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Study of structural features and thermodynamic parameters, determining the chromatographic behaviour of drug–cyclodextrin complexes

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Abstract

The chromatographic behaviour of host–guest inclusion complexes was studied, in order to predict the optimal conditions for their accurate analysis and overcome the significant analytical errors generated by the presence of cyclodextrins. Complexes of tolfenamic acid and ketoprofen with β-cyclodextrtin (βCD), 2-hydroxypropyl-βCD (HPβCD) and methyl-βCD (MeβCD) prepared in different molar ratios, were studied. Since the drug release from cyclodextrins' complexes is a prerequisite for its accurate quantitation, several parameters affecting the dissociation during the analysis were evaluated. In an attempt to explain the drug release mechanism from cyclodextrins, during HPLC analysis, the possible correlation of the NMR structural findings with the binding constants and the thermodynamic quantities of complexation were examined, in relation to their chromatographic behaviour. Finally, the presence of the solvation spheres around the supramolecules, which affect the complex stability, is suggested to be crucial for our chromatographic findings. Particularly, entropy change in the system is considered the most critical factor, determining the time required for dissociation of drug–cyclodextrin complexes, during drug quantitation.

Keywords: Ketoprofen; Tolfenamic acid; CDs; Dissociation; Thermodynamics; Binding constant

1. Introduction

The stability of drug–cyclodextrin (drug–CD) complexes, as a binary equilibrium, is extensively investigated and most studies focus on the binding constant (K_c) and the related thermodynamic parameters (ΔC_p, ΔH, ΔS, and ΔG). Nevertheless, the chromatographic behaviour of complexes remains unpredictable. It is difficult to find information about multicomponent systems with several coexisting equilibria, such as chromatographic systems including host–guest complexes. Interactions involved in these systems can be hydrophobic, electrostatic, hydrogen bonds, etc. and contribute to the thermodynamic profile of the host–guest system.

Several parameters, which are considered to be significant for chromatographic separations, e.g., ionic interactions and lipophilicity, have been extensively studied in the literature. However, the well known rules, that generally govern the chromatographic performance, provide incomplete interpretation of the observed phenomena during the analysis of drug–CD complexes or stereoselective analyte–selector interactions. The quite autonomous drug–CD binary interactions can be affected by the presence of other components, which consist part of multiple equilibria. In detail, supramolecular chromatography can be characterized not only by the classical interactions between the solutes, the stationary phase and the mobile phase components but also, by further interactions of all the above with the complexed guest and free host molecules.

In such a system, the addition of any interfering component can modify the already existing equilibrium. As a result, significant differences in the adsorption, partitioning and exclusion phenomena arise and the overall chromatographic behaviour changes.

Cyclodextrins are known to alter the absorptivity of guest molecules [1–4] therefore, analytical methods that are based on the spectrophotometric data, present accuracy problems [5–7]. These inaccuracies have generated the need for specific analytical methods able to determine the drugs as free molecules. The results derived from our experiment are presented in Table 1.

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Table 1
Relative errors (%) for ketoprofen and tolfenamic acid CD complexes calculated during UV analysis

Drug–CD molar ratio	Relative errors (%)		
	βCD	MeβCD	HPβCD
Tolfenamic acid			
1:0.5	+1.0	–	−2.1
1:1	+1.6	+0.8	–
1:2	+3.1	+1.6	+1.8
1:6	−2.4	−3.9	−3.1
1:10	−6.8	−4.7	−5.5
1:20	−7.8	−8.4	−6.3
1:40	−11.0	−14.7	−8.6
Ketoprofen			
1:1	+16.1	+4.6	+3.7
1:2	+37.6	+0.7	−1.2
1:40	+20.8	−8. 6	−5.1

The purpose of this work was to investigate the chromatographic behaviour of host–guest complexes. Two widely prescribed non-steroidal anti-inflammatory drugs, tolfenamic acid (TO) and ketoprofen (KT), with structural similarities were selected and their complexes with βCD, 2-hydroxypropyl-βCD (HPβCD), and methyl-βCD (MeβCD) were prepared and studied in mixtures containing different drug–cyclodextrin molar ratios. During drug quantification with a common HPLC method [8,9], the measured analytical relative errors for TO and KT varied from +3.1 to −14.7% and from +37 to −8%, respectively (the relative error (%) of the method is defined as $E_r\% = (E_r/\mu) \times 100 = [(x_i - \mu)/\mu] \times 100$, where x_i is the calculated concentration of the drug and μ the theoretical concentration).

Our previous investigations, which resulted in specific HPLC methods [5–7,10], demonstrated that the extent of drug release from the drug–CD complexes before detection, can be considered a crucial parameter, as the not dissociated fraction of complex causes the detected errors. In order to achieve drug release from CDs' cavity, complexation thermodynamics must be altered in such a way that the binding of any competitive compound to CDs is favoured and the drug's inclusion in the CDs' cavity unfavoured.

In the case of TO–CDs complexes the drug was released before the detection, by modification of the HPLC conditions [5]. On the contrary, in the case of strongly bound complexes, like KT–CDs, a chemical reagent, e.g., 1-adamantanol, which shows better binding affinity towards the CD cavity, must be used as displacer of the included drug [10].

Therefore, several parameters affecting the release from the CDs complexes, during the analysis, were evaluated. The effect of complexes' structure on the dissociation time, in relation to the relative magnitude of thermodynamic parameters was interpreted by evaluating the chromatographic behaviour.

Specific RP-HPLC methods, for the quantitation of the free drug molecules, were applied for the analysis of the samples collected during the phase solubility experiments. K_c values, characterizing the prepared drug–CD complexes, were determined over a wide range of temperatures. From the comparison of the 26 calculated K_c values it can be concluded that they provide insufficient information to explain the observed differences in the dissociation characteristics and to predict the necessary sample handling for an accurate analysis.

For better understanding the decomplexation mechanism, the thermodynamic quantities of complexation (ΔH and ΔS) were calculated with the aid of van't Hoff plots. Furthermore, in an attempt to find a rational explanation for the diverged properties among the prepared complexes, their structural features were studied by ^{1}H NMR spectroscopy techniques and the effect of the supramolecular structures on the chromatographic performance was, then, interpreted.

2. Experimental

2.1. Chemicals

Tolfenamic acid [3-[(3-chloro-2-methylphenyl)aminobenzoic] acid and ketoprofen [(*RS*) 3-benzoyl-α-methylbenzenacetic acid] were supplied by the courtesy of ELPEN Ph. (Athens, Greece). KT was used as a racemic mixture of the two enantiomers. βCD, randomly methylated βCD and 2-hydroxypropyl-βCD (molar degree of substitution 0.8) were purchased from Sigma (St Louis MO, USA). HPLC grade methanol and water were purchased from Carlo Erba (Modena, Italy). The mobile phases were vacuum filtered and degassed through 0.45 μm pore PTFE membranes with the aid of the Millipore filtration system, from Millipore (Milford, Massachusetts, USA). The buffers, used during the development of the methods, were prepared with phosphate salts. Deuterated solvents were obtained from Merck (Darmstadt, Germany). The extraction cartridges, employed for solid-phase extraction (SPE) technique were 3 ml/500 mg isolute-NH_2 (amino propyl, IST, UK).

2.2. Equipment

2.2.1. HPLC

Experiments were carried out using a Waters high-performance liquid chromatograph (Alliance 2690 Separation Module), equipped with a photodiode array detector, a constant temperature oven and an autosampler. Millenium 32 was used as software facility. The chromatographic columns were: a Spherisorb ODS2 125 mm × 4.0 mm (5 μm particle size) and a LiChrospher C_{18} 150 mm × 4.6 mm (10 μm particle size), both purchased from MZ (Mainz, Germany).

2.2.2. SPE

For the extraction of KT, IST Vacmaster (International Sorbent Technology, UK) was used and the glass chamber was vacuum controlled via manometer. The pressure was

regulated to 0.5 bar as to achieve a constant flow rate of 1.0 ml min^{-1}.

2.2.3. ^1H NMR spectroscopy

^1H NMR spectra were recorded on a Brucker DRX 400 MHz spectrometer. The probe temperature was regulated at 298 K. All chemical shifts were related to external standard sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) 1% (w/w). The two-dimensional (2D) rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectra were acquired with 256 number of scans and mixing time 250 ms at transmitter attenuation 65.0 dB.

3. Methods

3.1. Preparation of the complexes

For the preparation of the drug–CD complexes aliquots of two aqueous solutions, containing around 3.93×10^{-5} M KT and 1.88×10^{-4} M TO, respectively, were mixed with solutions containing appropriate quantities of βCD, HPβCD and MeβCD in order to obtain final solutions of drug–cyclodextrin molar ratios 1:1, 1:2, and 1:40. The concentration of KT in the final solutions was around 1.97×10^{-5} M and of TO around 1.69×10^{-4} M.

The inclusion complexes of TO with βCD, HPβCD, and MeβCD were stirred at constant temperature until equilibration. The complexation process with MeβCD and HPβCD was completed faster than with βCD. Correspondingly KT–MeβCD and KT–HPβCD solutions were shaken at constant temperature for only 24 h, instead of 7 days needed for the equilibrium of KT–βCD to be reached. In all cases the achieved equilibrium was determined by measurements with UV Spectroscopy.

3.2. High-performance liquid chromatography methods

3.2.1. Tolfenamic acid

The HPLC conditions applied for TO quantitation were as follows: the column was a 5 μm LiChrospher C_{18} 150 mm × 4.6 mm regulated at 30 °C, the mobile phase consisted of methanol and 40 mM phosphate buffer pH 3.2 (90:10, v/v), the flow rate was 2.0 ml min^{-1} and the detector wavelength was set at 286 nm.

3.2.2. Ketoprofen

The complex samples were pre-treated with 1-adamantanol, which was used as a competitive agent. Then the samples were loaded on SPE amino-propyl cartridges and free KT was extracted. Further details on the method can be found in our previously published work [10]. The extracts were analysed with a routine HPLC method for KT determination. The HPLC conditions were as follows: the column was a 5 μm Spherisorb ODS2 125 mm × 4.0 mm regulated at 50 °C, the mobile phase consisted of methanol and 40 mM phosphate buffer pH 8.0 (40:60, v/v), the flow rate was 1.0 ml min^{-1} and the detector wavelength was set at 260 nm.

3.3. Phase solubility experiments

Phase solubility experiments were performed according to the technique described by Higuchi and Connors [11]. In saturated aqueous mixtures of TO and KT, appropriate amounts of all the CDs studied, were added. The final concentrations of CDs varied from 5.4×10^{-4} M to 141×10^{-4} M in TO mixtures and from 5.0×10^{-3} M to 200×10^{-3} M in KT mixtures. Ten to twelve mixtures were prepared for each experiment. The mixtures were shaken in a constant temperature bath at various temperatures (±0.5 °C) for up to 3 days and aliquots of the equilibrated samples were filtered in vials already stored at the same temperature. Accurate volumes of the filtrate were diluted to the desired concentration range, before being injected into the chromatograph.

3.4. Determination of the binding constants

The binding constants of the studied complexes, in aqueous solutions, were calculated from the slope and the intercept of the linear part of the phase solubility diagrams [11], using the following equation:

$$K_c = \frac{S_t - S_0}{S_0\{[CD]_t - (S_t - S_0)\}} = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_t is the solubility of the complex, S_0 the solubility of free drug corresponding to the intercept of the solubility axis, and $[CD]_t$ is he concentration of the non-complexed CD.

The K_c values were also determined at 25, 30, 37, 45, and 52 °C.

3.5. Determination of thermodynamic quantities

The enthalpy and entropy changes of complexation (ΔH and ΔS), for the studied CD complexes of TO and KT were determined from the van't Hoff plots. The $\ln K_C$ values were plotted versus $1/T$. The ΔH quantities were calculated from the slope and ΔS from the intercept of the derived plots.

3.5.1. ^1H NMR spectroscopy

The ^1H NMR spectra of KT were recorded in mixtures of 9.96 ml 99.8% ^2H_2O and 40 μl NaO^2H 40%. ^1H NMR spectra of TO were recorded in mixtures of 9.9 ml 99.8% ^2H_2O and 10 μl of 99.5 NaO^2H 40%.

For the self-titration experiments [12], KT and TO sodium salt solutions were prepared. The concentration of the first KT solution was 0.5×10^{-3} M and was increased stepwise up to 5.8×10^{-3} M. Totally, 10–12 solutions were prepared.

Likewise, the concentration of the 10 TO solutions ranged from 0.5×10^{-3} M to 3.77×10^{-3} M.

4. Results

In the course of our studies, in the field of supramolecular non-covalent interactions [5–7,10], we focused on coexisting equilibria, which determine the drug release from CDs during the chromatographic analysis. In order to predict the optimal conditions for the HPLC analysis, the parameters affecting the extent of complexation/dissociation pattern were investigated.

4.1. The binding constants K_c of the complexes

In order to quantitate the complexation affinity of TO and KT complexes with different CDs, their apparent binding constants (K_c) at various selected temperatures were determined by phase solubility experiments (Table 2) and compared to the chromatographic data.

It can be suggested that the determined binding constants K_c, listed in Table 2, provide insufficient information for understanding the drug release properties and the detected analytical errors. Complexes having similar K_c values showed different behaviour in the chromatographic system. These differences cannot be explained without the knowledge of the complexes structure and the thermodynamic quantities involved.

4.2. The proposed supramolecular structures

In an attempt to elucidate the dissociation pattern occurred during the analysis, the structural features of TO and KT complexes with different CDs in aqueous solutions were studied with 1D ^{1}H NMR and 2D ROESY experiments [13,14].

4.2.1. Structure of tolfenamic acid–CD complex (Fig. 3):
4.2.1.1. 1D ^{1}H NMR. One-dimensional ^{1}H NMR Spectra of TO solutions containing different CDs, presented significant chemical shift changes, which depend on the CD derivative chosen. They ranged from $\Delta\delta = 0.06$ to 0.33 ppm, where $\Delta\delta = \delta_{free} - \delta_{complex}$. These results evidenced the drug–CD complexation, while the corresponding data from ROESY spectra facilitated the description of the supramolecular structure.

The following findings supported the complexation as well: the peaks corresponding to H-3 and H-5 of TO appear overlapped in the presence of equimolar quantities of TO and βCD. Strong upfield displacement of H-3 was caused by a threefold increase of CD concentration. Furthermore, the protons H-4′ and H-5′ of free TO appeared as multiplet, whereas in the presence of βCD as separate peaks.

Although shielding effects were expected due to molecular encapsulation, the measured changes of chemical shift values for most TO protons revealed a strong deshielding effect upon complexation with CD. This rather unexpected phenomenon can be interpreted by the possible dissociation of TO dimers. The dimerization phenomena of TO were studied through 1D ^{1}H NMR self-titration experiments. The dimers' formation induced strong shielding displacements of TO protons due to magnetic anisotropic phenomena caused by the neighbouring aromatic rings. The aromatic protons displacements ($\Delta\delta = \delta_{37.7} - \delta_{5.0}$) detected, ranged from −0.13 to −0.46 ppm. Strong upfield shifts were observed also for TO methyl group protons ($\Delta\delta = -0.29$ ppm).

Upon complexation with CDs, dimer formation is not favoured since their volume does not allow insertion into the CD cavity. Therefore, the strong deshielding effect due to dimer dissociation overrules the shielding effects caused by inclusion in the CD cavity.

It is noteworthy, that during all the experiments in the presence of CDs, cross peaks appear between TO H-3 and the protons of its methyl group. This reveals that the conformation presented in Fig. 1B is favoured upon complexation with CD. During the complexation process, TO dimers undergo forced dissociation causing a deshielding effect to the involved protons. A supporting finding was that only H-3 of TO appears to be shielded upon complexation because of the neighbouring methyl group effect.

Table 2
The apparent binding constants K_c of ketoprofen and tolfenamic acid complexes, with various CDs, in different temperatures

Host	Guest	Ratio	K_c (M^{-1})[a]				
			25 °C	30 °C	37 °C	45 °C	52 °C
βCD	TO	1:1	460	296	235	184	–
HPβCD	TO	1:2	478	226	110	52	–
MeβCD	TO	1:1	156	81	74	42	–
βCD	KT	1:1	386	372	369	365	352
HPβCD	KT	1:2	–	1496	1293	551	247
MeβCD	KT	1:1	4636	2760	2701	2078	1427

[a] According to the results of phase solubility experiments.

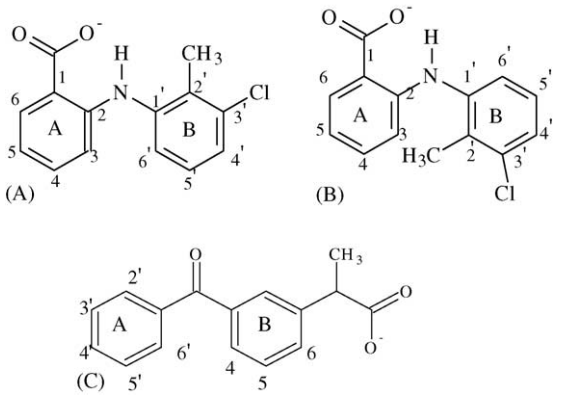

Fig. 1. Structures and numbering of tolfenamic acid anion in two conformations (A and B) and ketoprofen anion (C).

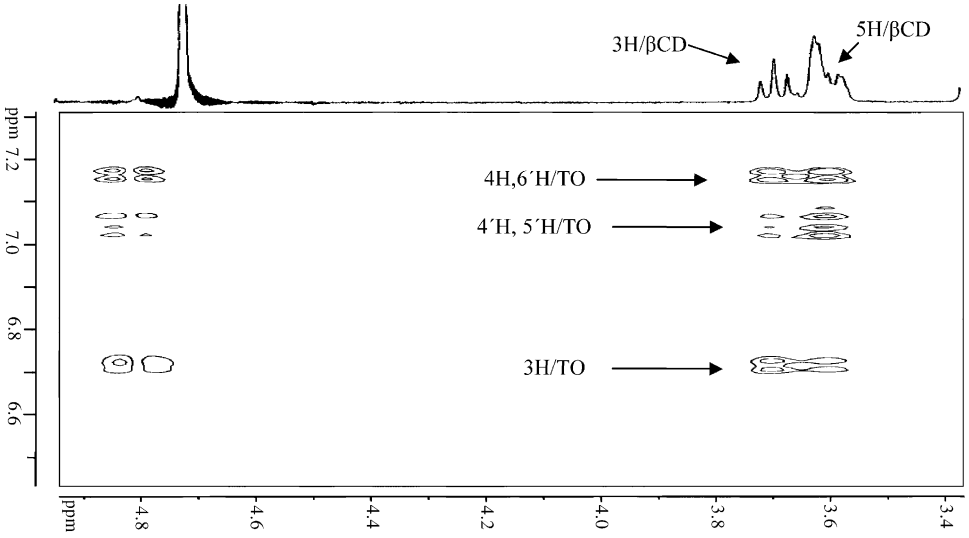

Fig. 2. Partial 2D ROESY spectrum of tolfenamic acid–βCD complex, in aqueous solution, with molar ratio 1:3.

4.2.1.2. 2D ^1H NMR. The 2D ROESY spectra of TO–βCD 1:1 ratio (Fig. 2) revealed cross peaks of all TO protons, except H-5 and H-6, with H-3 and H-5 of βCD cavity. In the case of 1:3 ratio, peaks of TO protons seem to have better resolution. Protons H-4′ and H-5′ produce cross peaks only with H-5 of CD cavity (narrow side), while H-6′, H-3 and H-4 show cross peaks with both H-3 and H-5 of βCD. It can be suggested that, the molecule enters CD from the secondary end and that, H-5 and H-6 protons of TO probably remain outside the cavity, as they present no interaction with the internal CD protons.

Furthermore, the rotation of ring A around the bond with the nitrogen atom can be hindered by the limited space available inside the CD cavity. Intramolecular hydrogen bond is formed between the two functional groups of TO, which does not seem to be hindered by complexation, whereas the participation of these groups in intermolecular interactions with CD should rather be excluded. A similar case is described for mefenamic acid [15].

All the abovementioned results demonstrate that the formed inclusion complexes are of 1:1 stoichiometry (Fig. 3), but 1:2 stoichiometry cannot be excluded [5]. From the NMR study of TO–HPβCD and TO–MeβCD, more shallow complexes of the same orientation can be concluded, which are not shown for brevity reasons.

4.2.2. Ketoprofen–CD complexes

4.2.2.1. 1D ^1H NMR. The peaks attributed to free KT to aromatic protons appeared overlapped and a more detailed assignment was facilitated by the presence of βCD, where the peaks appeared separated and easier to distinguish.

With the aid of 1D ^{1}H NMR self-titration experiments, dimerization effect was proven for KT. Concentration dependent, shielding effects of the aromatic protons region were detected ($\Delta\delta = \delta_{5.78} - \delta_{0.51} = 0.06$–$0.09$ ppm). The signals corresponding to methyl group protons were also found to be shielded and the calculated $\Delta\delta$ ($\delta_{5.78} - \delta_{0.5}$) was 0.03 ppm.

4.2.2.2. 2D ^1H NMR. An interesting finding from ROESY experiments of KT–βCD complex is the homomolecular cross peak between KT protons H-3′ and H-2, implying the existence of KT dimers, which do not dissociate upon complexation. From the ROESY experiments of drug–CD complexes the observed supramolecular interactions were studied in connection to the homomolecular interactions of KT dimers.

In the spectra of KT–βCD 1:1 ratio (Fig. 4), KT protons H-6, H-4 and H-5′ produced cross peaks with H-3 and H-5 of βCD, while H-4′ and H-5 presented cross peaks only with H-3 of βCD. Therefore, it could be concluded that, KT

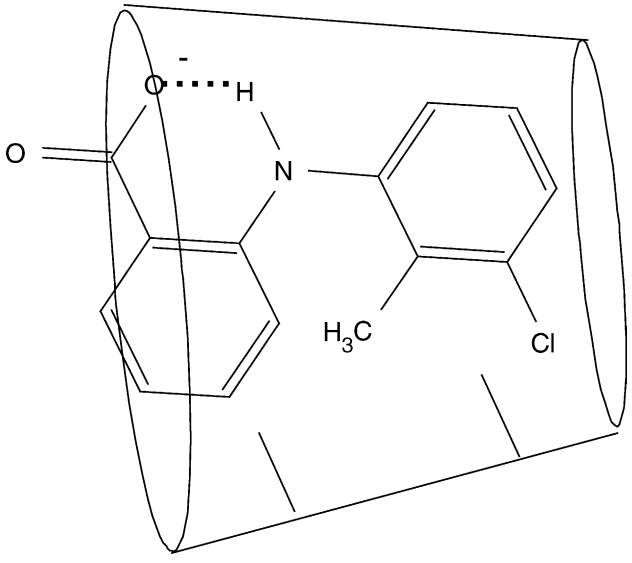

Fig. 3. The proposed structure of tolfenamic acid–βCD complex.

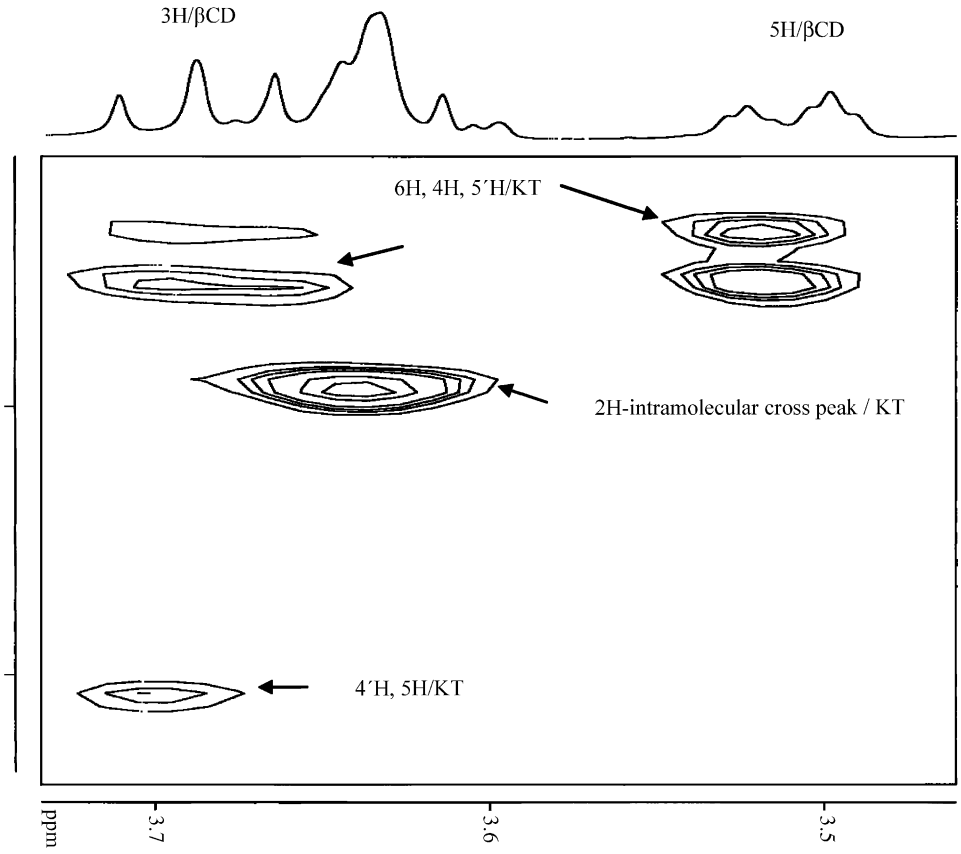

Fig. 4. Partial 2D ROESY spectrum of ketoprofen–βCD complex, in aqueous solution, with molar ratio 1:1.

molecules penetrate into the CD cavity from the secondary side. The reason that H-4′ of KT provides no cross peak with H-5 of the βCD could be that, this part of the molecule exits from the primary end of the CD and remains in contact with the solvent (Fig. 5).

An interesting assumption about the structure of KT dimers is, also, that the H-2 proton of KT lies outside the cavity, interacting with H-3′ of a second KT molecule. This inter-molecular interaction between KT protons H-3′ and H-2 may well explain an edge-to-face (T-shaped) dimer structure, but certainly not a face-to-face structure. Therefore, the second KT molecule may be located vertically (or by angle) to the level of ring B of the inserted KT molecule as well as ring B of the second KT molecule can retain its free-rotating ability. T-shaped dimers are characterized as low energy level structures and are therefore quite stable. The vertical position of the second KT molecule stabilizes also the whole supramolecular assembly. The presence of CD molecules does not seem to interfere with the KT dimer formation or dissociation (spectral data).

In addition, the peaks ascribed to the KT aromatic protons, in KT–HPβCD and KT–MeβCD spectra, appeared at different magnetic field values than those of KT–βCD complexes. Nevertheless, the observed cross peaks revealed more shallow complexes of the same orientation.

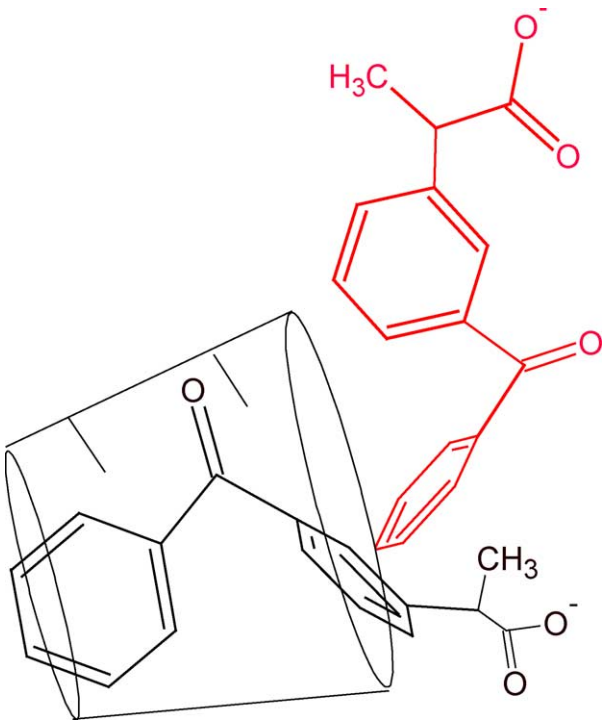

Fig. 5. The proposed structure of ketoprofen–βCD complex.

5. Discussion

5.1. Correlation between the complexes chromatographic behaviour and intermolecular interactions

Two complexes having similar K_c, as revealed from our repeated experiments, may dissociate at different time periods and under different conditions. A rational explanation for this phenomenon can be the dissimilar absolute values of their formation (k_1) and dissociation (k_{-1}) rate constants, as K_c expresses only the k_1/k_{-1} ratio. From this equation it can easily be concluded that the same K_c values can arise from rate constants (k) of enormously different magnitude.

The nature of intermolecular non-covalent binding forces between host and guest molecules could be the factor determining the chromatographic behaviour of the generated complexes in a given environment and might vary with any alteration of the analysis condition. It is expected that by modifying the chromatographic conditions (temperature, pH or hydrophobicity) the corresponding related supramolecular forces will be affected.

The magnitude of the effect that the above mentioned parameters exercise on the intermolecular interactions is evaluated in our experiments by calculating in all cases the recovery of the drug during the chromatographic analysis.

5.1.1. Tolfenamic acid complexes

During the chromatographic analysis of TO–CDs complexes, the application of increased column temperatures resulted to no significant changes at the calculated relative errors. The observations were similar also, by changing the pH of the mobile phase. This is probably due to the lack of inter-molecular hydrogen bonds between TO and cyclodextrin molecules, which is quite expected as a intra-molecular hydrogen bond is occupying all the available, for such a binding, groups of the inserted molecule.

Important changes were observed only with small variations of the organic modifier of the mobile phase. This can be attributed to the presence of hydrophobic interactions in the system, affected by the increasing concentration of an organic modifier, especially methanol.

5.1.2. Ketoprofen complexes

In the case of KT–HPβCD complexes, important changes at the calculated relative errors were observed when the pH of the mobile phase and the temperature of the column were changed. These results demonstrate the presence of multiple hydrogen bonds in the supramolecular structure. On the contrary small to negligible reduction of the errors was observed in the case of KT–βCD and KT–MeβCD complexes, which can be ascribed to differently bound complexes, characterized by the lack of significant number of hydrogen bonds.

During the chromatographic analysis of these complexes, changes of the organic phase concentration led to significant shift of the equilibrium towards free KT, suggesting that hydrophobic interactions were present.

It is noteworthy that, KT–βCD complexes were the most difficult to dissociate in comparison to all the other CD complexes studied. This can be probably due to the presence of hydrophobic interactions that stabilize better these complexes.

Table 3
Thermodynamic quantities of complexation calculated from various CD complexes of tolfenamic acid and ketoprofen

CD	Tolfenamic acid complexes		Ketoprofen complexes	
	ΔH^a	ΔS^b	ΔH^a	ΔS^b
βCD	−8.17	−15.44	−3.63	+0.94
MeβCD	−12.30	−31.10	−5.64	−2.32
HPβCD	−18.90	−51.80	−21.24	−53.83

[a] kcal mol^{-1} (1 cal = 4.1868 J).
[b] kcal mol^{-1} K^{-1}.

5.2. Thermodynamics of tolfenamic acid complexes

In an attempt to explain the drug release mechanism from CDs, the possible correlation of the structural findings with the thermodynamic quantities of complexation and consequently with the chromatographic behaviour, was examined. From the determined apparent binding constants, the related thermodynamic values were calculated, using the corresponding van't Hoff plots and are presented in Table 3. According to the deviations (relative errors) calculated during the HPLC method analysis, it was observed that TO–βCD complexes dissociate later than the corresponding MeβCD and HPβCD complexes. However when comparing the values of their binding constants, a different order can be observed: HPβCD > βCD > MeβCD.

The greater instability of HPβCD system (favouring the dissociation), compared to the other CDs, may be attributed to the greater negative ΔS value of this complex. The experimental findings revealed that, the values of ΔS correlate more efficiently with the stability of the complex than K_c does [16]. From the calculated errors it can be suggested that, the values of the binding constants are not definitive for the strength of complexation, but provide only an evaluation of the association affinity of the two molecules.

By the investigation of NMR data, it was revealed that TO penetrates deeper in the βCD cavity, less in the MeβCD cavity and the least in the HPβCD. The structures of the complexes examined above are in good agreement with the presumed decomplexation order. From the chromatographic results, the increased stability in the case of βCD could be attributed to more pronounced van der Waals forces presented in complexes and to probable formation of βCD dimers.

Furthermore an intra-molecular hydrogen bond between the carboxyl and the amino group of free TO molecule, which is maintained during the complexation, diminish the possible participation of these groups in suprarmolecular hydrogen bonds. This is also supported by the low K_c values of the TO–CD complexes and can easily interpret the rather mild

conditions necessary for the dissociation of TO, in comparison to KT.

5.3. Thermodynamics of ketoprofen complexes and solvation spheres

The solvation spheres created from solvent molecules around the assemblies strongly depend on the available functional groups. The loss of the solvation sphere of KT and the following reorganization of water molecules during the approach of host and guest molecules, affect the complexation and decomplexation kinetics and consequently the time required for equilibration. KT has pK_a 4.3 and in aqueous solution appears in ionic form. A solvation sphere, strongly bound around the ionized carboxyl group, is most probably reducing the interaction affinity between KT and CD. This phenomenon is more intense in the case of HPβCD due to the extended solvation sphere around its substituents. The less stable complexation with HPβCD detected during HPLC analysis was also confirmed by the NMR experiments.

In the case of the KT–βCD complex, the smaller negative ΔH value was measured and also the most favourable entropy change ($+\Delta S$), in comparison to the other CDs. Small negative ΔH of complexation can be connected to hydrophobic interactions. This kind of complexation is favoured even if enthalpy changes are small, because ΔS functions as the driving force for the association (entropy driven complexation). The increased $+\Delta S$ can be the result of better or deeper insertion of the molecule in the βCD cavity, a fact favoured by the lack of substituents, which might hinder the approach of KT molecule. The greater the entropy value, the more water molecules are displaced from the CD cavity upon complexation, which supports the previous conclusion.

The more efficient binding of KT with βCD in comparison to the other CDs is also demonstrated by the HPLC experiments, where the relative errors during KT recovery from KT–βCD complex are significantly greater. This complex is more difficult to dissociate, particularly when the KT–βCD ratio is greater than 1:1. Furthermore, the stabilization of this particular complex can be attributed to the presence of hydrophobic interactions. These suggestions were supported also by the great impact that the change of the mobile phase lipophilicity had on the equilibrium displacement towards the free KT [10].

In the case of KT–HPβCD complex, association is followed by high $-\Delta S$, which can be ascribed to multiple intermolecular hydrogen bond formation. This was expected, because of the existence of carboxyl group in KT molecule and hydroxyl groups in CD molecules. The supramolecular multiple hydrogen bond formation reduces the molecules mobility, causing a decrease of the system entropy and compensation of the enthalpy offer. The presence of these hydrogen bonds, was supported by the HPLC experiments, since a strong effect of pH on the equilibrium displacement was observed. Dissociation was found to be more efficient in the case of 1:2 molar ratios. It is well known that, when the extent of hydrogen bonding increases, the entropy value reduces, destabilizing thus the complexes [17,18]. KT–HPβCD complexes show the most unstable chromatographic profile. In addition to the high $-\Delta S$, the presence of hydrophobic interactions characterizes these complexes, a finding which conforms to the conclusions of Junquera and Aicart [19] who studied these complexes by potentiometry.

The determined binding constants values for the KT–MeβCD and KT–HPβCD complexes are quite dissimilar and therefore different dissociation behaviour would be expected. Nevertheless, the calculated relative errors are quite close. It can be concluded that the displacement of complexation equilibrium towards the free drug depends on the entropy change of the system [20]. In our experiments, the entropy change contributes to the better stabilization of the KT–MeβCD complex ($\Delta S = -2.32$ kcal mol^{-1} K^{-1}) than the KT–HPβCD complex ($\Delta S = -53.83$ kcal mol^{-} K^{-1}).

Furthermore, KT molecules have an ionized carboxyl-group, which is surrounded by strongly organized water molecules, susceptible to temperature changes. KT maintains its capacity of forming supramolecular hydrogen bond networks with the CDs. In the case of TO, the ionized carboxyl-group is occupied with an intra-molecular hydrogen bond (with the amino-group), thus inhibiting a) solvation spheres to be constructed around these groups and b) the formation of inter-molecular hydrogen bonds with the CDs. Therefore, the obtained KT complexes appeared more stable than TO complexes. The stability of KT complexes leads to slow drug–CD dissociation, which means that the time period necessary for dissociation is bigger than the time required for a chromatographic analysis. This contributes to the maintenance of the detected inaccuracies with the HPLC methods used, unless sample pre-treatment precedes. On the contrary, TO–CD complexes dissociate within the HPLC analysis time.

6. Conclusion

The binding constant K_c describes the extent of association, however provides insufficient information for the time required for the complex dissociation. Although the complexation efficacy depends on the binding constant of the inclusion complex, the “ultimate stability of complexation” (as defined by Rekharsky and Inoue [20]) is controlled by the entropy term.

Particularly, entropy change in the system is considered the most critical factor affecting the time required for dissociation of drug–CD complexes, during drug quantitation. This can be applied for the prediction of the enantiomers’ elution order.

Structural features of the created supramolecules, combined with the provided HPLC environment (environment leading to dissociation) determine their chromatographic profile.

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