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ORIGINAL ARTICLE

Spectroscopic investigation of β-cyclodextrin -metoprolol tartrate inclusion complexes

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Abstract Aqueous-solution complexes of β-cyclodextrin (β-CD) with metoprolol tartrate (MET) have been analysed with ^{1}H NMR and UV–vis spectroscopy. With ^{1}H NMR a [1:1] stoichiometry could be established for the β-CD-MET complex while its stability constant was determined with UV–vis spectroscopy. Powder diffraction data of a polycrystalline sample of the β-CD-MET complex show that a novel product has been formed, likely to be a β-CD-MET [1:1] inclusion complex. Also Hyperchem MM+ molecular-dynamics results suggest an inclusion complex and from ^{1}H NMR data it is inferred that probably the MET is docked in the CD with the formers methoxyethyl-benzene moiety in front.

Keywords Inclusion complexes · Cyclodextrin · Metoprolol · Cardiovascular adrenoreceptor antagonist · Powder diffraction · NMR · UV–vis · Stoichiometry · Stability constant

Introduction

Host/guest inclusion complexes are frequently used in industry to increase bioavailability, taste, and stability of hydrophobic drugs in aqueous solution and they have also been used as enzyme models. Although host/guest interactions have been analysed with many techniques, depending on the applications envisaged for the complex, most of these techniques are unable to discriminate between internal complexation (= inclusion) and external complexation [1]. In this respect, the analysis of ^{1}H NMR spectral data is particularly useful since from chemical-shift differences potential host–guest docking interactions may be inferred.

Cyclodextrins (CDs), see Fig. 1, are cyclic oligosaccharides that consist of units of α-D-(+)-glucopyranose (six, seven, or eight units, referred to as α-, β- or γ-CD, respectively). A CD molecule may accommodate in its hydrophobic cavity a docked-in guest molecule, so together they form a host–guest inclusion complex (IC) [2].

Racemic metoprolol tartrate [(R, S)-3[4-methoxyethyl] phenoxy)-1(isopropyl amino) propan-2-ol] tartrate; $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$; MET, see Fig. 2] is a cardioselective β_1-adrenoreceptor antagonist (β_1-blocker) and one of the most frequently drugs used in the treatment of cardio-vascular (related) diseases, e.g. hypertension, angina pictoris and cardiac dysrhytmias [3].

An important reason to study β-CD/MET ICs is potential enantiomeric separation. ICs of MET with carboxy-

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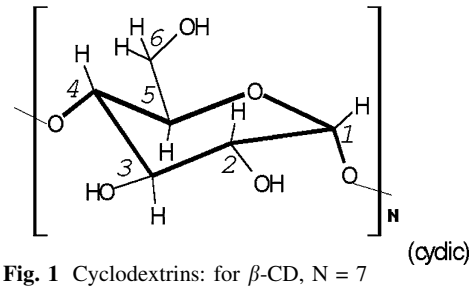

Fig. 1 Cyclodextrins: for β-CD, N = 7

methyl-β-CD (CMCD) have been studied for this reason [4]. Preliminary data [5] on usage of MET/β-CD complex in reversed-phase HPLC indicate the potential application of this complex as a kind of pre-column derivatization for enantiomeric separation of beta(1)-blockers. Quite recently, the influence of the CD cavity size on the MET/CD inclusion structure was reported [6, 7]. Thermodynamic parameters (the enthalpy and the entropy for the complexation) for the MET/β-CD system based on a Van't Hoff plot have also been reported [7].

In this paper the β-CD-MET complex is analysed with a combination of analytical techniques, ^{1}H NMR, UV–vis and X-ray powder diffraction (XRPD), and molecular modelling. It is shown that with these techniques the occurrence of an inclusion complex can be ascertained, the stoichiometry and stability constant of this guest–host inclusion complex can be determined and docking-in information regarding the guest molecule can be obtained.

Materials and methods

Synthesis

MET, obtained from ''*Helcor*'' (Baia Mare, Romania), is a water-soluble drug. β-CD (having ≤15% in water weight) was purchased from Merck (Germany) and was used without further purification.

A slurry of the host–guest inclusion complex of β-CD (host, *H*) and MET (guest, *G*), obtained by a kneading procedure, was dissolved in water. The solution was stirred 24 h at 40 °C, and subsequently kept isothermal at 50 °C for 2 days. Finally, a polycrystalline precipitate was obtained by slow cooling to room temperature until all water had evaporated.

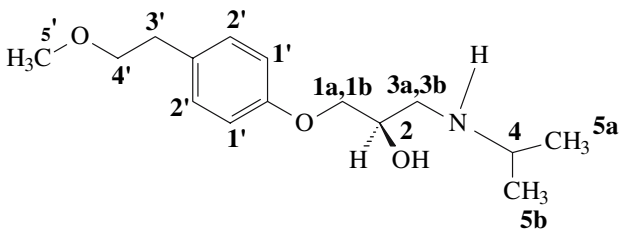

Fig. 2 Metoprolol molecule

Aqueous solutions of the β-CD-MET complex were prepared from millimolar (mM) mother solutions of *G* and *H* in D_2O, while taking the molecular water present in β-CD into account. Two 5 mM starting solutions of β-CD and of MET in D_2O (ROMAG, Turnu Severin, Romania), respectively, were prepared. On basis of these mother solutions, several mixtures of constant volume were prepared, having different molar ratios of *G* and β-CD. The sum of the total concentration $M = ([\beta\text{-CD}]_t + [G]_t)$ (the subscript t refers to the total concentration) was kept constant at 5 mM The molar ratio of *G*, $r_G = [G]_t/([\beta\text{–CD}]_t + [G]_t)$ was varied from 0 to 1 in steps of at 0.1.

X-ray powder diffraction data collection

A high-resolution XRPD pattern was recorded at beamline BM01B of the Swiss-Norwegian CRG at the European Synchrotron Radiation Facility (ESRF, Grenoble) with a fixed wavelength of 0.79942 Å at room temperature ($T \sim 301$ K). Data collection was carried out in a continuous scan mode from 1.03 to 35.485°2θ using a filled capillary (diameter 0.7 mm.) that was rotated during exposure. After data collection the scan was binned at 0.005°2θ.

^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded for all solutions with a *Bruker Avance 400* spectrometer after 15 min of equilibration. The spectrometer was operated at 400.13 MHz, with the following parameters: 16384 data points, pulses of 90°, 2s delay between scans (32 scans) and a digital resolution of 0.588 Hz/point. Chemical shifts were expressed in parts per millions (ppm) relative to those of the HOD signal located at 4.32 ppm.

UV–vis spectrocopy

Six aqueous mixtures were prepared from a solution of 0.2 mM MET and six solutions of increasing β-CD concentration: 0.01; 0.015; 0.020; 0.03; 0.04; 0.06 and 0.08 M. The aequous mixtures were stored at room temperature for 12 h before collecting UV–vis spectra using standard quartz cells.

The stoichiometry of the inclusion complexes

The stoichiometry of the complex was obtained by using the well-known continuous variation method, i.e. Job's method [8]. Let us assume that a complex C, having the [1:n] guest:host stoichiometry, is formed:

$$G + n\beta\text{-CD} \rightleftarrows \text{C} \tag{1}$$

The symbol δ_f will represent the chemical shift of a proton of a given species X (X = G, β-CD) when they are found to be free in solution; δ' the observed chemical shift when the observed species is found in the presence of the other; δ_C the chemical shift of a proton in the IC, the last one can not be measured experimentally but can be calculated. The following differences are also defined:

$$\Delta\delta_{\text{obs}} = \delta_f - \delta' \tag{2}$$

$$\Delta\delta_C = \delta' - \delta_C \tag{3}$$

If there is a rapid exchange between the free and bound states for the X species, each proton will give a unique, averaged signal. In this case, the product of $\Delta\delta_{obs}$ by the total concentration of the analyzed substance, $[X]_t$, i.e, $\Delta\delta_{obs}[X]_t$, will be proportional to [C].

By plotting $\Delta\delta_{obs}[X]_t$ vs. r_G one obtains a curve, its maximum indicating the value of r_G at which the maximum concentration of the complex is achieved. This value is related to n by [9, 10]:

$$r_G^{\max} = 1/(1+n) \tag{4}$$

that allows the determination of the stoichiometry of the obtained inclusion complexes.

Association constant determination by UV–vis spectrophotometry

Various methods for the determination of the association constant have been described in literature, using techniques such as [11–14]: conductometric titration, potentiometric and spectroscopic methods, solubility studies, etc. Although the association constant K_C can be derived from ^{1}H NMR data, using chemical-shift differences, its value will depend on the proton taken into consideration. Therefore, we prefer the use of UV–vis spectroscopy to determine the stability constant.

In order to determine the stability (association) constant, the difference in absorbance between free MET and the β-CD-MET IC was determined at 284 nm. The stability constant K_C for the β-CD-MET IC was calculated in agreement with the Scott equation [15]:

$$\frac{[\text{MT}] \cdot [\text{CD}]}{d} = \frac{1}{K_C \cdot \varepsilon} + \frac{[\text{CD}]}{\varepsilon} \tag{5}$$

where d is the change in absorbance between free MT (MET) and β-CD-MET and ε represents the difference in the corresponding molar absorptivities.

Molecular modeling

Guest molecules can have interaction with the hosting CD via the latters OH groups, by Van der Waals forces, electrostatic forces and hydrogen bonding [16]. The interaction may be external but also internal. In the latter case, the guest enters the CD cavity, thus forming an inclusion complex. To assess the precise type of complex, a starting model was built by positioning the MET molecule at the larger side of the β-CD cavity. The geometry of the complex was optimized in vacuum using the molecular- mechanics algorithm of the HyperChem software [17]; the details are given elsewhere [18]. The well-known MM+ (with the Polak–Ribière conjugate gradient) method was used to minimize the energy of the structures until a RMS gradient lower than 0.015 kcal mol^{-1} Å^{-1} was obtained.

Results and discussion

X-ray powder diffraction analysis

A comparison of the experimental powder diffraction patterns for MET, β-CD and the β-CD-MET inclusion complex (Fig. 3.) points out that the 2θ-positions of the diffraction maxima in the β-CD-MET pattern differ from those in the patterns of MET and β-CD, thus implying that indeed a novel compound has been formed with lattice parameters that differ from those of the starting compounds. Attempts to determine the crystal structure of this inclusion complex on the basis of the powder data are in progress but are hampered by the presence of a large amorphous halo in the pattern (Fig. 3.)

The stoichiometry of the β-CD-MET inclusion complex

^{1}H NMR spectra for several aqueous solutions of β-CD and MET are presented in Figs. 4a and b. The degree of interaction between different protons of the β-CD and MET can be estimated by plotting the chemical shifts of the β-CD and G vs. r_G. From analysing the results of applying Job's method to the complexes (Figs. 5, 6), it was established that MET forms a [1:1] stoichiometric complex with β-CD.

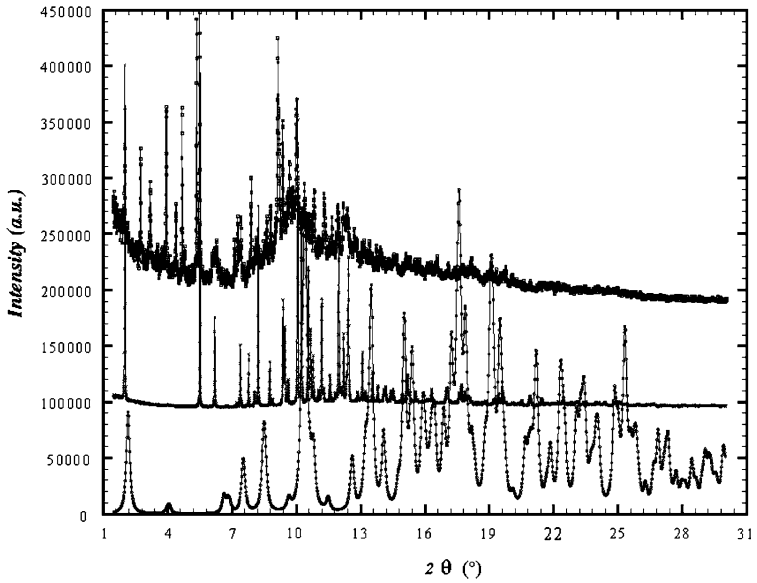

Fig. 3 Powder diffraction patterns for: β-CD (bottom), MET (middle) and the inclusion complex (top), respectively.

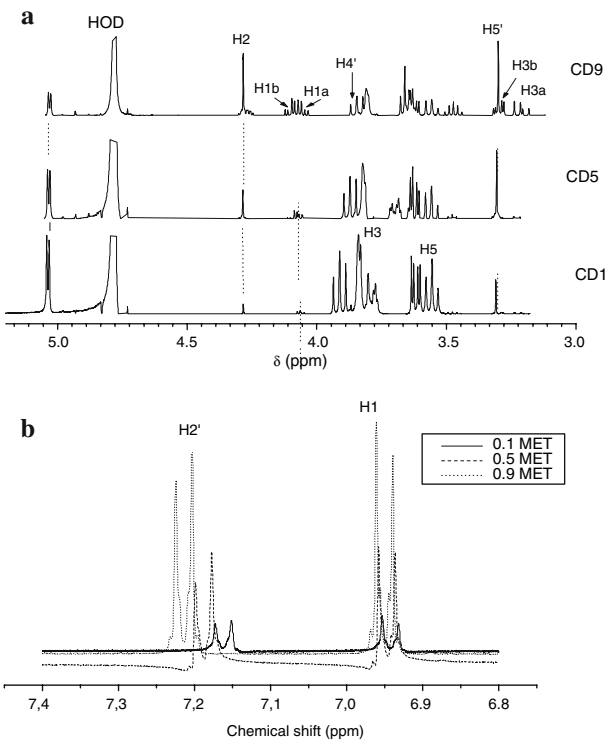

Fig. 4 (**a**) ^{1}H NMR spectra for different molar ratios of β-CD and MET. Legend: r = 0.1 for CD1; r = 0.5 for CD5 and r = 0.9 for CD9. (**b**) ^{1}H NMR spectra for different molar ratios of β-CD and MET. Legend: r_{MET} = 0.1; r_{MET} = 0.5 and r_{MET} = 0.9

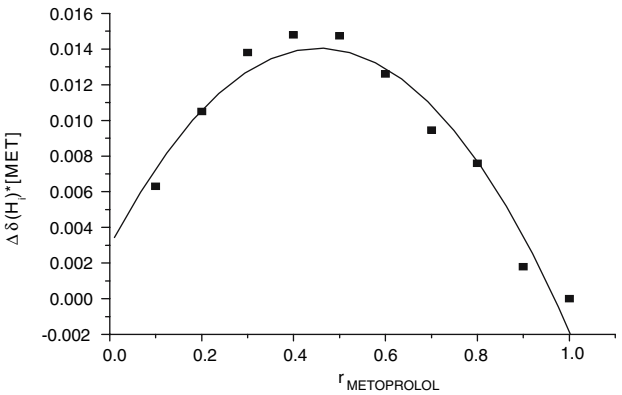

Fig. 5 Job's plot for MET (vs. the MET molar fraction) (■: H1′). Origin B-spline curve was fitted to the experimental points [19]

The stability constant from UV–vis data

The UV–vis spectra of aqueous solutions of MET and the [1:1] β-CD-MET mixture (Fig. 7) show that in presence of β-CD the UV absorption maximum is shifted to a longer wavelength with a concomitant increase of the intensity. Using Scott's equation, for the [1:1] β-CD-MET complex a K_C value of 250 (±19) M^{-1} for was obtained. This is comparable to stability constants K_C for (R)-and (S)-MET that have been reported [4] as being 288 and 262 M^{-1}, respectively, both with CMCD as host molecule, and 440 M^{-1} for β-CD [7], respectively. The last value, derived from ^{1}H NMR data, strongly depends on the protons taken into consideration [20]. As stated earlier, we prefer the value obtained by using Scott's equation, as a global inclusion process value. Fig. 8

The β-CD MET inclusion process

The ^{1}H NMR data for the protons in the cyclodextrin in β-CD-MET show significant chemical shifts for the protons H3 and H5 that are situated inside the CD torus. Significant

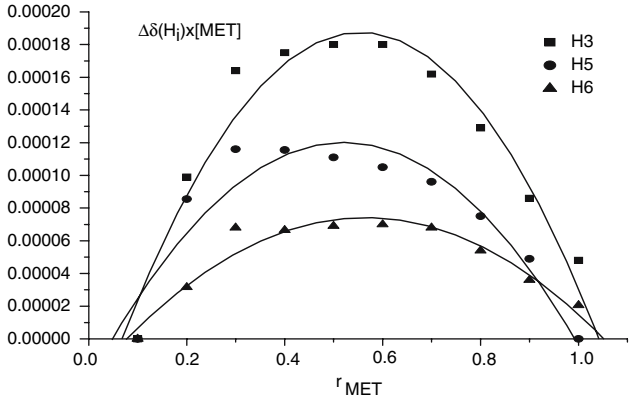

Fig. 6 Job's plots for β-CD (vs. the molar fraction of MET) (■: H3; •: H5; ▲: H6). Origin B-spline curves were fitted to the experimental points [19]

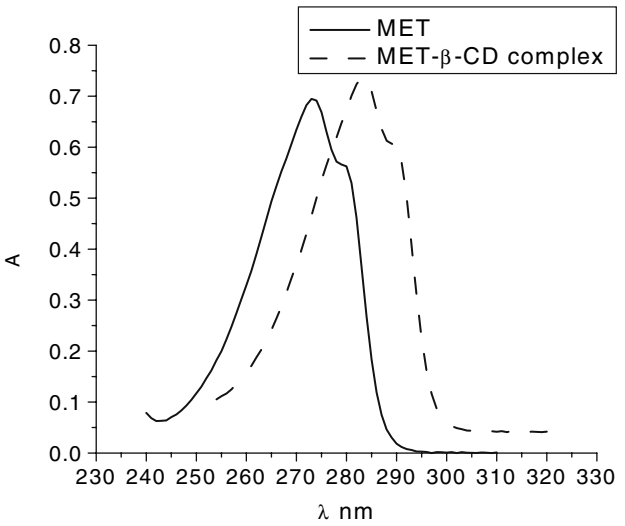

Fig. 7 UV spectra of MET and MET/β-CD complex

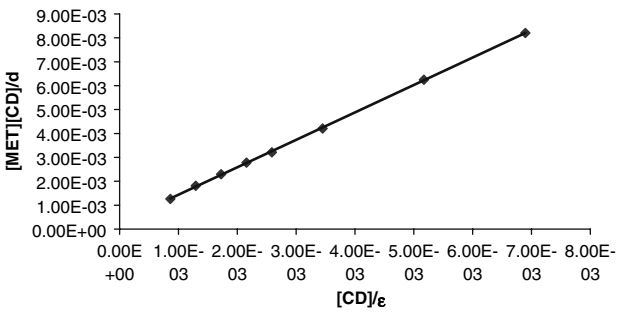

Fig. 8 The stability constant determination for the β-CD-MET complex

chemical shifts are also observed for the protons H5′, H4′ and H3′ and for the protons that are bonded to MET's benzene ring. From this it can be inferred that MET enters the CD cavity with this moiety.

The results of the geometry optimisation in vacuum using the MM+ molecular mechanics calculations, shown in Fig. 9, are in line with the ^{1}H NMR data. Obviously, an inclusion compound is formed although it cannot be decided whether the MET enters the CD-cavity at the primary or secondary side [7] because of the limitations of the approximations, (i) a system in vacuum was used and (ii) the influence of the water molecules has been neglected.

Conclusions

From an analysis of the ^{1}H NMR data, a [1:1] stoichiometry was established for the aqeuous β-CD-MET system. The stability constant of the β-CD-MET complex was calculated as 250 M^{-1} from UV–vis data. This value is of the same order of magnitude as reported for MET/CMCD complex. Thus, NMR and UV–vis spectroscopy can be used to derive satisfactory both the stoichiometry and the association constant of inclusion complexes.

On the basis of X-ray powder diffraction data, it has become clear that the MET and β-CD form a novel crystalline compound, likely to be an [1:1] inclusion complex, as inferred from^{1}H NMR and molecular modelling data.

An analysis of the chemical shifts observed in the ^{1}H NMR spectra led to the tentative conclusion that the methoxyethyl-benzene moiety of MET probably enters the cyclodextrin torus when forming the β-CD-MET inclusion complex. Molecular mechanics calculations are consistent with this model but a more detailed interpretation is not possible due to the MM+ approximations used. More work needs to be done to establish the nature [16] of the forces implied in the inclusion process and in particular the

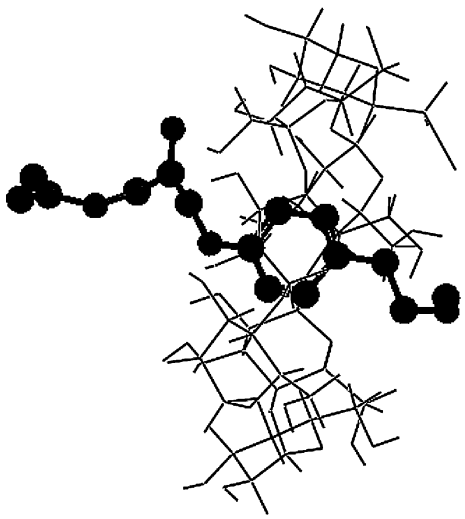

Fig. 9 The structure of the β-CD-MET complex obtained with the Hyperchem software

expulsion processes of the intracavity water molecules must be taken into account [21].

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References

1. Moloney, G., Craik, D.J., Irkouder, M.N., Nero, T.L.: Synthesis, NMR studies and conformational analysis of oxazilidine derivatives of the beta-adrenoreceptor antagonists metoprolol, atenolol and timolol. J.C.S. Perkin Trans. **2**, 199–206 (1998).
2. Szjejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998).
3. Ficarra, R., Ficarra, P., Di Bella, M.R., Raneri, D., Tommasini, S., Calabrò, M.L., Gamberini, M.C., Rustichelli, C.: Study of beta-blockers/beta-cyclodextrins inclusion complex by NMR, DSC, X-ray and SEM investigation. J. Pharm. Biomed. Anal. **23**, 33–40 (2000).
4. Park, K.L., Kim, K.H., Jung, S.-H., Lim, H.M., Hong, Ch.H., Kang, J.S.: Enantioselective stabilization of inclusion complexes of metoprolol in carboxymethylated β-cyclodextrin. J. Pharm. Biomed. Anal **27**, 569–576 (2002).
5. Radulovic, D.M., Karljikovic-Rajic, K.D., Lucic, B.M., Vujic, Z.B.: A preliminary study of beta-cyclodextrin/metoprolol tartrate inclusion complex for potential enantiomeric separation. J. Pharm Biomed. Anal. **24**, 871–876 (2001).
6. Bratu, I., Gavira-Vallejo, J.M., Hernanz, A.: ^{1}H-NMR study of the inclusion processes for α- and γ-cyclodextrin with fenbufen. Biopolymers **77**, 361–367 (2005).
7. Ikeda, Y., Hirayama, F., Arima, H., Uekama, K., Yoshitake, Y., Harano, K.: NMR spectroscopic characterization of metoprolol/cyclodextrin complexes in aqueous solution: cavity size dependency. J. Pharm. Sci. **93**, 1659–1671 (2004).
8. Job, P.: Formation and stabitity of inorganic complexes in solution. Ann. Chem. **9**, 113–203 (1928).
9. Bogdan, M., Farcas, S.I., Bojita, M.: Nuclear magnetic resonance study of naproxen and ibuprofen interaction with beta-CD in solution. Studia Univ. Babes-Bolyai, Ser. Phys. Special issue 115–120 (2000).
10. Bratu, I., Gavira-Vallejo, J.M., Hernanz, A., Bogdan, M., Bora, Gh.: Inclusion complex of fenbufen with β-cyclodextrin. Biopolymers-Biospectroscopy **73**, 451–456 (2004).
11. Saenger, W.: Cyclodextrin inclusion compounds in research and industry. Angew. Chem. Int. Ed. Engl. **92**, 343–361 (1980).
12. Li, S., Purdy, W.C.: Cyclodextrins and their applications in analytical chemistry. Chem. Rev. **92**, 1457–1470 (1992).
13. Junquera, E., Aicart, A.: Potentiometric study of the encapsulation of ketoprophen by hydroxypropyl-β- cyclodextrin. Temperature, solvent, and salt effects. J. Phys. Chem. B **101**, 7163–7171 (1997).
14. Tran, Ch.D., De Paoli Lacerda, S.H.: Determination of binding constants of cyclodextrins in room-temperature ionic liquids by near-infrared spectrometry. Anal. Chem. **74**, 5337–5341 (2002).
15. Scott, R.L.: Some comments on the Benesi-Hildebrand equation. Rec. Trav. Chim. **75**, 787–789 (1956).
16. Liu, L., Guo, Q.-X.: The driving forces in the inclusion complexation of cyclodextrins. J. Inclus. Phenom. Macro. Chem. **42**, 1–14 (2002).
17. HyperChem^{TM} Release 4, Hypercube, Inc. (1994), Waterloo, Ontario N2l 3X2, Canada.
18. ''Computational Chemistry'', Hypercube, Inc. Waterloo, Ontario N2l 3X2, Canada (1994).
19. Origin® 7.0, OriginLab Corporation, Northhampton, MA 01060, USA.
20. Zubiaur, M., Jaime, C.: Complexation between tert-butyl ketones and β-cyclodextrin. Structural study by NMR and MD simulations. J. Org. Chem. **65**(24), 8139–8145 (2000).
21. Gavira, J.M., Hernanz, A., Bratu, I.: Dehydration of β-cyclodextrin: An IR ν (OH) band profile analysis. Vib. Spectrosc. **32**(2), 137–146 (2003).