 7 and 8, respectively. The thermal curve of SMT was typical of a crystalline anhydrous substance, with a sharp fusion endothermic event occurring at 198°C corresponding to the melting point of the drug, followed by an exothermal event attributable to its thermal decomposition, as can be observed by comparison with the TG curve ( $\Delta m=52\%$ ). Both  $\beta$ CD and M $\beta$ CD lost water at temperatures between 25°C and 100°C ( $\Delta m=11\%$  and 7%, respectively), with decomposition taking place above 300°C as evidenced by the mass loss observed in the TG curves ( $\Delta m=77\%$  and 83%, respectively).

The curves of the physical mixtures of SMT/ $\beta$ CD and SMT/M $\beta$ CD presents two events, the first of which corresponds to the dehydration band of CD ( $\Delta m=9\%$  and 3%, respectively), and the second one corresponding to the melting point of SMT, which is reduced and to some extent displaced in the case of M $\beta$ CD system at (198°C and 193°C, respectively). The peak reduction may be explained by the small SMT content, but its displacement is supposed to be caused by an interaction with the CD during the heating

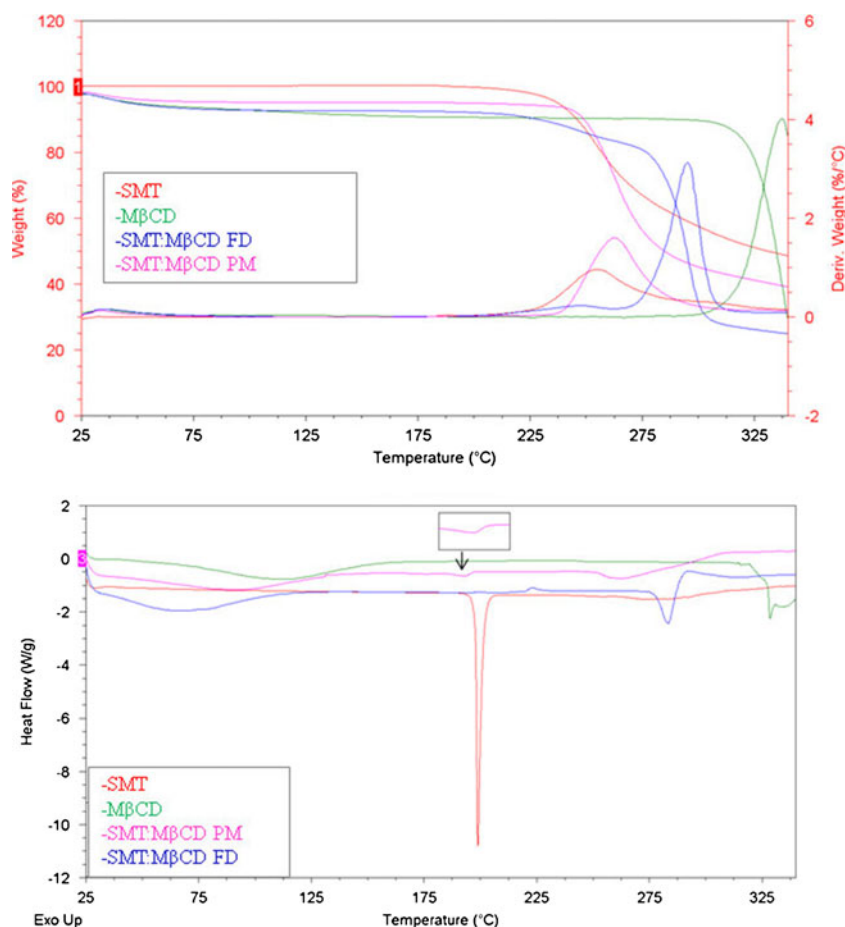
phase. Finally, decomposition takes place above 200°C ( $\Delta m=42\%$  and 56%, respectively).

Regarding the results obtained for the systems prepared by freeze-drying method, the DSC curve shows that the endothermic peak of pure SMT at 198°C is not present, indicating that the melting event did not take place, which might be due to changes in the crystalline form of the solid or to the formation of an inclusion complex. Furthermore, the thermogravimetric profile shows an initial loss of weight corresponding to the dehydration of the complexes ( $\Delta m=4\%$  and 5%, respectively), followed by a decomposition phase that starts above 200°C ( $\Delta m=66\%$  and 67%, respectively).

Taking into account the results obtained by the different techniques in solid state, it is possible to confirm the formation of inclusion complexes between SMT and both CDs when the systems were prepared by applying a freeze-drying method. The amorphous nature of both samples was also confirmed by means of the previously discussed techniques.

### CONCLUSIONS

The freeze-drying method employed in this work to prepare the system in solid state resulted in real inclusion of the drug in the CD cavity proved by IR and DSC studies. Simultaneous analysis of complexation behavior using different



**Fig. 8.** DSC and TG curves of M $\beta$ CD (green), SMT (red), SMT/M $\beta$ CD physical mixture system (SMT/M $\beta$ CD PM; pink) SMT/M $\beta$ CD freeze-drying system (SMT/M $\beta$ CD FD; blue)

methods obviously has a lot of advantages. Indeed, analogies found among the SMT/ $\beta$ CD and SMT/M $\beta$ CD interactions in aqueous solution and in the solid state suggest similar patterns of complexation mechanisms and inclusion modes of the guest molecule in the host cavity. The solubility of SMT was improved by inclusion complexation with CDs. This satisfactory behavior of the SMT/CD complex is potentially useful for its future application in pharmaceutical dosage formulations. Previous studies in our group (14,15) have demonstrated that CDs can form complexes with SDZ and SMR, which are frequently administered as a “triple sulfa drug” with SMT. Therefore, this might have relevant applications in the preparation of an oral dosage product containing complex forms of these three drugs, which could also lead to better bioavailability.

#### ACKNOWLEDGMENTS

The authors thank the Fondo para la Investigación Científica y Tecnológica (FONCYT) Préstamo BID PICT 1376, the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT), and the Consejo Nacional de Investigaciones Científicas y Tecnológicas de la Nación (CONICET) for financial support. We also thank Ferromet S.A. (agent of Roquette in Argentina) for its donation of  $\beta$ CD

and M $\beta$ CD. We are grateful to Dr. Gloria Bonetto for NMR measurements and for her helpful discussion of the  $^1\text{H}$  NMR spectra, and to Dr. Paul Hobson, native English speaker, for revision of the manuscript.

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