PHARMACEUTICAL TECHNOLOGY

Complexation of Sulfonamides With β-Cyclodextrin Studied by Experimental and Theoretical Methods

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Received 20 August 2009; revised 10 November 2009; accepted 17 November 2009

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22062

ABSTRACT: The complex formation between three structurally related sulfonamides (sulfadiazine (SDZ), sulfamerazine (SMR), and sulfamethazine (SMT)) and β-cyclodextrin (β-CD) was studied, by exploring its structure affinity relationship. In all the cases, 1:1 stoichiometries were determined with different relative affinities found by phase solubility (SDZ:β-CD > SMR:β-CD > SMT:β-CD) and nuclear magnetic resonance (NMR) (SMT:β-CD > SMR:β-CD > SDZ:β-CD) studies. The spatial configurations determined by NMR were in agreement with those obtained by molecular modeling, showing that SDZ included its aniline ring into β-CD, while SMR and SMT included the substituted pyrimidine ring. Energetic analyses demonstrated that hydrophobicity is the main driving force to complex formation. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3166–3176, 2010

Keywords: sulfonamides; solubility; cyclodextrins; complexation; phase solubility studies; NMR spectroscopy; molecular modeling

INTRODUCTION

Sulfonamides comprise a large family of compounds described first in 1935 to exhibit antibacterial activity and being clinically used since 1968.[1] From then on, several types of additional pharmacological applications have been reported, including antitumor, anticarbonic anhydrase, diuretic, hypoglycemic, antithyroid, or protease inhibitory activity,[2] sulfonamides constituting a family of drugs widely used in human and animal medicine. Although the therapeutic potential of such compounds is fully described, suboptimal physicochemical properties, such as low water solubility, represent an important concern as to their therapeutic use which still needs to be further addressed. Sulfadiazine, sulfamerazine, and sulfamethazine (SDZ, SMR, and SMT, respectively, Fig. 1) exemplify the widespread use of sulfonamide drugs, frequently administered as a "triple sulfa drug" formulation.[3–5] These three molecules are structurally closely related, with an aniline ring (ring A, Fig. 1) bond through a sulfonamide moiety to a substituted pyrimidine ring (ring B, Fig. 1).

A strategy frequently adopted to enhance drug solubility constitutes the complexation with cyclodextrins (CDs).[6,7] β-cyclodextrin (β-CD, Fig. 2), formed by seven units of glucopyranoses with α-1,4 bonds, being the most widely used CD in the pharmaceutical field. It exhibits a truncated-cone shape with a hydrophobic inner and a hydrophilic outer surface, capable of including molecules to form inclusion complexes, and enhancing properties such as solubility, bioavailability and stability. Therefore, previous reports demonstrated that the formation of complex between several sulfonamides and CDs exhibited improved aqueous solubility compared to that of the free drugs,[8–11] and thus may have implication on its therapeutic applicability.

In this work, the complex formation between SDZ, SMR, and SMT with β-CD was studied in detail with the objective of understanding the basis of their structure–affinity relationship. Hence, the affinity and three-dimensional structures of the corresponding complexes were determined by experimental techniques such as phase solubility studies and nuclear magnetic resonance. In addition, molecular modeling techniques, including molecular docking and molecular dynamics, were applied and correlated with experimental results.

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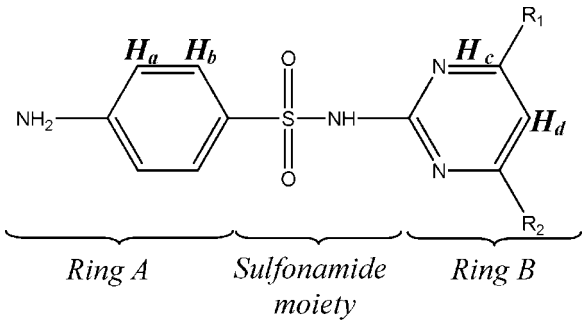
Journal of Pharmaceutical Sciences, Vol. 99, 3166–3176 (2010)



SDZ: R1, R2 = H

SMR: R1=H R2= CH_3

SMT: R1, R2= CH_3

Figure 1. Chemical structure of sulfadiazine (SDZ), sulfamerazine (SMR), and sulfamethazine (SMT).

MATERIALS AND METHODS

Materials

Pure sulfadiazine and sulfamerazine and sulfamethazine sodium salt were obtained from Parafarm (Argentina), while sulfamethazine base was obtained from neutralization with HCl and subsequent recrystalization. D_2O 99.9 at.% D, used in spectroscopic studies was purchased from Sigma®, while β-CD ($M_W = 1135$) was kindly supplied by Roquette (Lestrem, France). All other materials and solvents were of analytical reagent grade. A Milli-Q Water Purification System (Millipore®) (Bedford, Massachusetts) generated the water used in solubility studies.

Solubility Studies

The effects of β-CD on the solubility of each sulfonamide were studied as follows: an excess of each sulfonamide was added to water containing different amounts of β-CD (0–13.2 mM). The resulting suspensions were sonicated in an ultrasonic bath for 1 h and placed in a 25.0 ± 0.1°C thermostatized water bath for 72 h. After equilibrium was reached, the suspensions were filtered through a 0.45 μm 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 K_c values for the corresponding guest:β-CD complex, were calculated from the slope of the phase-solubility diagrams and S_0 according to Eq. (1):[12]

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

The quantitative determinations of each sulfonamide were performed spectrophotometrically (Shimadzu UV-Mini 1240 spectrophotometer) at 264, 263, and 262 nm for SDZ, SMR, and SMT, respectively. The stability of the three studied sulfonamides was determined in water at 25°C, no drug degradation was found after 72 h of incubation.

NMR Studies

All experiments were performed on a Bruker Advance II High Resolution Spectrometer, equipped with a Broad Band Inverse probe (BBI) and a Variable Temperature Unit (VTU). The spectra were measured at 298 K and obtained at 400.16 MHz, with the chemical shift of the residual solvent at 4.8 ppm used as an internal reference. $\Delta\delta$ in the ^1H chemical shifts for β-CD and each sulfonamide originated due to their complexation were calculated using the following equation:

$$\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}}$$

The K_c of the inclusion complex in solution was determined by applying Scott's equation (Eq. 2):[13]

$$\frac{[\beta - CD]_t}{\Delta\delta_{obs}} = \frac{[\beta - CD]_t}{\Delta\delta_c} + \frac{1}{K_c \Delta\delta_c} \quad (2)$$

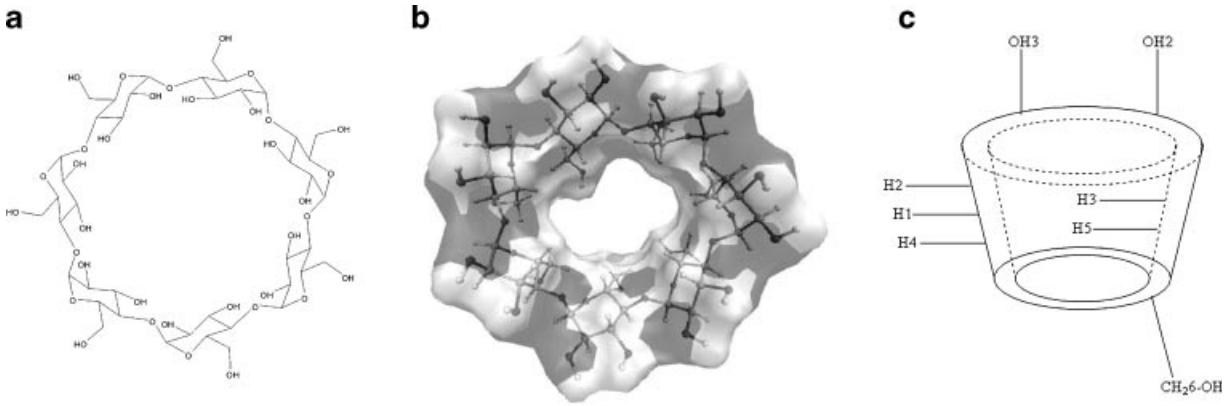


Figure 2. β-cyclodextryn molecule: (a) chemical structure, (b) three-dimensional structure showing the hydrophobic cavity and (c) hydrogen notation used for NMR studies.

where $[\beta\text{-CD}]_t$ is the total β-CD concentration; $\Delta\delta_{obs}$ is the difference between the chemical shift of free drug and the corresponding chemical shift of drug in the presence of β-CD at each concentration; $\Delta\delta_c$ is the difference between the chemical shift of a pure sample of the complex and its free components. By plotting $[\beta\text{-CD}]_t/\Delta\delta_{obs}$ against $[\beta\text{-CD}]_t$, K_c could be calculated from the intercept obtained by a least-squares linear analysis. In this experiment, the concentration of each drug was kept constant at 1 mM, while β-CD concentration was varied from 5 to 10 mM for the D_2O.

2D ROESY: Drug inclusion into the β-CD cavity was studied by two-dimensional rotating frame Overhauser experiments (2D ROESY), with spinlock for mixing phase sensitive, using $180x\ 180 - x$ pulses for polarization transfer. The spectra were measured with a relaxation delay of 2 s, p15 pulse for ROESY spinlock of 20 ms and l4, spinlock loop, $(p15/p25 \times 2) = 400$. Before Fourier transformation, the matrix was zero filled to 4096 (F2) by 2048 (F1) and Gaussian apodization functions were applied in both dimensions. Working concentrations of each drug and β-CD were 1 mM in D_2O.

Molecular Modeling Procedures

Initial structures of SDZ, SMR, and SMT were built using Gabedit software,[14] after which a conformational search was carried out in order to obtain the minimum energy conformation. The systematic search was conducted applying a semiempirical method (AM1), with the final minimum energy conformation being optimized using an *ab initio* (HF-6-31G*) method. Conformational search and optimization procedures were performed using Gaussian98 software,[15] with the results being processed with Molden v.4.7 software.[16] The initial structure of β-CD was obtained from the Cambridge Structural Database (code BCDEXD10).

For the molecular docking procedure, restrained electrostatic potential (RESP) fitted charges were assigned to SDZ, SMR, and SMT, while Glycamm04 force field charges implemented in the Amber9 software package were assigned for β-CD.[17] Autodock v3.05 was used to accomplish the docking runs,[18] by applying a Lamarckian Genetic Algorithm (LGA) to generate the population evaluated, using the following parameters: a population of 150 individuals, a maximum of 250,000,000 evaluations and 5000 generations as an end criterion. An elitism value of 1 was selected, with a probability of mutation and crossing over of 0.02 and 0.8, respectively. Thirty docking runs were assayed, with the lowest energy cluster conformation being selected for subsequent molecular dynamics assays.

The Amber9 software package was used for the molecular dynamics studies.[17] Atomic charges for SDZ, SMR, SMT, and β-CD were identical to those used for the molecular docking procedures described above. The complexes obtained by molecular docking were solvated with a preequilibrated TIP3P octahedric water box, after which an initial minimization of the solvent followed by a minimization of the whole system was carried out. These minimized systems were heated to 300 K for 20 ps, followed by a 100 ps equilibration run. Production runs were performed for 20 ns, using a timestep of 2 fs and under constant pressure and temperature, with the SHAKE algorithm being applied to constrain bonds involving hydrogens.

Hydrogen bonds and energetic components were analyzed using the Ptraj and Molecular Mechanics Poisson-Bolzmann Surface Area (MM-PBSA) modules of Amber9, respectively.[19] MM-PBSA analyses were applied over the entire trajectory (20 ns), with individual snapshots sampled every 10 frames. The corresponding structures were visualized using VMD v.1.8.6 software.[20] Molecular dynamics simulations were computed at the Fimm Cluster resource (Parallab, Bergen Center for Computational Science, University of Bergen, Norway).

RESULTS AND DISCUSSION

Phase-Solubility Analysis

The corresponding phase-solubility diagrams (PSD) of each sulfonamide with β-CD in water are depicted in Figure 3, with the corresponding affinity constant (K_c), intrinsic solubility (S_0) and maximum solubility (S_{max}) values being calculated from each PSD (Tab. 1). As it can be seen, the three sulfonamides formed complexes with β-CD, demonstrated by the marked improvement in their solubility when the

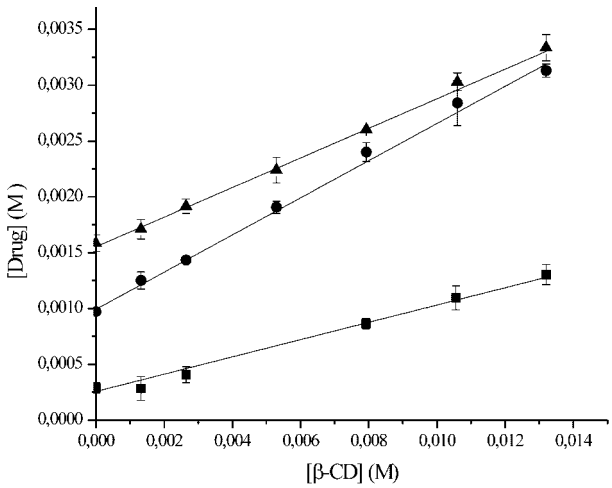


Figure 3. Phase-solubility diagrams of the SDZ:β-CD complex (■), SMR:β-CD complex (●), and SMT:β-CD complex (▲).

 DOI 10.1002/jps

Table 1. Data of the Phase-Solubility Studies of SDZ, SMR and SMT With β-CD

	K_c (M^{-1})	S_0 (mg/mL)	S_{max}[a] (mg/mL)
SDZ	282 ± 15	0.07 ± 0.01	0.33 ± 0.02
SMR	198 ± 22	0.26 ± 0.01	0.83 ± 0.02
SMT	101 ± 4	0.42 ± 0.02	0.93 ± 0.03

[a]Drug solubility in β-CD (13.2 mM) solutions.

macromolecule was added to the solution. In all the cases, A_L type solubility diagrams were obtained, in which a linear increase in solubility as a function of β-CD concentration and a slope value less than unity indicates a 1:1 stoichiometry.[7] Comparing the solubility parameters before (S_0) and after (S_{max}) β-CD addition, it can be seen that solubility increased to 4, 3.2, and 2.2 times for SDZ, SMR, and SMT, respectively, indicating that the complexation with β-CD can be an effective strategy to enhance the aqueous solubility of these drugs.

NMR Studies

Figure 4 shows the β-CD spectra obtained before and after complex formation with the three sulfonamide molecules, from which the corresponding chemical shifts (δ) and chemical shift displacements ($\Delta\delta$) were determined (Tab. 2). In all the cases, protons lying in the interior of the hydrophobic cavity (H_3 and H_5) exhibited displacements in their δ in the presence of the corresponding guest molecule, confirming the formation of an inclusion complex. For the SDZ:β-CD complex, downfield displacements were found for both H_3 and H_5, suggesting the insertion of an electronegative moiety into the β-CD hydrophobic cavity, which produces the deshielding effects observed. For the SMR:β-CD complex, a downfield and an upfield displacement was found for H_3 and for H_5, respectively, which may result from the partial inclusion of an electronegative functional group, with a deshielding effect only on H_3. When the SMT:β-CD complex was analyzed, both protons showed marked upfield displacements, indicative of the inclusion of an electron rich moiety rather than of an electronegative group. In addition, $\Delta\delta$ corresponding to the protons located in the outer surface of β-CD (H_1, H_2, and H_4) were also modified, which may be due to a conformational rearrangement in the host molecule.[21–23]

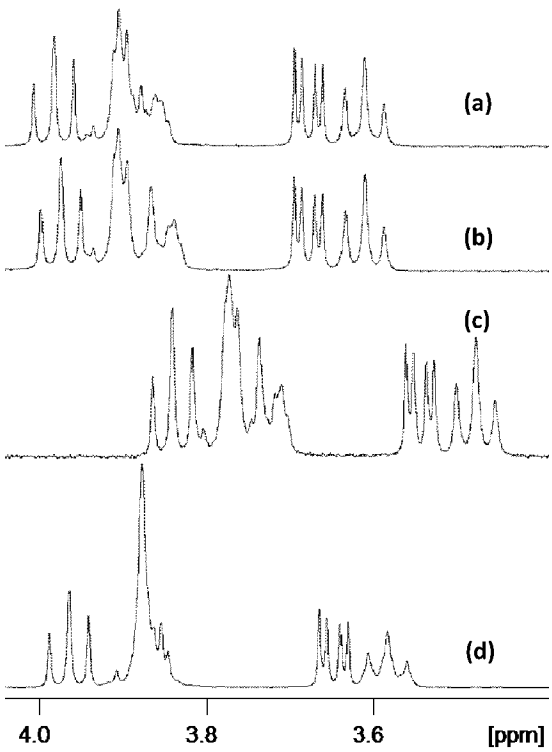


Figure 4. Spectra (a) SDZ:β-CD complex, (b) SMR:β-CD complex, (c) SMT:β-CD complex, (d) β-CD free.

When the $\Delta\delta$ for the three sulfonamide molecules were analyzed (Tab. 3, spectra not shown), it can be seen that almost all proton resonances were modified upon complexation, through which both upfield and downfield displacements were found. For SDZ, downfield displacements were observed for H_b and H_c, probably due to van der Waals interactions between SDZ and β-CD, suggesting the insertion of the central portion of this molecule into the β-CD cavity. For SMR, the most marked displacements corresponded to protons located in ring B (H_c, H_d and H_{CH_3}), with both downfield and upfield displacements suggesting the establishment of van der Waals interactions and the proximity of atoms rich in π electrons such as glycosidic linkage oxygens, respectively, all of which support the inclusion of ring B into the hydrophobic cavity. From the $\Delta\delta$ corresponding to the SMT:β-CD complex, we founded that protons located in ring B as well as those located in ring A exhibited significant upfield displacements, it does not allow the identification of the portion of the molecule inserted into the β-CD cavity.

In order to calculate K_c values for each sulfonamide:β-CD complex, Scott's plots (Fig. 5) were determined on the basis of the $\Delta\delta$ of H_d. The corresponding calculated values were 231, 368, and 826 M^{-1} for SDZ, SMR, and SMT, respectively. When K_c obtained by NMR studies were compared to those determined by PSD, significant differences were found, with PSD diagrams showing an increase in affinity in the order SDZ > SMR > SMT, while NMR studies showed an inverse fashion (SMT > SMR > SDZ). The discrepancies between the K_c values determined by different experimental methods have been reviewed extensively by Loftsson et al.[24] with experimental data being inconclusive so as to unequivocally determine the origin of this phenomena. Probably, the fact that

Table 2. Chemical Shifts for the Protons of β-Cyclodextrin in the Free State and in the Complex

Proton	β-CD Free (ppm)	SDZ:β-CD[a] (ppm)	Δδ (ppm)	SMR:β-CD[b] (ppm)	Δδ (ppm)	SMT:β-CD[c] (ppm)	Δδ (ppm)
H_1	5.0765	5.0965	0.0200	5.0956	0.0191	5.0404	−0.0361
H_2	3.6554	3.6767	0.0203	3.6773	0.0219	3.6200	−0.0354
H_3	3.9729	3.9839	0.0110	3.9747	0.0018	3.9047	−0.0682
H_4	3.5908	3.6102	0.0194	3.6097	0.0189	3.5509	−0.0399
H_5	3.8579	3.8633	0.0054	3.8482	−0.0097	3.7804	−0.0775
H_6	3.8850	3.9002	0.0152	3.9002	0.0152	3.8486	−0.0364

[a] [SDZ]:[β-CD] = 1:1.
[b] [SMR]:[β-CD] = 1:1.
[c] [SMT]:[β-CD] = 1:1.

PSD studies require the use of saturated drug solution in contrast to NMR assays, where the drug under study is completely dissolved, may explain the differences observed.

2D ROESY experiments were carried out to confirm the inclusion of each sulfonamide into the β-CD cavity. Figure 6 shows partial contour plots of 2D ROESY spectra for the three studied systems. For SDZ:β-CD (Fig. 6a), it can be observed that H_a protons of SDZ correlate with the inner protons of β-CD (H_5), indicating the formation of an inclusion complex in which the ring A of SDZ is deeply inserted into the hydrophobic cavity. For SMR:β-CD and SMT:β-CD complexes (Fig. 6b and c, respectively), intermolecular cross-peaks between H_b protons of both guest molecules and H_3 protons of β-CD were found, evidencing again the formation of an inclusion complex but suggesting that the ring A of SMR and SMT may not be so deeply inserted into the hydrophobic cavity as for SDZ.

Computational Studies

Conformational Analysis

Three-dimensional structures of SDZ, SMR, and SMT were built, after which energy minimizations were performed. In order to assess the relative position of ring A with respect to ring B, the dihedral angle defined by the sulfonamide bond was rotated and the resulting energy analyzed (Fig. 7). As shown, two minimum energy conformers were found for each molecule, both of them corresponding to the eclipsed position between the hydrogen bond to the nitrogen atom and both oxygen atoms in the sulfonamide moiety, which established intramolecular hydrogen bonds responsible for the higher stability of these two conformers. Similar results were recently reported for SDZ when conformational properties were studied by means of experimental and high-level theoretical methods.[25]

Molecular Docking

Considering that the minimum energy conformers obtained are the most likely conformations in solution, docking procedures were applied by restraining the rotation of the sulfonamide bond of the two minimum energy conformers described previously, allowing the rotation of all other rotatable bonds. Figure 8 shows the three-dimensional structures of the corresponding complexes obtained from docking assays, in which ring B of the three sulfonamide molecules is deeply inserted into the β-CD hydrophobic cavity. Similar docking poses and energy were also observed for the two conformers analyzed.

Further inspection of the docked conformations shows that in all cases ring A is placed in a coplanar position with respect to the β-CD cavity and perpendicular to its axis, allowing the establishment of hydrogen bonds between the hydroxyls of the macromolecule wide rim and the primary amine and sulfonamide moiety of the guest molecule. Hence, the electrostatic component of the affinity seems to be originated mainly in the interaction between the guest molecule and the hydroxyls situated in the wide

Table 3. Chemical Shifts for the Protons of SDZ, SMR, and SMT in the Free State and in the Complex

Proton	SDZ (ppm)	SDZ:β-CD[a] (ppm)	Δδ (ppm)	SMR (ppm)	SMR:β-CD[b] (ppm)	Δδ (ppm)	SMT (ppm)	SMT:β-CD[c] (ppm)	Δδ (ppm)
H_a	6.8317	6.8302	−0.0015	6.8595	6.8289	−0.0306	6.8380	6.7602	−0.0778
H_b	7.6916	7.7061	0.0145	7.7294	7.7383	0.0089	7.7374	7.7211	−0.0163
H_c	8.3406	8.3515	0.0109	8.1681	8.2053	0.0372	—	—	—
H_d	6.8820	6.8792	−0.0028	6.8040	6.8071	0.0031	6.7181	6.6902	−0.0279
H_{CH_3}	—	—	—	2.3853	2.4008	0.0155	2.3474	2.3326	−0.0148

[a] [SDZ]:[β-CD] = 1:1.
[b] [SMR]:[β-CD] = 1:1.
[c] [SMT]:[β-CD] = 1:1.

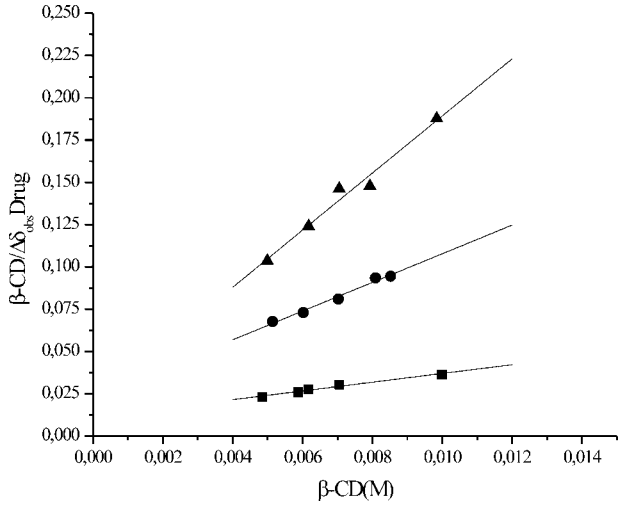


Figure 5. Scott's plots for proton H_d of SDZ (■, $r^2 = 0.969$), SMR (●, $r^2 = 0.979$), SMT (▲, $r^2 = 0.969$) with increasing concentrations of β-CD.

rim of β-CD, whereas the hydrophobic contribution results from the inclusion of ring B. This would preliminarily suggest that SMT should exhibit the highest affinity within the series of the compounds studied due to the higher hydrophobicity of the dimethylpyrymidine moiety compared to that of methylpyrimidine and pyrimidine in SMR and SDZ, respectively. When the docked energies obtained were compared, it can be seen that the complex SMR:β-CD is more stable than SDZ:β-CD (−9.14 and −8.70 kcal/mol, respectively), in agreement with SMR enhanced lipophilicity. However, for SMT:β-CD (−8.86 kcal/mol), no additional contribution to the stability of the complex was noted after a second methyl moiety was introduced into ring B. Considering that β-CD is maintained rigid during the docking procedure, the lower docked energy founded for SMT:β-CD may be originate in a steric hindrance when a second methyl group is introduced.

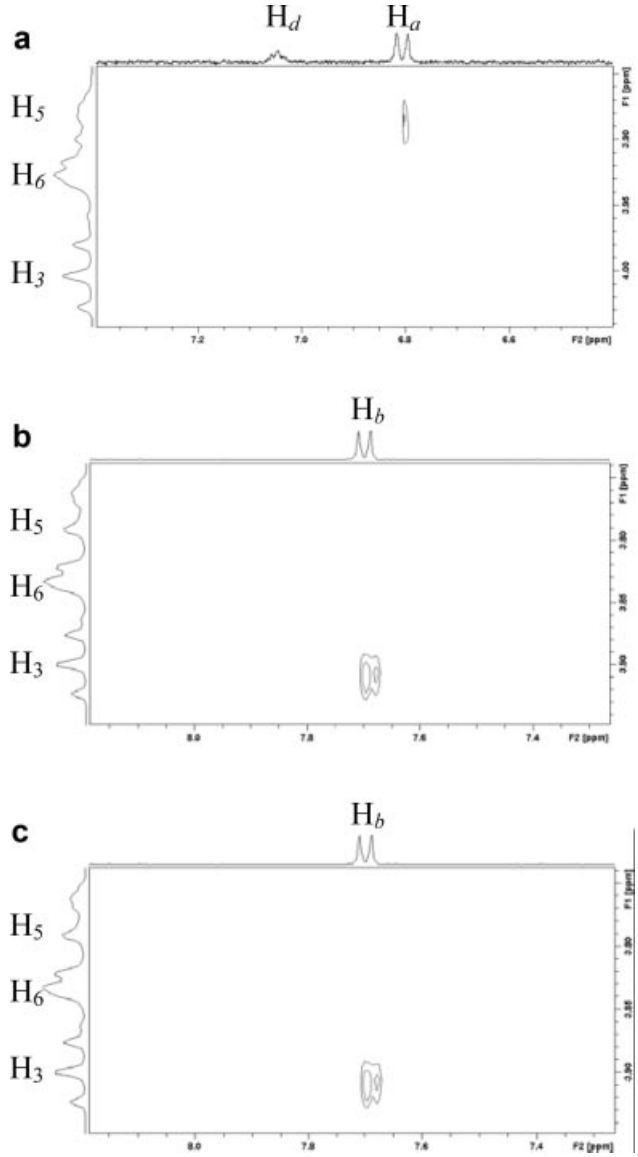


Figure 6. Partial contour plot of 2D ROESY spectrum of (a) SDZ:β-CD complex, (b) SMR:β-CD complex, (c) SMT:β-CD complex.

Molecular Dynamics

The complexes obtained by molecular docking were solvated, heated, and equilibrated, after which extensive simulation runs (20 ns) were performed. In order to study the structure of the complex over time, the establishment of intermolecular hydrogen bonds was analyzed. Table 4 shows the most relevant hydrogen bonds formed between SDZ and β-CD. It should be noted that when the initial structure obtained by molecular docking is solvated, heated, and equilibrated, the guest molecule moves from the initial docked position, by which the sulfonamide moiety is inserted into the β-CD cavity early in the simulation (Fig. 9,1.a). During this movement, the amine function in ring A is oriented towards the bulk solvent, where hydrogen bonds are formed with water molecules and maintained during the entire simulation (mean frequency 46.4%). Simultaneously, part of ring A is buried into the cavity through the wide rim, while ring B is partially excluded from the β-CD in the opposite site of the macromolecule (Fig. 9,1.a). In a second cluster of conformations, ring A is deeply buried into the cavity, with ring B almost completely excluded from the hydrophobic cavity and the sulfonamide moiety establishing hydrogen bonds with hydroxyls of the narrow rim (Fig. 9,1.b). Thus, a dynamic equilibrium between these two conformations is achieved, by which the pattern of hydrogen bonds formed can be observed (Tab. 4). For SDZ, and as evidenced by the hydrogen bonding frequency, the conformation cor-

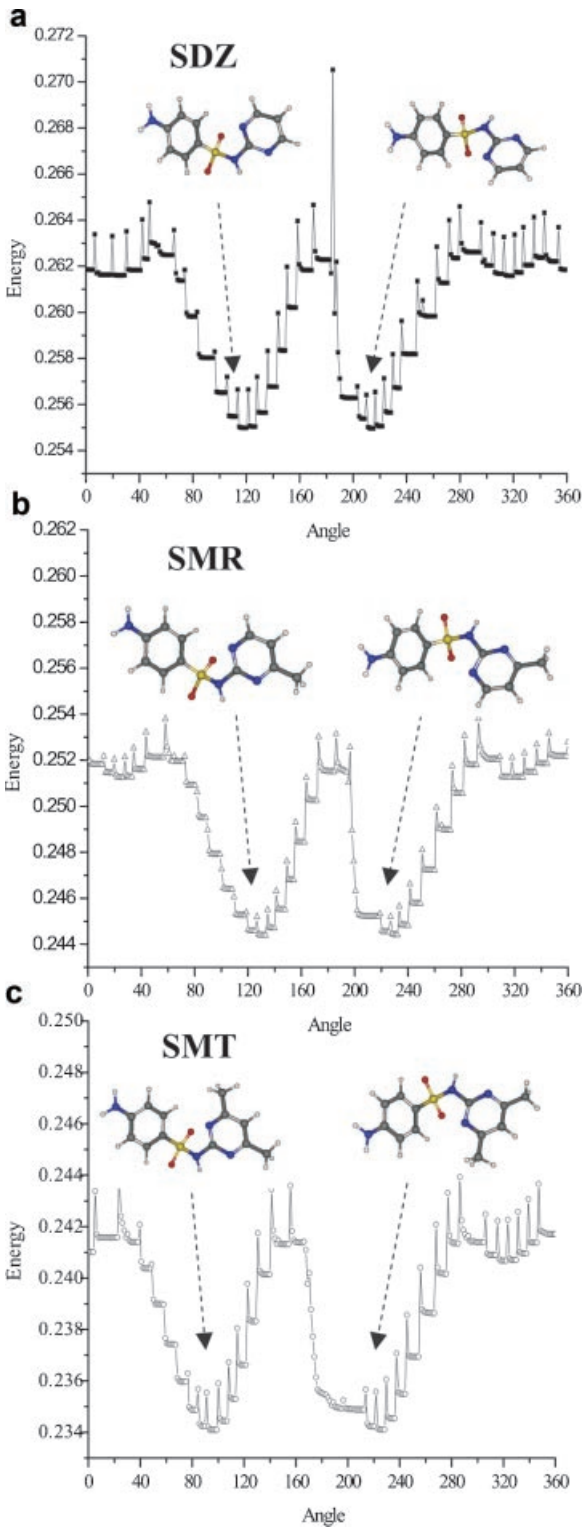


Figure 7. Energetic profiles obtained for the conformational analyses of SDZ, SMR, and SMT.

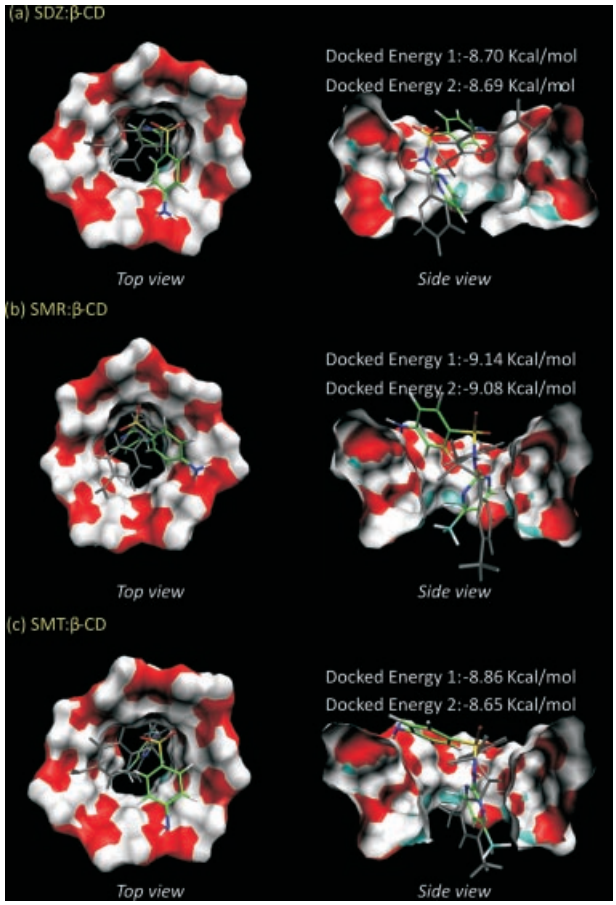


Figure 8. Structure of the complexes between SDZ, SMR, and SMT with β-CD as obtained by molecular docking procedures.

responding to the first cluster (Fig. 9,1.a) is clearly predominant. This model is consistent with the downfield displacements of protons H_3 and H_5 found in the corresponding ^{1}H NMR spectra (Tab. 2, Fig. 3), probably caused by the deshielding produced by the electronegative sulfonamide moiety. Previous reports dealing with the binding of SDZ to hydroxypropil-β-CD also suggested the insertion of the ring A into the hydrophobic cavity, in agreement with the complex conformation properties described in this work.[8]

Table 5 summarizes the most relevant hydrogen bonds detected for the SMR:β-CD complex. As found for SDZ, during the heating and equilibration phase the sulfonamide moiety in SMR is buried into β-CD cavity (Fig. 9,2.a), with the primary amine moiety establishing sustained hydrogen bonds with water molecules throughout the trajectory (mean frequency 55.3%). A second cluster of conformations is formed when ring B is reinserted into the β-CD cavity, with the sulfonamide moiety and ring A being extruded and exposed to the bulk solvent (Fig. 9,2.b). As evidenced by the hydrogen bond analysis, an equilibrium between these two spatial arrangements is established, with the second cluster (Fig. 9,2.b) being highly predominant. In this case, the insertion of ring A into the hydrophobic cavity was not observed, since this would encompass the extrusion of ring B on the narrow rim of the macromolecule which, considering its high hydrophobicity, would represent an unfavor-

 DOI 10.1002/jps

Table 4. Hydrogen Bonds Between SDZ and β-CD

ns	Acceptor → Donor	Frequency (%)	Distribution
2–3	O1 (SDZ) → OH (CYC)	15.7	[.XXXX-]
	O2 (SDZ) → OH (CYC)	10.4	[o-oo.]
3–4	O (CYC) → NH (SDZ)	5.8	|. . oo |
4–5	O (CYC) → NH (SDZ)	7.7	| -oo- |
8–9	O (CYC) → NH(SDZ)	47.6	| -ooxoooxxooooox|
	O (CYC) → NH2 (SDZ)	24.2	| .- -..--ooo-.oo--|
9–10	O (CYC) → NH(SDZ)	30.3	|ooooxxxxoo- |
	O (CYC) → NH2 (SDZ)	17.9	|o-o---oo-. |
17–18	O1 (SDZ) → OH (CYC)	5.1	| .. o- |

Frequency notation (%): . = 5–20; - = 20–40; o = 40–60; × = 60–80; * = 80–95; @ = 95–100.

able event in terms of energetic contribution. The predominant cluster of conformations in which the sulfonamide moiety is not deeply included is consistent with the upfield and downfield displacement observed for H_5 and H_3, respectively, since the deshielding effect is produced on H_3 while H_5 experiments a shielding by the high electronic density of the ring B.

Table 6 shows the hydrogen bond interactions as founded in the trajectory for the SMT:β-CD complex. In this case, after the initial heating and equilibration, hydrogen bonds between the sulfonamide and the hydroxyls of the wide rim of β-CD are maintained with high frequency, indicating that this moiety is not deeply buried into the β-CD cavity. In this conformation, ring B is inserted into the β-CD hydrophobic cavity, maximizing van der Waals interactions. Additionally, a low frequency was observed for the conformations in which the sulfonamide moiety is included into the cavity and ring B is excluded through the narrow rim, which may be due to the high hydrophobicity of ring B. This model is in agreement with the upfield displacements founded for H_3 and H_5, which may be originate in the shielding produced by ring B.

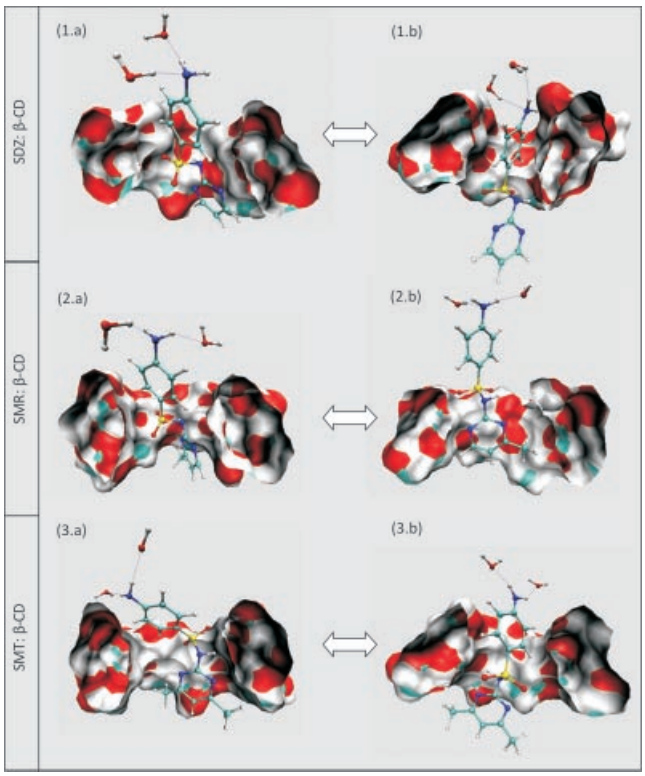


Figure 9. Hydrogen bonds and three-dimensional structures obtained for each sulfonamide:β-CD complexes.

Energetic Analyses

Table 7 lists the energetic components as determined for the three complexes studied using the MM-PBSA methodology. As it can be seen, the major contribution to the complex stability arises from the van der Waals component, comparable to SDZ and SMR (−30.10 and −30.02 kcal/mol, respectively) and slightly higher for SMT (−31.82 kcal/mol). Significant differences were observed in the electrostatic components, with its contribution to affinity increasing in the order SMT > SMR > SDZ (−10.84, −8.18, and −7.13 kcal/mol, respectively).

The polar contribution to solvation (PB_{cal}) is of utmost importance in the formation of β-CD complexes, and may be decisive in the overall affinity observed.[6,26] As it can be seen in Table 7, PB_{cal} was positive for the three complexes studied and increased in the order SMT > SMR > SDZ, indicating that the energy required to solvate the SMT:β-CD complex is higher than that for SMR:β-CD and SDZ:β-CD (29.16, 25.64, and 25.57 kcal/mol, respectively). In contrast, the total interaction energy (PB_{tot}) was negative for the three cases, which indicates that the formation of the corresponding complexes is thermodynamically favorable. Thus, the theoretically calculated affinity increased in the order SMT:β-CD > SMR:β-CD > SDZ:β-CD, with energy required for solvation being compensated by intermolecular van der Waals and electrostatic interac-

Table 5. Hydrogen Bonds Between SMR and β-CD

ns	Acceptor → Donor	Frequency (%)	Distribution
1–2	O1 (SMR) → OH (CYC)	13.3	| x* |
2–3	O2 (SMR) → OH (CYC)	11.3	|. .. .. |
	O1 (SMR) → OH (CYC)	6.8	| -o - . . |
3–4	O1 (SMR) → OH (CYC)	33.9	| --**x-*x*oo |
7–8	O2 (SMR) → OH (CYC)	6.9	|-- o . - |
8–9	O1 (SMR) → OH (CYC)	28.9	| xoo***x*|
	O2 (SMR) → OH (CYC)	9.7	| .*- o.|
9–10	O1 (SMR) → OH (CYC)	75	|**x***o -*xx**@x|
	O2 (SMR) → OH (CYC)	12	|.- ..- .. -. -.-.|
10–11	O1 (SMR) → OH (CYC)	61.4	|*x**xo**--ooxo. |
13–14	O2 (SMR) → OH (CYC)	9.8	| .xx- |
14–15	O2 (SMR) → OH (CYC)	6.7	| -o--
	O1 (SMR) → OH (CYC)	5.5	| xo
15–16	O2 (SMR) → OH (CYC)	7.2	| -o .. . |
17–18	O1 (SMR) → OH (CYC)	7.8	| ---x
19–20	O2 (SMR) → OH (CYC)	57	|ooxxoxxxxxxoxxxx|

Frequency notation (%): . = 5–20; - = 20–40; o = 40–60; × = 60–80; * = 80–95; @ = 95–100.

tions. When the conclusions drawn from the energetic analysis were compared with experimental results, we observed that affinity predicted by PB_{tot} was consistent with K_c values obtained by NMR studies, whereas affinities expected from the energetic payoff to solvate the complex (PB_{cal}) were in agreement with K_c values found by PSD. As stated, discrepancies between the affinities determined by different methods have been previously reported, and on the basis of our experimental and theoretical results, it seems feasible that differences in affinities calculated by PSD and NMR were produced by a phenomenon related to the solvation energies in the overall complex formation process.

Table 6. Hydrogen Bonds between SMT and β-CD

ns	Acceptor → Donor	Frequency (%)	Distribution
0–1	O1 (SMT) → OH (CYC)	73.7	|xxoxxx****xx**x |
	O2 (SMT) → OH (CYC)	31.8	| -oo o xooooxx..
1–2	O1 (SMT) → OH (CYC)	54.8	|x*@**o*o*x* .x|
	O2 (SMT) → OH (CYC)	29.1	|..xx oooxx.o. .x|
2–3	O1 (SMT) → OH (CYC)	42.9	| -**ox..x*o. |
	O2 (SMT) → OH (CYC)	17.6	| xx-**|
6–7	O1 (SMT) → OH (CYC)	21.7	|oxo**x. |
	O2 (SMT) → OH (CYC)	21.1	|*x-xx-. -. . |
8–9	O1 (SMT) → OH (CYC)	11	| oo-oo|
11–12	O1 (SMT) → OH (CYC)	8.5	| .*x|
12–13	O1 (SMT) → OH (CYC)	68.5	|oo*x**x**x-*ox-x|
	O2 (SMT) → OH (CYC)	40.5	|x--*- oxxo-xoo . o.
13–14	O1 (SMT) → OH (CYC)	63.7	|o.*xxxoxoxox**x--|
	O2 (SMT) → OH (CYC)	25.4	| . - -xx.--o-o-o|
14–15	O1 (SMT) → OH (CYC)	62.4	|x-xoxx**-*o.ooxx*|
	O2 (SMT) → OH (CYC)	23.3	|. -.. .x-oo-o -x|
15–16	O1 (SMT) → OH (CYC)	61.3	|x-o*xx***-xo--**x|
	O2 (SMT) → OH (CYC)	31.6	|*oox-- oo--o o- . -
16–17	O1 (SMT) → OH (CYC)	13.7	|xx- |

Frequency notation (%): . = 5–20; - = 20–40; o = 40–60; × = 60–80; * = 80–95; @ = 95–100.

DOI 10.1002/jps

Table 7. Energetic Component Analysis Using the MM-PBSA Methodology

	SDZ	SMR	SMT
E_{Ele}	−7.13	−8.18	−10.85
E_{VdW}	−30.10	−30.02	−31.82
E_{gas}	−37.23	−38.03	−42.67
PB_{sur}	−3.63	−3.73	−4.00
PB_{cal}	25.57	25.64	29.16
PB_{tot}	−15.29	−16.11	−17.51

CONCLUSIONS

The aqueous solubility of the three sulfonamides assayed was improved by the formation of 1:1 inclusion complexes with β-CD. NMR studies and molecular modeling techniques were in agreement with the K_c values of the three compounds studied for β-CD, indicating the following trend: SMT > SMR > SDZ. This tendency could be attributed to the hydrophobicity enhancement of ring B due to the addition of the methyl groups, giving rise a molecule with enhanced affinity. Hence, for the SDZ:β-CD complex, the inclusion of ring A represented the predominant conformation, while for both SMR:β-CD and SMT:β-CD complexes, inclusion of ring B was energetically favored. From this work, we can conclude that the formation of the inclusion complex between β-CD and SDZ, SMR, and SMT suggest an effective strategy to enhance the solubility of the three drugs.

ACKNOWLEDGMENTS

The authors thank Fondo para la Investigación Científica y Tecnológica (FONCyT), préstamo BID 1728/OC-AR PICT 1376, Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT), and 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 β-cyclodextrin, and also Dr. Petter Bjorstad (Research Director at Bergen Center for Computational Science (BCCS)) for kindly providing our access to BCCS computing resources.

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 DOI 10.1002/jps