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Sulfadiazine/hydroxypropyl-β-cyclodextrin host–guest system: Characterization, phase-solubility and molecular modeling

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Abstract—In this work we prepared and characterized an inclusion complex of the dihydropteroate synthase inhibitor sulfadiazine (SDZ) in 2-hydroxypropyl-β-cyclodextrin (HPBCD). From the phase-solubility diagram we observed an increase in the water solubility of the drug, calculating a binding constant of 1879 M^{-1}. The inclusion mode involves a NH_2-in orientation of the drug in the HPBCD cavity, according to the 2D NMR (ROESY) data and confirmed by molecular modeling using the semiempirical PM6 and RM1 methods.

1. Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii*, an intracellular parasite that can infect all warm-blooded animals.[1–3] The infection can remain in a latent and asymptomatic state, reactivating in immunocompromised patients and causing cerebral inflammation (toxoplasmic encephalitis), a condition prone to result in seizures and death.[4] The conventional treatment is based on mixtures of antiparasitic drugs including sulfadiazine (4-amino-*N*-(2-pyrimidinyl) benzenesulfonamide) (SDZ), an inhibitor of the essential metabolic enzyme dihydropteroate synthase. SDZ can induce severe allergic reactions in many patients, as in a study of 29 patients, ~54% abandoned treatment.[5,6]

It is well described that the use of drug carriers may improve the treatment efficacy and reduce side effects, by solubility increase and transport/release of the drug to specific sites.[7–9] Concerning the drawbacks associated with the SDZ treatment, the use of a suitable carrier could increase its water solubility, potentially reducing the side effects by the use of lower dosages. On the other hand, the reduction in the dosage of SDZ and other pharmaceuticals has become important also in the environmental context, since nowadays they are referred to as a class of emerging contaminants—endocrine disruptors.[10] Recent investigations have shown some evidence that substances of pharmaceutical origin are not eliminated during wastewater treatment, which is an increasing concern due to their continuously growing use.[11]

The standard toxoplasmosis therapy also includes pyrimethamine, a poor water soluble dihydrofolate reductase (DHFR) inhibitor, and recently we reported its inclusion complex with cyclodextrin derivatives improving the water solubility of the drug.[12] Cyclodextrins (CDs), cyclic oligosaccharides formed by glucopyranose units linked by α-(1,4) glycosidic bonds,[13,14] are among the most studied potential drug carriers. There are three main natural forms of cyclodextrins, differing in the number of glucopyranose units: α-(6 units); β-(7 units), and γ-(8 units). The special feature of the CDs is the ability of forming inclusion complexes by the accommodation of molecules with apolar structures inside cavity.[15,16] Inclusion complexes of native and modified

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CDs with many different species have been widely described in the fields of supramolecular chemistry, pharmacology, food science, cosmetics, catalysis, chromatography, etc.[17–23] 2-hydroxypropyl-β-cyclodextrin (HPBCD) is a hydroxyalkylated CD derivative that combines a relatively high water solubility with a low toxicity and a satisfactory inclusion ability.[24,25] Several commercial formulations are composed of cyclodextrin inclusion complexes, evidencing the importance of the field.[26–29]

The use of inhibitors of essential enzymes is the base of treatment of several other parasitic diseases such as malaria and cryptosporidiosis,[30] indicating that the use of this family of drugs is of broad interest. For instance, Li et al. reported the inclusion complex of trimethoprim, another DHFR inhibitor, with β-cyclodextrin[31] and Romero and co-workers prepared dimeric sulfadiazine nanocarriers.[32]

In this work we studied the formation of inclusion complexes of SDZ with HPBCD by phase-solubility techniques, molecular modeling by semiempirical and molecular mechanics methods and characterized the complexes by thermal analysis and 2-D NMR spectroscopy (ROESY).

2. Results and discussion

2.1. Phase-solubility

The phase-solubility diagram of the system HPBCD/SDZ, Figure 1, evidences that the solubility of the drug increased linearly with increasing HPBCD concentration. This diagram can be classified as A_L type and according to the model proposed by Higuchi and Connors[33] it can be related with the formation of a soluble inclusion complex. The apparent stability constant ($K_{1:1}$), was calculated from the linear fit of the curve according to the following equation:

$$K_{1:1} = \frac{\text{Slope}}{\text{So}(1 - \text{Slope})},$$

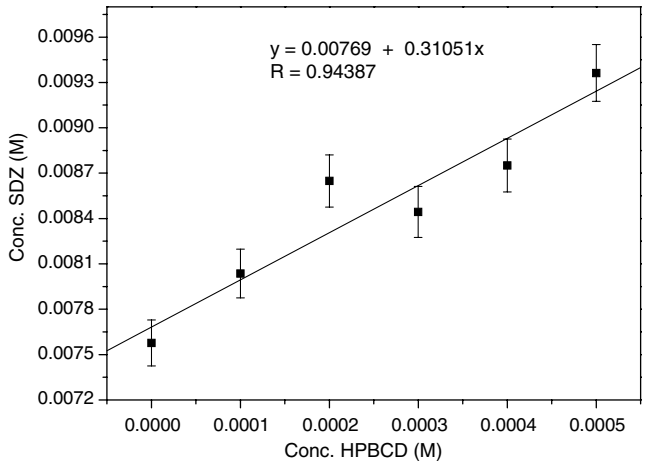

Figure 1. Phase-solubility diagram for the HPBCD/sulfadiazine host–guest system.

where Slope is the value found in the linear regression and So is the aqueous solubility of the drug at pH 7 (So = 40 mg L^{-1}) in the absence of HPBCD, determined spectrophotometrically by Lazaro.[34] The value calculated for $K_{1:1}$ was 1879 M^{-1} suggesting the occurrence of favorable interactions, since in general the association constants of drugs to CDs are reported in the range 50–2000 M^{-1}.[8] The constant found here is very similar to that reported for pyrimethamine complexation with HPBCD, ($K_{1:1}$ = 1900.6 M^{-1}).[12] This finding can be further explored in the treatment of toxoplasmosis since the most common therapy is based on a mixture of these two drugs.

2.2. ^{1}H NMR spectroscopy

^{1}H one- and two-dimensional (^{1}H–^{13}C HSQC and ^{1}H–^{1}H ROESY) spectra were measured in order to confirm the inclusion of sulfadiazine in HPBCD and also to characterize the binding mode.[35] Owing to the observation of superimposed signals in HPBCD ^{1}H spectrum, an HSQC measurement was obtained, Figure 2, in order to provide a tentative assignment. This spectrum is in good agreement to that reported previously by Zoppetti and co-workers.[36] As commercial HPBCD contains a mixture of hydroxypropyl β-CD derivatives with different substitution degrees, a special characteristic of this spectrum is the presence of signals spread over a range of δ values. Figure 3a shows the ^{1}H spectrum for HPBCD below 5.5 ppm. In Figure 3b a zoom from 6 to 8.7 ppm evidences the SDZ signals: δ = 6.628 ppm (d, Ha), δ = 6.933 ppm (t, Hd), δ = 7.717 ppm (d, Hb), and δ = 8.411 ppm (d, Hc), see Figure 3c and d for proton identification.

The two-dimensional ^{1}H–^{1}H ROESY spectrum of the inclusion complex (Fig. 4) shows that Ha protons of the *p*-substituted aniline ring of sulfadiazine correlate both with H-3 and H-5 protons of HPBCD, apparently a slightly stronger correlation with H-3. As cross peaks correlate protons with separation lower than 4.0 Å in space, the observed correlations indicate that the aniline ring is included in the CD cavity. As H-3 protons are closer to the secondary CD rim, the inclusion mode

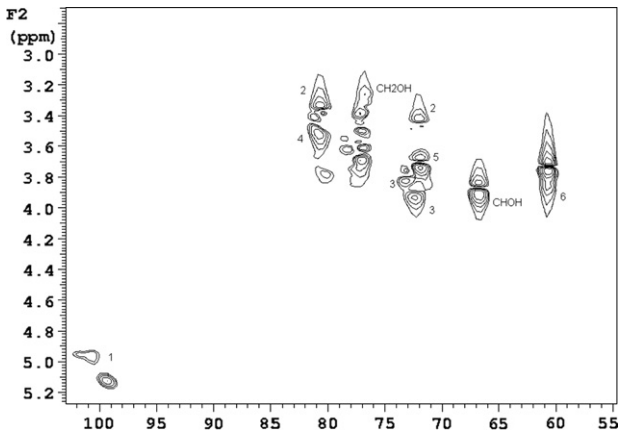

Figure 2. HSQC spectrum of HPBCD with a tentative assignment.

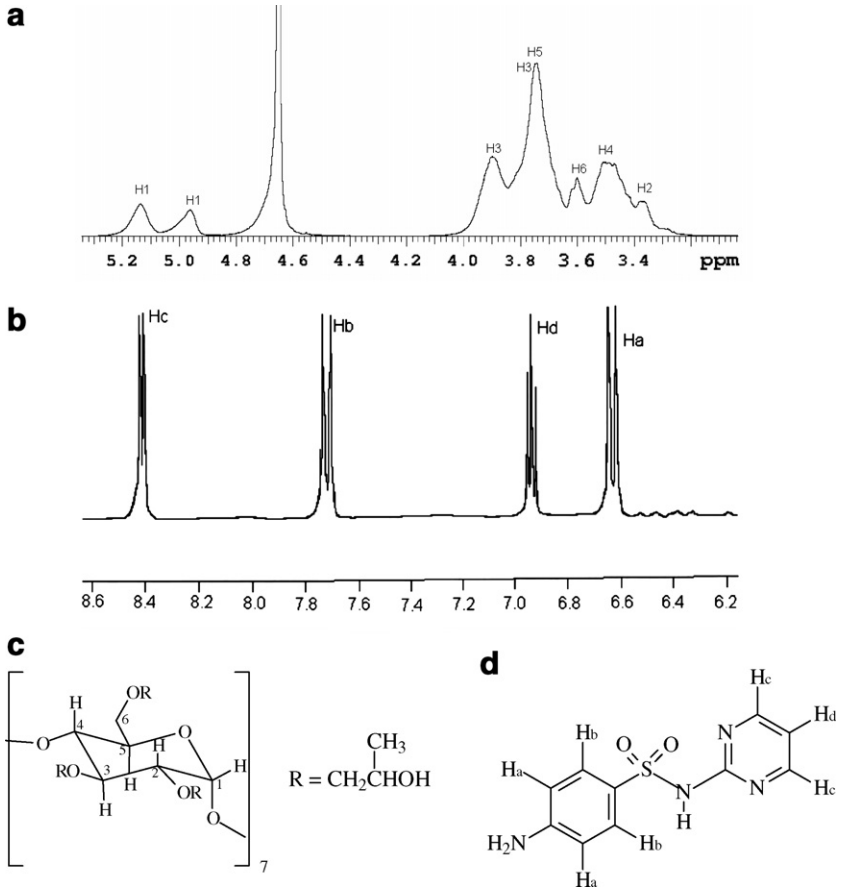

Figure 3. (a) ^{1}H NMR spectrum of the HPBCD in D_2O showing the region of HPBCD signals; (b) ^{1}H NMR spectrum of the SDZ in CD_3OD showing the region of sulfadiazine signals; (c) identification of HPBCD protons from the glucopyranose units; (d) identification of sulfadiazine protons.

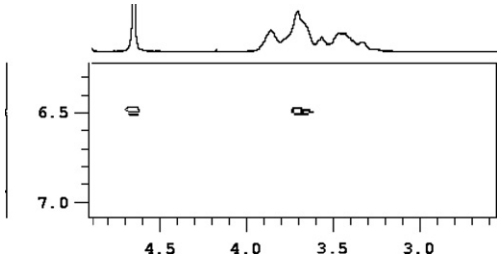

Figure 4. ^{1}H ROESY spectrum of the HPBCD/sulfadiazine system in D_2O at 298.1 K.

may involve a relatively low penetration degree in the cavity.

2.3. Thermal behavior and X-ray diffraction of the inclusion complex

Figure 5(a–d: DSC, e–h: TG) shows thermal analysis results for the samples studied. Free SDZ starts to melt with decomposition at 259 °C, as can be observed by a comparison of the TG and DSC curves, Figure 5a and 5e. HPBCD loses water at temperatures slightly above 100 °C and decomposes above 269 °C, as evidenced by the mass loss in the TG curve, Figure 5f. The physical mixture of HPBCD and SDZ apparently contains only

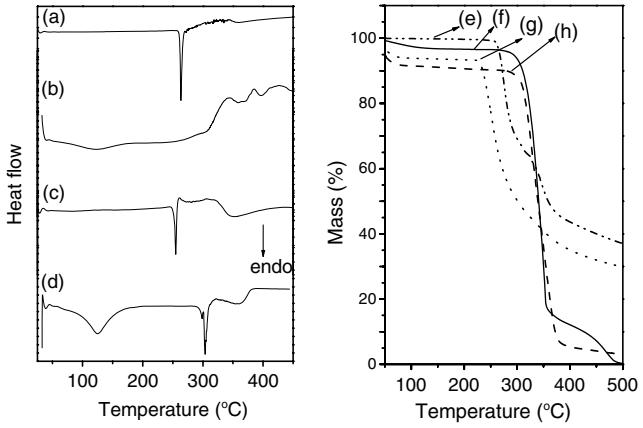

Figure 5. DSC (a–d) and TG (e–h) (N_2, 10 °C/min) curves for (a and e) sulfadiazine; (b and f) HPBCD; (c and g) HPBCD/sulfadiazine 1:1 (mol proportion) physical mixture, and (d and h) HPBCD/sulfadiazine inclusion complex.

the free species, as indicated by the endothermic peak due to melting of SDZ at 255 °C (Fig. 5c), followed by the decomposition of both compounds, represented by a continuous mass loss in the TG curve (Fig. 5g). Finally, apart from a broad endothermic peak near 100 °C due do water loss, the melting peak of free SDZ is absent in the DSC curve of the lyophilized sample (Fig. 5d).

The curve also presents a sharp peak at 310 °C, absent from the curve of free SDZ. We suggest that this event may be related to the melting of non-included drug[37] or to the beginning of decomposition, since the peak at 310 °C coincides with the beginning of mass loss in the TG curve (Fig. 5h).

Powder XRD patterns allowed us to examine the medium and long range ordering of the materials.[38] In contrast to the amorphous character of HPBCD, Figure 6a, free sulfadiazine is a crystalline solid, Figure 6b. The XRD pattern of the physical mixture confirmed that it contains both species as isolated solids, as the diffractogram presents SDZ peaks in addition to the amorphous halo of HPBCD. Finally, the lyophilized inclusion complex has an amorphous structure, probably as a result both the structure of HPBCD and of the lyophilization process and evidence the absence of SDZ crystalline particles.

2.4. Molecular modeling

The structures of the inclusion complex in the two orientations considered were initially optimized by the MM3 method, with the guest aligned with the centroid axis of the CD cavity. An energy map representing the energy variation of the system with SDZ entrance as well as dihedral between SDZ rings for each orientation is also shown in Figure 7. For the NH_2-out orientation the energy depended on both factors resulting in a 2-D energy map, in contrast to the NH_2-in, for which the energy was found to be dependent only on the distance. This difference may arise from the influence of the NH_2 group orientation outside the cavity, for the NH_2-out mode. The distance between the SDZ amino group and the closest hydroxyl group from HPBCD for NH_2-in orientation of the inclusion complex was 2.2 Å, suggesting the existence of a hydrogen bonding. On the other hand, a distance of 3.2 Å separating the closest HPBCD hydroxyl and the SDZ molecule was found for the NH_2-out orientation.

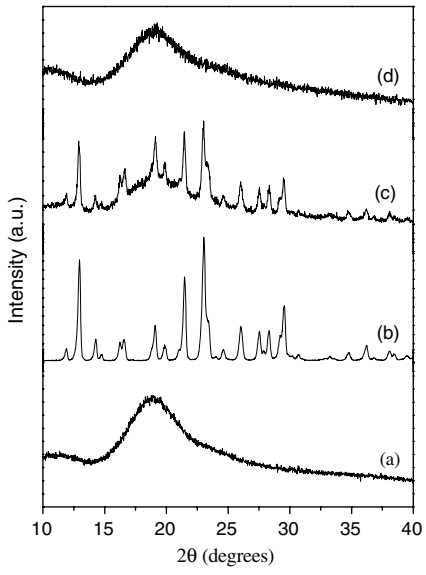

Figure 6. Powder X-ray diffractograms (Cu-κα) for: (a) HPBCD; (b) sulfadiazine; HPBCD/sulfadiazine 1:1 (mol proportion) physical mixture; (d) HPBCD/sulfadiazine inclusion complex collected by lyophilization.

After this initial procedure, the energy minimization was carried out without constraint using the semiempirical PM6[39] and RM1[40] methods, which yielded the enthalpies of formation for both orientations in addition to Gibbs free energy and entropy variation (NH_2-in and NH_2-out, Table 1). According to calculation the NH_2-out orientation was favored over NH_2-in in vacuum, in contrast to the overall orientation of the SDZ molecule in the HPBCD cavity evidenced by the ROESY measurement (NH_2-in). On the other hand, when a continuum medium with the water dielectric constant is considered, an inversion in the most stable inclusion orientation is observed for both methods. The value of ΔG calculated for the formation of the complex pointed out a spontaneous process, both in vacuum and in water as continuum medium. Alternatively enthalpy of the complex was calculated from the vibrational energy, leading to positive ΔG values. Positive ΔG values can be observed for calculations performed in vacuum, as reported previously by Piel and co-workers,[41] suggesting the role of the elimination of water molecules from the cavity.

The optimized host–guest molecular structures for the complexation of HPBCD with sulfadiazine in the two binding orientations obtained by PM6 and RM1 calculations (vacuum and water continuum medium) are shown in Figure 8. The aniline ring has a very low penetration degree in the cavity for the vacuum NH_2-in structures from both methods, in contrast to the higher penetration for the vacuum NH_2-out structures. This fact can account for the preferred NH_2-out orientation in vacuum from both methods. When the aqueous medium is considered, an increased penetration degree can be observed for the aniline ring in the NH_2-in orientation from both methods, stabilizing the configuration in comparison to the NH_2-out, probably as a result of favorable interactions. In general a very good agreement was observed between the optimized NH_2-in orientations (continuum medium) and experimental ROESY data, in which protons from the aniline ring are closer to the secondary face.

3. Conclusions

In conclusion, encapsulation of sulfadiazine in 2-hydroxypropyl-β-cyclodextrin causes a linear increase of the drug solubility with increasing host concentration. This is an important finding since sulfadiazine is widely used in the treatment of parasitic diseases, both in humans and in animals. Characterization of the inclusion mode by 2D NMR spectroscopy, in addition to theoretical calculations by the AM1 method, pointed out that the preferred orientation involves inclusion of the aniline ring into the CD cavity.

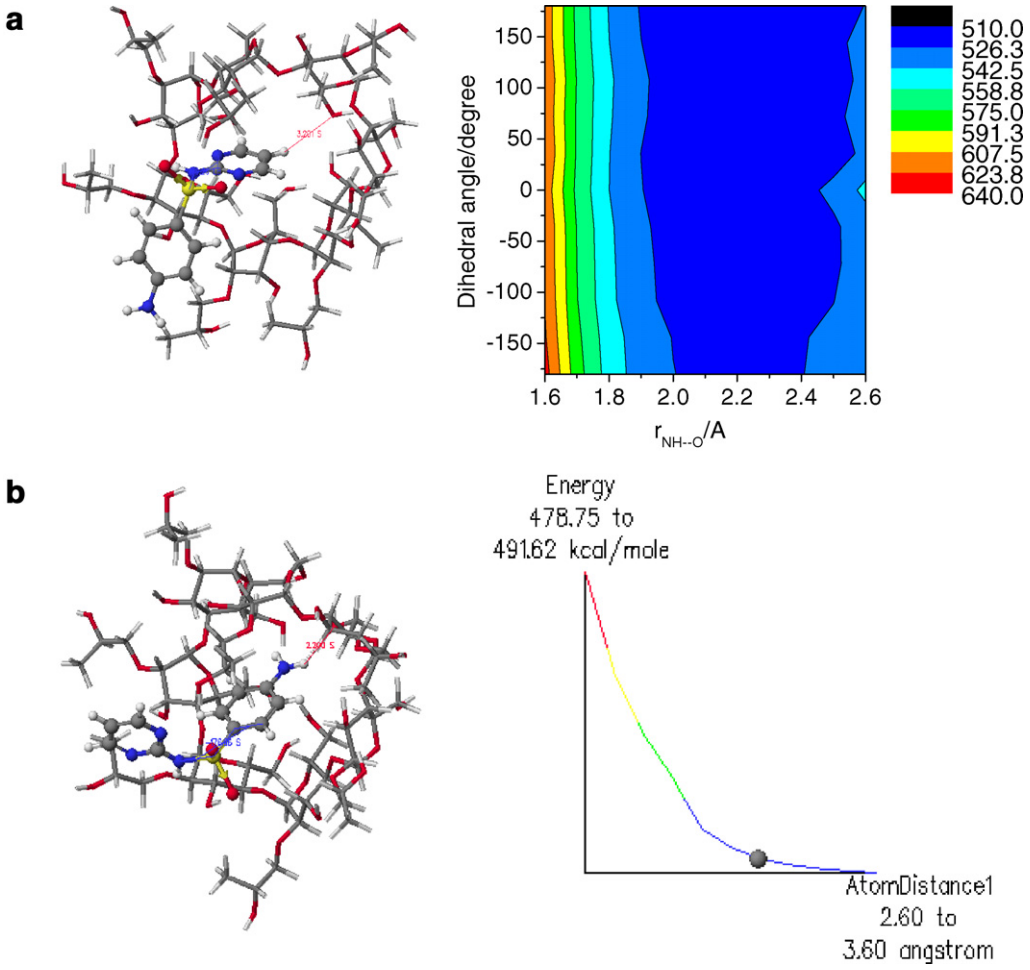

Figure 7. Minimum energy structures and energy maps for the two orientations considered, by the MM3 method: (a) NH_2-out with 2D conformational map, showing the energy dependence of the system with SDZ entrance in HPBCD cavity and with the dihedral angle between the two SDZ rings; (b) NH_2-in with 1D conformational map. The energy increases following the color sequence blue–green–yellow–red.

Table 1. Enthalpies of formation ($\Delta\Delta_f H$), enthalpy of complexation from vibrational energy including zero-point energy corrections ($\Delta H^{vib(0)}$), entropy variations (as $T\Delta S$) and Gibbs free energy (ΔG_1 from $\Delta\Delta_f H$ and ΔG_2 from $\Delta H^{vib(0)}$) at 298 K upon the inclusion complexation of HPBCD with SDZ

Method			$\Delta\Delta_f H$ (kcal/mol)	$\Delta H^{vib(0)}$ (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG_1 (kcal/mol)	ΔG_2 (kcal/mol)
PM6	Vacuum	NH_2-in	−1952.25	−8.77	−19.41	−1932.84	10.64
		NH_2-out	−1964.19	−9.55	−19.54	−1944.65	9.99
	Water	NH_2-in	−2023.88	−14.81	−22.39	−2001.49	7.58
		NH_2-out	−2014.29	−15.07	−26.83	−1987.46	11.76
RM1	Vacuum	NH_2-in	−1898.53	−19.87	−25.29	−1873.24	5.42
		NH_2-out	−1906.13	−21.03	−26.15	−1879.98	5.12
	Water	NH_2-in	−1956.28	−26.34	−30.23	−1926.05	3.89
		NH_2-out	−1933.36	98.03	−24.56	−1908.80	122.60

4. Experimental

4.1. Materials

2-hydroxypropyl-β-cyclodextrin (FW = 1540) and sulfadiazine (FW = 250.28) were purchased from Sigma and used as received. Other reagents and chemicals were of analytical reagent grade. All experiments have been carried out using ultrapure water (MILLI Q).

4.2. Preparation of inclusion complexes

The inclusion complexes were prepared by the suspension method.[12] Briefly, SDZ and HPBCD were mixed in water in the molar proportion 1:1 and stirred at room temperature for 48 h protected from light to prevent degradation of the molecules. After this period the solid residue was separated by centrifugation at 15,000 rpm for 15 min and the upper liquid layer was filtered over

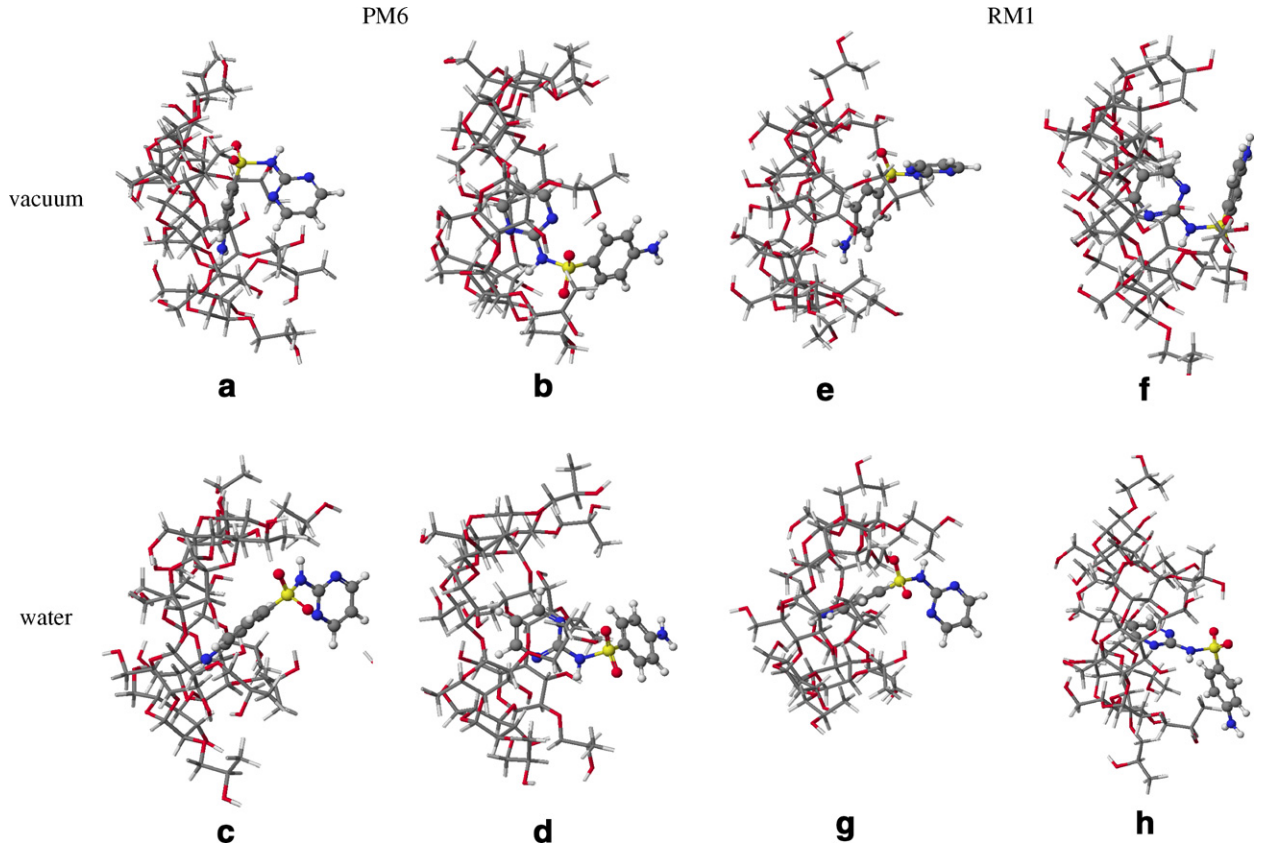

Figure 8. Complex SDZ/HPBCD in two orientations: NH_2-in (a, c, e, g) and NH_2-out (b, d, f, h) from PM6 and RM1 methods calculated in vacuum and in water (continuum medium).

0.45 μm Millipore membrane. The solution was then dried by lyophilization for the solid inclusion complex to be collected.

A physical mixture was also prepared to test for possible inclusion by grinding together a 1:1 molar mixture of HPBCD/SDZ for 5 min with a small amount of water (the minimum amount to form a slurry) in an agate mortar.

4.3. Phase-solubility diagram

The phase-solubility diagram was studied according to the method proposed by Higuchi and Connors.[33] A series of HPBCD solutions was prepared with increasing concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 mM. A constant mass of SDZ, in fivefold molar excess relative to the highest concentrated HPBCD solution, was added to each solution and the suspensions were stirred for 48 h in dark conditions. After this period, all suspensions were centrifuged and the supernatants were filtered over 0.45 μm Millipore membranes. The absorbance at 258 nm (ε = 13,066 ± 211 L mol^{-1} cm^{-1}[34]) was then recorded for each solution after 1:100 dilution in a Perkin-Elmer Lambda 45 UV/vis spectrophotometer.

4.4. Characterization of the complexes

Thermal analyses (thermogravimetry—TG and differential scanning calorimetry—DSC) were recorded using TA Instruments models 2960 and 2010, respectively, both with 10 °C/min heating rate and under 100 mL/min N_2 flow. Powder X-ray diffraction (XRD) was measured in a Rigaku diffractometer using Cu-κα (λ = 1,5460 Å) with 40 mA, 40 kV, and scanning rate of 3°/min. All NMR spectra were obtained in a Varian INOVA spectrometer at 500 MHz. ^1H spectra for SDZ and HPBCD were obtained in CD_3OD and D_2O, respectively. HSQC spectrum was obtained in D_2O using relaxation delay 1.5 s and acquisition time 0.232 s. Rotating-frame overhauser effect spectroscopy (ROESY) experiment was carried out with D_2O as solvent, relaxation delay 2.0 s, and mixing time = 2.00 ms.

4.5. Molecular modeling-geometry optimization

In the present work, two different inclusion orientations were considered. An orientation in which NH_2 points toward the narrower HPBCD rim (NH_2-in) and another in which the pyrimidine ring points toward the narrower rim (NH_2-out). The inclusion complex was emulated by entering the guest molecule from one end of the HPBCD molecule and then letting it pass through the host molecule by steps and the dihedral angle between rings change for each step using the MM3 method. The minimum structure found was optimized, without any constraint, with semiempirical PM6[39] and RM1[40] methods implemented within the Cache worksystem 6.1[42] program. Consideration of solvent effects was accomplished with the use of conductor like screening model (COSMO)[43] model implemented within CaChe, with the water dielectric constant (78.4).

Acknowledgments

The authors dedicate this work to Prof. Oswaldo Luiz Alves, from LQES, IQ-Unicamp, on his 60th birthday. Authors are grateful to Prof. I. O. Mazali from IQ-Unicamp. E. K. B. V. acknowledges CAPES for fellowship.

References and notes

1. Sensini, A. *Clin. Microbiol. Infect.* **2006**, *12*, 504.
2. Sukthana, Y. *Trends Parasitol.* **2006**, *22*, 137.
3. Montoya, J. G.; Liesenfeld, O. *Lancet* **2004**, *363*, 1965.
4. Walker, M.; Zunt, J. R. *Clin. Infect. Dis.* **2005**, *40*, 1005.
5. Anderson, A. C. *Drug Discovery Today* **2005**, *10*, 121.
6. Klepser, M. E.; Klepser, T. B. *Drugs* **1997**, *53*, 40.
7. Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045.
8. Loftsson, T.; Hreinsdóttir, D.; Masson, M. *Int. J. Pharm.* **2005**, *302*, 18.
9. Nasongkla, N.; Wiedmann, A. F.; Bruening, A.; Beman, M.; Ray, D.; Bornmann, W. G.; Boothman, D. A.; Gao, J. *Pharm. Res.* **2003**, *20*, 1626.
10. Chen, M.; Ohman, K.; Metcalfe, C.; Ikonomou, M. G.; Amatya, P. L.; Wilson, J. *Water Qual. Res. J. Can.* **2006**, *41*, 351.
11. Babic, S.; Mutavdzic, D.; Asperger, D.; Horvat, A. J. M.; Kastelan-Macan, M. *Cromatographia* **2007**, *65*, 105.
12. Araujo, M. V. G.; Vieira, E. K. B.; Lazaro, G. S.; Conegero, L. S.; Ferreira, O. P.; Almeida, L. E.; Barreto, L. S.; Costa, N. B., Jr.; Gimenez, I. F. *Bioorg. Med. Chem.* **2007**, *15*, 5752.
13. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.
14. Martin Del Valle, E. M. *Process Biochem.* **2004**, *39*, 1033.
15. Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803.
16. Ludden, M. J. W.; Reinhoudt, D. N.; Huskens, J. *Chem. Soc. Rev.* **2006**, *35*, 1122.
17. Harada, A. *Acc. Chem. Res.* **2001**, *34*, 456.
18. Loftsson, T.; Brewster, M. E. *J. Pharm. Sci.* **1996**, *85*, 1017.
19. Gao, H.; Wang, Y. N.; Fan, Y. G.; Ma, H. B. *Bioorg. Med. Chem.* **2006**, *14*, 131.
20. Buschmann, H. J.; Schollmeyer, E. *J. Cosmetic Sci.* **2002**, *53*, 185.
21. Beni, S.; Szakacs, Z.; Csernak, O.; Barcza, L.; Noszal, B. *Eur. J. Pharm. Sci.* **2006**, *29*, 340.
22. Jullian, C.; Miranda, S.; Zapata-Torres, G.; Mendizabal, F.; Olea-Azar, C. *Bioorg. Med. Chem.* **2007**, *15*, 3217.
23. Easton, C. J.; Lincoln, S. F. *Chem. Soc. Rev.* **1996**, *25*, 163.
24. Gould, S.; Scott, R. C. *Food Chem. Toxicol.* **2005**, *43*, 1451.
25. Castronuovo, G.; Niccoli, M. *Bioorg. Med. Chem.* **2006**, *14*, 3883.
26. Brewster, M. E.; Loftsson, T. *Pharmazie* **2002**, *57*, 94.
27. Duchene, D.; Wouessidjewe, D.; Poncel, G. *J. Controlled Release* **1999**, *62*, 263.
28. Irie, T.; Uekama, K. *J. Pharm. Sci.* **1997**, *86*, 147.
29. Davis, M. E.; Brewster, M. E. *Nat. Rev. Drug Disc.* **2004**, *3*, 1023.
30. Gangjee, A.; Kurup, S.; Namjoshi, O. *Curr. Pharm. Des.* **2007**, *13*, 609.
31. Li, N.; Zhang, Y.-H.; Wu, Y.-N.; Xiong, X.-L.; Zhang, Y.-H. *J. Pharm. Biomed. Anal.* **2005**, *39*, 824.
32. Prieto, M. J.; Bacigalupe, D.; Pardini, O.; Amalvy, J. I.; Venturini, C.; Morilla, M. J.; Romero, E. L. *Int. J. Pharm.* **2006**, *326*, 160.
33. Higuchi, T.; Connors, K. A. *Adv. Anal. Chem. Instrum.* **1965**, *4*, 117.
34. Lazaro, G. S. Master Thesis, UFS (2006).
35. Schneider, H.-J.; Hacket, F.; Rudiger, V. *Chem. Rev.* **1998**, *98*, 1755.
36. Torri, G.; Bertini, S.; Giavana, T.; Guerrini, M.; Puppini, N.; Zoppetti, G. *J. Inclusion Phenom. Macrocyclic Chem.* **2007**, *57*, 317.
37. Corti, G.; Capasso, G.; Maestrelli, F.; Cirri, M.; Mura, P. *J. Pharm. Biomed. Anal.* in press, doi:10.1016/j.jpba.2007.07.018.
38. Liu, Y.; Chen, G.-S.; Chen, Y.; Lin, J. *Bioog. Med. Chem.* **2005**, *13*, 4037.
39. Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209.
40. Rocha, G. B.; Freire, R. O.; Simas, A. M.; Stewart, J. J. P. *J. Comput. Chem.* **2006**, *27*, 1101.
41. Barillaro, V.; Dive, G.; Bertholet, P.; Evrard, B.; Delattre, L.; Eric, Z.; Piel, G. *Int. J. Pharm.* **2007**, *342*, 152.
42. CAChe 6.0—Fujitsu, Ltd, Chiba, Japan, 2000.
43. Klamt, A.; Schüürmann, G. *J. Chem. Soc., Perkin Trans. 2* **1993**, 799.