J. Chil. Chem. Soc., 50, N° 3 (2005), págs: 591-596

PHYSICO-CHEMICAL CHARACTERIZATION OF THE INCLUSION COMPLEX BETWEEN A 2-PROPEN-1-AMINE DERIVATIVE AND β-CYCLODEXTRIN

ANA O. DE SOUZA[A*], JOEL B. ALDERETE[B], ADELAIDE FALJONI-ALARIO[C], CÉLIO L. SILVA[A] AND NELSON DURÁN[D,E]

[a]Laboratorio de Bioquímica e Biofísica, Instituto Butantan, Avenida Vital Brasil 1500, CEP 05503-900, São Paulo, S.P., Brazil; [b]Departamento de Orgánica, Casilla, 3-C, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, Chile; [c]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, 05508900, São Paulo, S.P.; Brazil; [d]Departamento de Físico-Química, Instituto de Química, Universidade Estadual de Campinas, 13083-970, Campinas, S.P., Brazil; [e]NCA, Universidade de Mogi das Cruzes, CEP 08780-911, Mogi das Cruzes, S.P., Brazil.

Dirección para correspondencia

ABSTRACT

Inclusion complexes and physical mixtures were prepared with isomeric mixture of E/Z (50:50) of 3-(4'-bromo-[1,1'-biphenyl]-4-yl)-3-(4-bromophenyl)-N,N-dimethyl-2-propen-1-amine (BBAP) and β-cyclodextrin (β-CD) in different proportions. In this study, theoretical calculations using Molecular Mechanics MM+ force field were applied to predict the structures of the inclusion complexes formed by interaction of BBAP and b-cyclodextrin. Circular dichroism, differential thermal analysis (DTA), X-ray diffraction and ^{13}C CP/MAS NMR methods were used to characterize the inclusion complexes and provide information about the stoichiometry of the inclusion complexes. The combined spectroscopy techniques indicate the formation of a complex of BBAP/β-CD in the molar proportion of 1:1 and 1:2 by co-evaporation and no complexation was detected in the physical mixture of the compounds.

Keywords β-cyclodextrin, inclusion complexes, 2-propen-1-amine

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides formed from 6, 7 and 8 D-(+)-glucopyranose units linked by α-1,4 linkages which are obtained from enzymatic starch degradation. These CDs are named α, β and γ, respectively, and have a relatively hydrophobic central cavity and a hydrophilic outer surface.[1] A number of guest molecules form stable host-guest inclusion complexes with cyclodextrins.[2]This

may alter many physicochemical properties of the included molecule[3] and the inclusion complexes can be exploited in the pharmaceutical field to enhance the stability, solubility[4,5] and bioavailability of various drugs.[6] CDs are able to entrap poorly soluble drug molecules of appropriate size and polarity in its cavity to form reversible noncovalent inclusion complexes. Hydrophobic forces and Van der Waals interactions are considered to be the major forces involved in inclusion complex formation.[7]

3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-3-(4-bromophenyl)-N,N -dimethyl-2-propen-1-amine (isomeric mixture - E/Z 50:50) (BBAP figure 1A) is a lipophilic compound with poor solubility. Although it has promising pharmacological activities against *Trypanosoma cruzi*, *Leishmania amazonensis* and mycobacteria[8-11] the cytotoxicity of this drug is known.[12] In order to improve the biological activity and decrease the cytotoxicity of BBAP, the primary aim of this study was to prepare and characterize a physical mixture and an inclusion complex of BBAP with β-CD. Theoretical calculations, differential thermal analysis (DTA), X-ray powder diffractometry, induced circular dichroism (ICD) and solid state ^{13}C NMR spectroscopy were used to characterize the inclusion complexes obtained from co-evaporated systems and the physical mixtures.

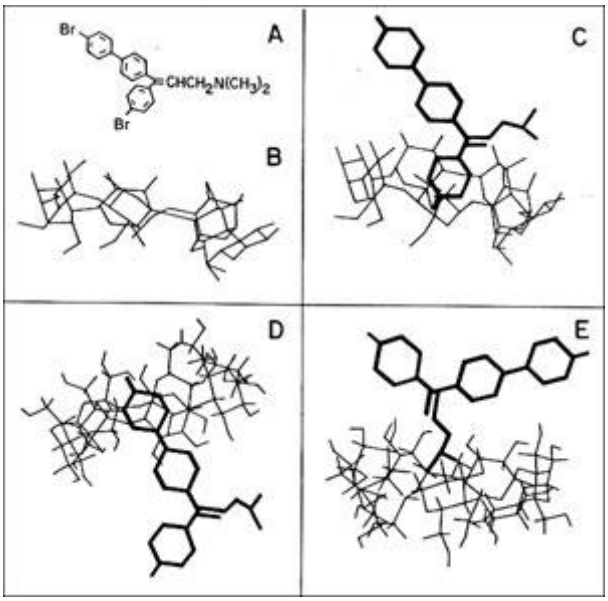

Figure 1. Structure of isomeric mixture of E/Z (50:50) of 3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-3-(4-bromophenyl)-N,N-dimethyl -2-propen-1-amine (BBAP) (**1A**); β-CD (**1B**) and theoretical calculation by Hyperchem force field for inclusion complex BBAP/β-CD 1:1 by the phenyl ring moiety (BBAP-1/β-CD) (**1C**); by the biphenyl ring moiety (BBAP-2/β-CD 1:1) (**1D**) and by N,N-dimethylamine moiety (BBAP-3/β-CD 1:1) (**1E**).

MATERIALS AND METHODS

Materials

The synthesis, purification and characterization of BBAP were performed as previously published.[12] β-CD was purchased from Sigma and was maintained in a desiccator until use. All the other reagents were of analytical grade.

Theoretical calculations

A molecular mechanics study was performed using the MM+ force field as implemented in the HyperChem software.[13] The energies of the complex structures between β-CD and BBAP were minimized considering the molecules in the gaseous state. The geometry optimization was performed to energy convergence with a RMS gradient less than 0.1 kcal $Å^{-1}$ mol^{-1}. Several different orientations of BBAP in the complex were considered. These complexes were designed as: BBAP-1/β-CD for the inclusion of phenyl moiety, BBAP-2/β-CD for the inclusion of biphenyl moiety and BBAP-3/β-CD for the inclusion of N,N-dimethylamino moiety (Figure 1). The complexes of 1:2 stoichiometry were designed as BBAP-12/β-CD, BBAP13/β-CD and BBAP-23/β-CD, where the numbers indicate those moieties included in the b-CDs (Figure 2). The BBAP/β-CD interaction was evaluated taking into account the energy involved in these process.[14]

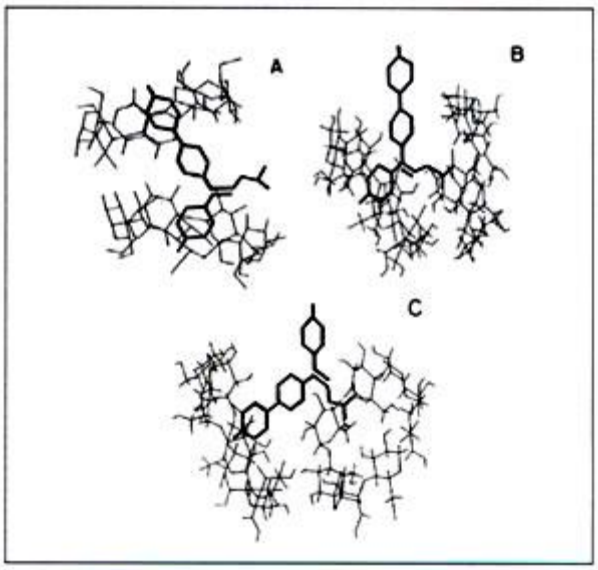

Figure 2. Theoretical calculation by Hyperchem force field for inclusion complex BBAP/β-CD 1:2 by the phenyl and biphenyl ring (BBAP-12/βCD - figure **2A**), by the phenyl and N,N-dimethylamine moiety (BBAP-13/βCD - figure **2B**); by the biphenyl and N,N-dimethylamine moiety (BBAP-23/βCD - figure **2C**).

Induced circular dichroism (ICD)

Induced circular dichroism (ICD) spectra were recorded on a Jasco-720 spectropolarimeter (10 nm/min and 8s response) using a 30 mm cell. BBAP was solubilized in ethanol at 4.5 mmol L^{-1} and 30 µL were added to 3 mL of water to obtain its spectra. To BBAP/β-CD 1:1, 1:2 and 1:3 spectra, 27, 54 and 81 µL of β-CD (5 mmol L^{-1}) were added to the first solution of BBAP, respectively. The sample temperature in the measuring compartment was kept at 25°C. The spectra for BBAP/β-CD were subtracted from those containing β-CD in water, averaged and subjected to Fourier filtering to suppress noise. The emission spectra scans were recorded between 190 and 400 nm. ICD signals were transferred to a computer for data processing and are expressed in ellipticity (deg).

Physical mixtures

BBAP and β-CD in the molar proportion of 1:1 and 1:2 were uniformly and gently mixed with care to avoid any grinding action in the mixture. They were dried under vacuum at room temperature for 6 hours and stored in a desiccator over silica.[15]

Co-evaporate of BBAP and β-CD

The co-evaporated system was prepared to obtain solid inclusion complexes between BBAP and β-CD in the molar ratios of 1:1 and 1:2. BBAP and β-CD were totally dissolved in a mixture of water/ethanol (30:70). After at least two hours after stirring the homogeneous mixture, the solvent was evaporated at 80°C under reduced pressure using a rotatory evaporator. The preparations were completely dried under high vacuum at room temperature and maintained in a desiccator until use.

Differential Thermal Analysis (DTA)

Samples (15 mg) were weighed in the pans and scanned at 10°C/min in the range from 30 to 500°C. The thermal behaviors of the raw materials BBAP and b-CD, as well 1:1 and 1:2 physical mixtures, and complexes were investigated by differential thermal analysis (DTA). The DTA scans were carried out using a computerized Perkin Elmer DTA/TGA under a dynamic N_2 purging gas atmosphere (constant rate of 50 $cm^3\ min^{-1}$).

X-ray powder diffraction

X-ray powder diffraction patterns of the BBAP, b-CD and the BBAP/β-CD preparations were measured in the 2θ range of 5-50° using a Shimatzu X-ray diffractometer. The target was a Cu-tuber (Ni-filter), 35 kv, 15 mA and the detector was a proportional counter with a voltage of 17 kv.

Solid state ^{13}C NMR

Cross polarization magic angle spinning (CPMAS) ^{13}C NMR spectra were recorded at resonance frequency of 75.5 MHz on a BRUKER 300 spectrometer at 25 ^0C, 90^0 ^1H-pulse, 2.0 ms contact time and 4 KHz spinning rate.

RESULTS AND DISCUSSION

Theoretical calculations

The complexed molecules can be included totally or partially in the β-CD cavity and many studies have focused on the ability of CDs to include guests of varying size in different stoichiometric ratios.[16,17] In aqueous solution the inner cavity is occupied by water molecules, which can be readily displaced by guest molecules of lower polarity by hydrophobic effect. For a host-guest complex to be formed, the guest must penetrate at least partially into the cavity. The inclusion phenomenon involves weak interaction such as hydrogen bonding and Van der Waals forces rather than chemical bonding.[18]

The molecular mechanics study of the complexation between BBAP (figure 1A) and β-CD (figure 1B) in the molar proportion 1:1 and 1:2 using the MM+ force field suggested a possible formation of the inclusion complexes of BBAP/β-CD. The formation of the inclusion complex BBAP/β-CD can be represented by the following global process:

$$g + nH \longrightarrow H_n :G$$

Where n=1,2 for 1:1 and 1:2 stoichiometry, respectively, G=BBAP and H=β-CD.

$$\Delta E_c = E_{Hn :G} - [E_G + nE_H]$$

The energy change associate with the formation of inclusion complex, without considering solvent interactions, is given by the equation:

ΔE_c can be defined in two terms in which one is taking place in the guest molecule (ΔE_G), and the other one takes place in the host molecule (ΔE_H) and the mutual interactions between the guest and host molecules (ΔE_I). Thus, the components of E_c can be expressed by:[19]

$$\Delta E_G = E_{G(in\ complex)} - E_{G(isolated)}$$

$$\Delta E_H = E_{nH\ (in\ complex)} - nE_{H(isolated)}$$

$$\Delta E_I = E_{nH:G\ (in\ complex)} - E_{nH\ (in\ complex)} - E_{G(in\ complex)}$$

Where n=1,2 for complexes of 1:1 or 1:2 stoichiometry.

The energies for each isolated species and the most stable complex are displayed in Table 1. The interaction of the BBAP/β-CD 1:1 with inclusion of the phenyl ring of BBAP releases 94.5 kcal/mol (Figure 1C), the inclusion of the biphenyl ring releases approximately 97.3 kcal/mol (Figure 1D) and the inclusion of N,N-dimethylamino moiety requires 95.1 kcal/mol (Figure 1E). Table 2 shows that in all cases $E_c < 0$ which suggests a possible formation of inclusion complexes. The mutual host-guest interactions are the main factors for the stabilization of the inclusion complex between BBAP and β-CD. In the case of BBAP/β-CD 1:2 (BBAP-12/β-CD figure 2A, BBAP-13/β-CD figure 2B, BBAP-23/β-CD figure 2C), the values of total energy change (ΔE_C) are more negative than those of the corresponding BBAP/β-CD 1:1

complexes (BBAP-1/β-CD, BBAP-2/β-CD, BBAP-3/β-CD), which is indicative of more stable structures.Thus, the formation of inclusion complexes with 1:2 stoichiometry is favored. In particular, Table 2 shows that BBAP-12/β-CD structure (*i.e.*, the complex that possesses both biphenyl and phenyl groups included in the β-CD cavity) is largely favored. Therefore, the inclusion of both chromophoric groups in the β-CD cavities should induce changes in the ICD spectrum. Thus, an axial inclusion of the chromophoric group should be expressed as a change in the sign of the ICD bands.[20]The theoretical calculations and the spectroscopic data presented represent the formation of the BBAP-12/β-CD complex, in which the phenyl and biphenyl rings are included. The calculated energy of the 1:3 complex formation using this theoretical method was extremely high, relative to the other complexes, indicating the instability of this complex (E_C = + 6.2 Kcal mol^{-1}).

Table 1. Total energy (Kcal mol^{-1}) for BBAP and β-CD in free state and for BBAP/β-CD inclusion complexes.

Species		Energy
Isolated compounds	BBAP	21.3
	β-CD	83.9
Complex 1:1	BBAP-1/β-CD	94.5
	BBAP-2/β-CD	97.3
	BBAP-3/β-CD	95.5
Complex 1:2	BBAP-12/β-CD	157.9
	BBAP-13/β-CD	163.7
	BBAP-23/β-CD	172.0

BBAP-1/β-CD inclusion complex BBAP/β-CD 1:1 by the phenyl ring moiety;
BBAP-3/β-CD 1:1 inclusion complex BBAP/β-CD 1:1 by the biphenyl ring moiety;
BBAP-3/β-CD 1:1 inclusion complex BBAP/β-CD 1:1 by N,N-dimethylamine moiety;
BBAP-12/βCD inclusion complex BBAP/β-CD 1:2 by the phenyl and biphenyl ring;
BBAP-13/βCD inclusion complex BBAP/β-CD 1:2 by the phenyl and N,N-dimethylamine moiety;
BBAP-23/βCD inclusion complex BBAP/β-CD 1:2 by the biphenyl and N,N-dimethylamine moiety;

Table 2. Total energy change (Kcal mol^{-1}) and their components in the formation of BBAP/β-CD inclusion complexes

Inclusion Complex	ΔE_C	ΔE_G	ΔE_H	ΔE_I
BBAP-1/β-CD	-10.6	2.7	2.0	-15.3
BBAP-2/β-CD	-7.9	2.5	2.0	-12.4
BBAP-3/β-CD	-10.1	1.3	2.5	-13.8
BBAP-12/β-CD	-31.3	1.5	-1.6	-31.4
BBAP-13/β-CD	-25.4	1.7	-3.8	-23.3
BBAP-23/β-CD	-16.9	1.5	2.4	-21.0

BBAP-1/β-CD inclusion complex BBAP/β-CD 1:1 by the phenyl ring moiety;
BBAP-2/β-CD 1:1 inclusion complex BBAP/β-CD 1:1 by the biphenyl ring moiety;
BBAP-3/β-CD 1:1 inclusion complex BBAP/β-CD 1:1 by N,N-dimethylamine moiety;
BBAP-12/βCD inclusion complex BBAP/β-CD 1:2 by the phenyl and biphenyl ring;
BBAP-13/βCD inclusion complex BBAP/β-CD 1:2 by the phenyl and N,N-dimethylamine moiety;
BBAP-23/βCD inclusion complex BBAP/β-CD 1:2 by the biphenyl and N,N-dimethylamine moiety;

Induced circular dichroism (ICD)

In the case of ICD, the inclusion of an achiral molecule leads to circular dichroism signals due to its interaction with the chiral environmental of the CD.[16] ICD provides information on the complexed guest without interference from the signal of the guest free in aqueous solution. Additionally, it has the advantage that the optical activity of a compound may be more specific than absorption, because its determination can only be interfered by another chiral compound. Frequently, the guest/CD complexes are formed with different stoichiometries.[16]

BBAP in an aqueous solution showed one strong negative band near 210 nm, whereas no bands were observed in the spectrum of β-CD as expected (not reported in Figure 3). For BBAP/ β-CD 1:1 there is a positive band around 220-240 nm and to BBAP/ β-CD 1:2 interaction there is a slightly positive band around 218 nm and a negative sign at 224-270 nm. No changes on 257-320 region were observed. This behavior can be related to the perturbation of the electronic transitions of the guest molecule caused by inclusion into the asymmetric cavity of cyclodextrin.[21] The theoretical study and ICD were important to define the preparation of the inclusion complexes in the molar proportion of 1:1 and 1:2.

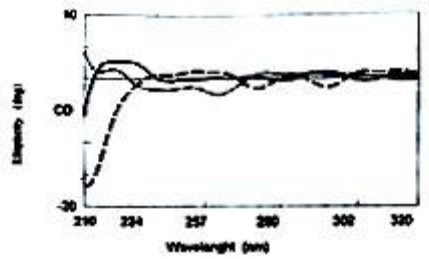

Figure 3. Induced circular dichroism to BBAP 45 μmol L^{-1} (- - -), BBAP/β-CD 1:1 (-) and BBAP/β-CD 1:2 (....).

X-Ray diffraction

For the white solid obtained from the co-evaporated systems (1:1 and 1:2), there was a significant evidence of BBAP/β-CD interaction due to the formation of an inclusion complex (Figure 4). For either 1:1 or 1:2 systems, almost all the peaks of β-CD disappeared or were attenuated due to the guest/host complexation. BBAP is an amorphous solid with an ability to induce amorphization of β-CD. This effect was previously observed by the action of the amorphous carrier Me-β-CD on Naproxen[22] or on Ketoprofen.[23] As illustrated in the figure, this phenomenon was more intense for the BBAP/β-CD 1:1 inclusion complex than that 1:2 complex.

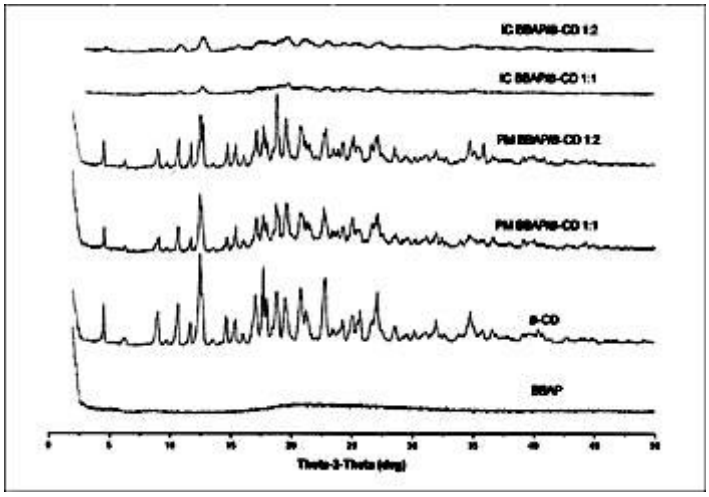

Figure 4. X-Ray powder Diffraction to BBAP, β-CD, physical mixtures (PM) and inclusion complexes (IC) in the molar ratios BBAP/β-CD 1:1 and BBAP/β-CD 1:2. Inclusion complexes were prepared in H_2O/EtOH and distillated at 80°C and high vacuum.

Differential Thermal Analysis (DTA)

The inclusion complexes BBAP/β-CD (1:1 and 1:2) and the physical mixtures of BBAP and β-CD showed a similar DTA behavior (Figure 5). For β-CD, an endothermic effect was recorded, with a peak around 330°C, consuming 3.5°C/g of the sample (endothermic process). BBAP showed a slight exothermic and endothermic process around 101°C and 300 °C, respectively.

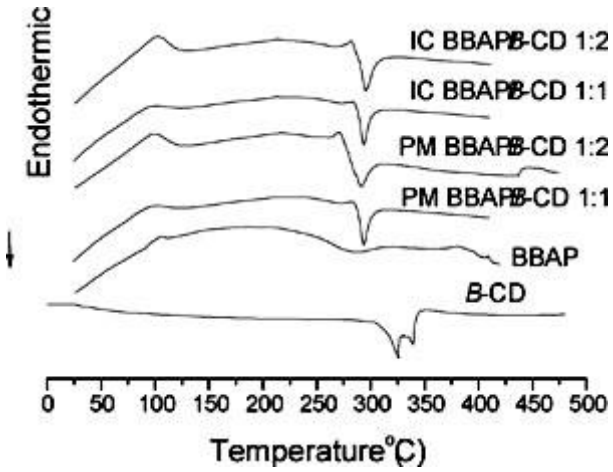

Figure 5. Differential Thermogram Analysis (DTA) to BBAP, β-CD, physical mixtures (PM) and inclusion complexes in the molar ratios BBAP/β-CD 1:1 and BBAP/β-CD 1:2. Inclusion complexes were prepared in H_2O/EtOH and distillated at 80°C and high vacuum.

For the physical mixtures of BBAP and β-CD and for the inclusion complexes BBAP/β-CD, there were a slight sign of exothermic process around 101°C which is due to the melting point of BBAP. The endothermic peak of the β-CD was shifted from 330°C to around 300°C to the inclusion complex and to physical mixtures, which could be a consequence of interaction between the components in the complexes.

^{13}C CPMAS NMR

Figure 6 shows the ^{13}C CPMAS NMR spectra of 1:1 BBAP/β-CD (a), 1:2 BBAP/β-CD (b) inclusion complexes and their respective physical mixtures in molar proportions of 1:1 (c) and 1:2 (d). The b-CD NMR signals are in agreement with the previously reported ones.[24] The appearance of multiple resonance for the b-CD glucopyranose carbons, in the physical mixtures, is indicative of the coexistence of different structural arrangement in solid β-CD. Whereas in the inclusion complexes (spectra (a) and (b)) the multiplicity of signals for each β-CD carbon is reduced, however the centers of the multiple resonance for β-CD carbons do not present significant chemical shift changes. Also, it is possible observe a marked broading of the signals associated to aromatic carbons of BBPA (120-140 ppm region) in both inclusion complexes. Thus, ^{13}C CPMAS NMR spectra show significant differences between the physical mixtures and inclusion complexes. In particular, the lost of multiple

structure of β-CD resonance signals by inclusion complexation also has been observed in other inclusion complexes in solid state.[24,25]

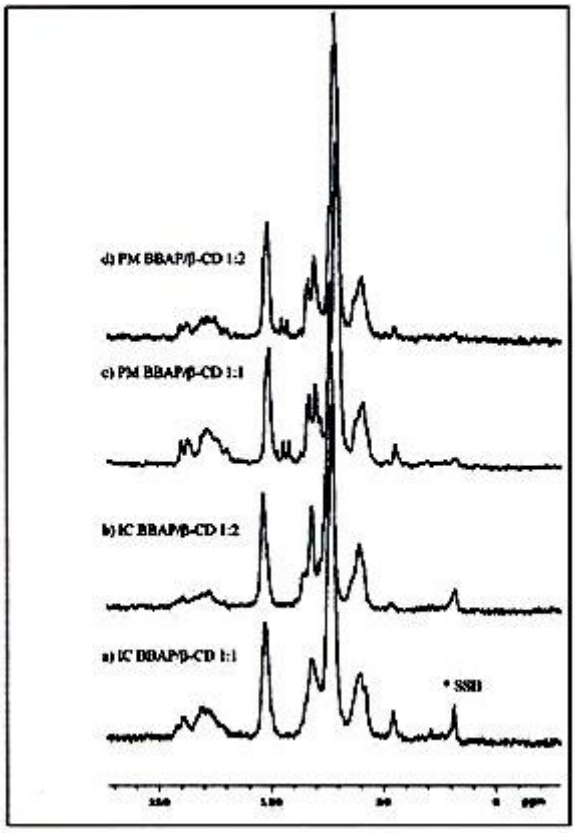

Figure 6. ^{13}C CPMAS spectra of inclusion complexes (IC) and physical mixtures (PM) in the molar ratios BBAP/β-CD 1:1 and BBAP/β-CD 1:2.

CONCLUSIONS

The complexation of BBAP into the β-CD cavity was predicted by theoretical calculations and demonstrated by ICD and ^{13}C CPMAS NMR. The preparation of the inclusion complexes in ethanol or in a mixture of water and ethanol gave solids with similar physical aspects after elimination of the solvent by two different co-evaporation methods.

The combined spectroscopy techniques indicate the formation of a complex of BBAP/b-CD in the molar proportion of 1:1 and 1:2 by co-evaporation. The results confirmed the presence of free crystalline b-CD in the physical mixtures, indicating no complexation of the drugs in this formulation.

ACKNOWLEDGEMENTS

Financial support from CNPq, PRONEX, FAPESP and DIUC (grant 200.023.025-1) is gratefully acknowledged.

REFERENCES

[1] D. C. Bibby, N. M. Davies, I. G. Tucker. Int. J. Pharm. **197**, 1 (2000). [Links]

[2] K.A. Connors. J. Pharm. Sci. **84**, 843 (1995). [Links]

[3] K. A. Connors, A. Paulson, D. J. Toledo-Velasquez. J. Org. Chem. **53**, 2123 (1988). [Links]

[4] G. Dollo, P. Le Corre, M. Chollet, F. Chevanne, M. Bertault, J-L Burgot, R. Le Verge. J. Pharm. Sci.**88**, 889 (1999). [Links]

[5] A. Mucci, L. Schenetti, M. A. Vandelli, B. Ruozi, F. Forni. J. Chem. Research (**S**) 414 (1999). [Links]

[6] S. Keipert, J. Fedder, A. Bohm, B. Hanke. Int. J. Pharm. **142**, 153 (1996). [Links]

[7] S. Nigam and G. Durocher. J. Photochem. Photobiol. A: Chem. **103**, 143 (1999). [Links]

[8] A. O. De Souza, D. N. Sato, D. C. G. Aily, N. Duran. J. Antimicrob. Chemother. **42**, 407 (1998). [Links]

[9] D. G. Pereira, S. L. De Castro, N. Durán. Acta Tropica **69**, 205 (1998). [Links]

[10] A. O. De Souza, R. R. Santos Júnior, J. F. Ferreira-Júlio, J. A. Rodrigues, P. S. Melo, M. Haun, D. N. Sato, N. Durán. Eur. J. Med. Chem. **36**, 843 (2001). [Links]

[11] A. O. De Souza, F. P. Hemerly, A. C. Busollo, P. S. Melo, G. M. C. Machado, C. C. Miranda, R. M. Santa-Rita, M. Haun, L. L. Leon, D. N. Sato, S. L. De Castro, N. Durán. J. Antimicrob. Chemother. **50**, 629 (2002). [Links]

[12] R. De Conti, S. M. N. Gimenez, M. Haun, R. A. Pilli, S. L. De Castro, N. Durán, N. Eur. J. Med. Chem. **31**, 915 (1996). [Links]

[13] M. B. M. De Azevedo, J. B. Alderete, A. C. S. Lino, W. Loh, A. Faljoni-Alario, N. Durán. J. Incl. Phenom. Macrocyclic. Chem. **37**, 67 (2000). [Links]

[14] D. Zhou, Y. Wu, Q. Xu, L. Yang, C. Bai, Z. Tan. J. Incl. Phenom. Macrocyclic. Chem. **37**, 273 (2000). [Links]

[15] H. O. Ammar, M. Ghorab, S. A. El-Nahhas, I. H. Emara, T. S. Makram. Pharmazie **54**, 142 (1999). [Links]

[16] A. Muñoz de la Pena, T. T. Ndou, J. B. Zung, I. M. Warner. J. Phys. Chem. **95**, 3330 (1991). [Links]

[17] V. K. Smith, T. T. Ndou, I. M. Warner. J. Phys. Chem. **98**, 8627 (1994). [Links]

[18] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. Pérez-Maseda. Anal. Chim. Acta **407**, 233 (2000). [Links][19] M. Ohashi, K. Kasatani, H. Shinohara, H. Sato. J. Am. Chem. Soc. **112**, 5824 (1990). [Links]

[20] K. Harata, H. Uedaria. Bull. Chem. Soc. Jpn. **48**, 375 (1975). [Links]

[21] J. A. Schellman. Acc. Chem. Res. **1**, 144 (1968). [Links]

[22] G. P. Bettinetti, A. Gonzzaniga, P. Mura, F. Giordano, M. Setti. Drug Dev. Ind. Pharm. **18**, 39 (1992). [Links]

[23] P. P. Mura, M. T. Faucci, P. L. Parrini, S. Furlanetto, S. Pinzauti. Int. J. Pharm. **179**, 117 (1999). [Links]

[24] L. Cunha-Silva, J.J.C. Teixeira-Dias. J. Phys. Chem. **106**, 3323 (2002). [Links]

[25] S. Lai, E. Locci, A. Piras, S. Porcedda, A. Lai, B. Marangiu. Carbohydr. Res. **338**, 2227 (2003). [Links]