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Solubilization of ibuprofen with β-cyclodextrin derivatives: Energetic and structural studies

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ABSTRACT

The aim of this work was to investigate the complexation of ibuprofen as model drug with various β-cyclodextrins (native β-cyclodextrin, hydroxypropyl-β-cyclodextrin with two different molar degrees of substitution, and methyl-β-cyclodextrin). Solutions of the commercially available β-cyclodextrins were prepared in phosphate buffer (73 mM). The pH value was adjusted to 7.4 and the solutions were isotonized with NaCl. A solution of ibuprofen was prepared in the same way. A thermal activity monitor was used for isothermal titration calorimetry (ITC). ^{1}H NMR analysis was employed to investigate the structures of the complexes. ITC analysis showed that each type of β-cyclodextrin had its characteristic values of both enthalpy and mass equilibrium constant for the complexation processes with the drug molecules. ^{1}H NMR spectroscopy of the complexes showed through significant differences in chemical shifts that the physical interaction between the cyclodextrins and ibuprofen molecules were also different, probably due to different three-dimensional arrangements of ibuprofen in the cyclodextrin cavity, induced by the different substituents bonded to the glucose rings. These differences were connected to the thermodynamic parameters of the complexes.

1. Introduction

An ever-increasing challenge for new drug substances is their poor solubility in physiologic fluids. Enabling technologies are necessary to transfer such substances into useful medicines. One of the most applied methods to increase the solubility of substances is incorporation into cyclodextrins [1]. Cyclodextrins (CD) are cyclic oligosaccharides consisting of (α-1,4)-linked α-D-glucopyranose units. They are frequently in use as food additives, and FDA acknowledges many types of CDs as GRAS for oral administration. Three ring-types are common, where α-CD is composed of six, β-CD of seven, and γ-CD of eight glucose units. Because of the chair conformation of the glucopyranose units, CD molecules are shaped like truncated cones, with a hydrophobic cavity inside and a hydrophilic surface outside. Several studies investigate the nature of the interaction between CDs and drug molecules [2–4]. Isothermal titration calorimetry (ITC) has successfully been applied to characterize the thermodynamic parameters of the complexation reaction [5–8]. Other studies have shown that nuclear magnetic resonance (NMR) spectroscopy can be used to investigate the nature of the interaction between CDs and the respective drug molecules [9]. Due to its chemical stability and low solubility, ibuprofen (IBP) is a model drug in a number of these studies [10,11]. Previous investigations have indicated that the benzyl-isobutyl-part of IBP (as well as of similar molecules) is the one incorporated into the CD cavity [12,13]. Most recently, the type of CD used, namely by the number of glucose units in the CD ring, has been found to influence the interaction with IBP in terms of energy, mass equilibrium constant, and structure of the complex [14]. However, the β-type CD with seven glucose units is used in many pharmaceutical preparations. In the past, the relatively low solubility of β-CD itself in water and in any aqueous systems has limited its usefulness for pharmaceutical formulations. Therefore, for β-CDs, highly soluble-derivatives have been developed, which are substituted at specific groups of the glucose rings. Some of these products are now commercially available (Fig. 1). To our knowledge, the influence of substituent groups on the complexation of drug compounds in terms of energy of interaction and the structure of the complexes have not been investigated yet. In the present work, both ITC and NMR have been used to study the impact of different substituent groups of commercially available CDs on the complexation with IBP. The purpose of this study was to evaluate thermodynamic parameters and relate them to the respective structure of the complex for a better understanding of the systems. This is regarded as an important step

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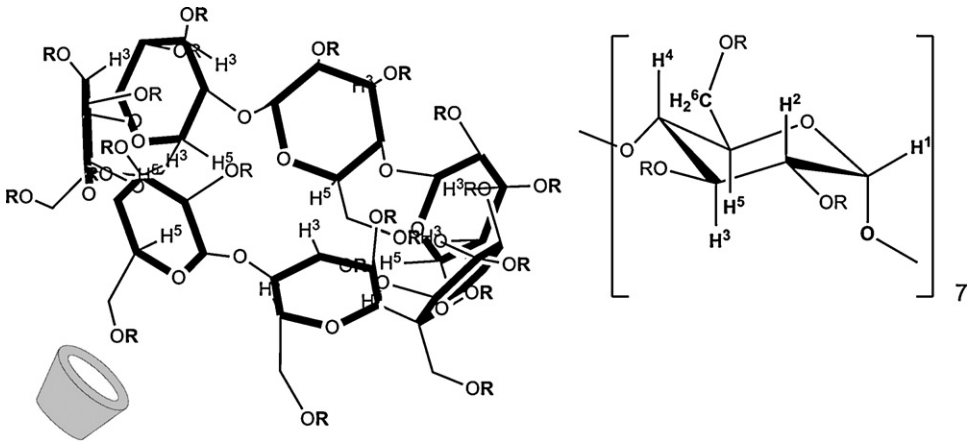

Fig. 1. Structure of β-CD (R: –H), HP-β-CD (R: $-CH_2CHOHCH_3$), and M-β-CD (R: $-CH_3$).

towards optimized IBP/CD formulations, and will have impact on the complexation of other drug molecules as well.

2. Materials and methods

2.1. Materials

In this work, ibuprofen (IBP; (*RS*)-2-(4-(2-methylpropyl)phenyl) propanoic acid; Ph. Eur. Grade; Caeleo, Caesar & Lorenz GmbH, Hilden, Germany) was used as a model drug in the complexation studies with a commercially available beta-cyclodextrin (β-CD), and 3 of its derivatives, namely hydroxypropyl-beta-cyclodextrin (HP-β-CD),with a molar degree of substitution (MS, defined as number of substituent per glucose unit) of 0.62 and 0.87, respectively, and methyl-beta-cyclodextrin (M-β-CD 0.57). All cyclodextrins were a kind gift from Roquette Freres (Lestrem, France). Sodium-di-hydrogen-phosphate and di-sodium-hydrogen-phosphate (Sigma Aldrich Chemie GmbH, Steinheim, Germany) were used for buffering the solutions at pH 7.4 and the isotonicity was adjusted using sodium chloride (Sigma Aldrich Chemie GmbH, Steinheim, Germany).

2.2. Preparation of the solutions

Twenty milliliters of a sodium di-hydrogen phosphate solution (2.55%, w/v) were mixed with 80 ml of a di-sodium hydrogen phosphate solution (1.55%, w/v), forming a buffer solution with a phosphate concentration of 73 mM. Both ibuprofen and cyclodextrin were dissolved in this mixture in order to obtain a concentration of 1 mM and 100 mM, respectively. The pH of the solutions was measured with a SympHony pH meter SB70P (Thermo Fischer Scientific, Waltham, USA) and if necessary adjusted to 7.4 ± 0.01 with sodium hydroxide. Osmolality of the solutions was measured with a Semimicro Osmometer K7400 (Herbert Knauer GmbH, Berlin, Germany) and if necessary adjusted by adding NaCl to 286 ± 1 mOsm/kg.

2.3. Dissolution studies

For each CD the stoichiometry of the complex was determined by solubility studies at 25 °C. Ibuprofen was added in excess to 8 solutions of different concentrations of the respective cyclodextrins in the phosphate buffer. After shaking for 5 days in a thermostatic water bath at 25 °C (Julabo SW 23, Julabo Labortechnik GmbH, Seelbach, Germany), the solutions were centrifuged using a 5804 R-centrifuge (Eppendorf AG, Hamburg, Germany) at $9 \times g$ for 10 min. Concentrations of IBP in the supernatants were detected by UV analysis performed with a Genesis 10 UV/VIS scanning system (Thermo Electron Corporation, Cambridge, UK) at 265 nm.

2.4. Isothermal titration calorimetry

The calorimetric analyses were performed using a 2277 Thermal Activity Monitor, TAM, (Thermometric, Järfälla, Sweden). Three milliliters of titrant solution (1 mM ibuprofen in phosphate buffer prepared as described in Section 2.2) were placed in each of two 5 ml steel vessels (sample and reference) at 25 °C. A 250 μl Hamilton syringe (Hamilton, Bonaduz, Switzerland) filled with the titration solution (CD 100 mM in phosphate buffer) was inserted in the 612 Lund Syringe Pump (Thermometric). Sixteen drops of CD solution (12 μl each) at 45 min intervals were used to complete the titration of the ibuprofen solution. Each titration experiment was performed in three replicates (until the deviation in heat effect for each drop was ≤5%). The absolute values of enthalpy (ΔH) and mass equilibrium constants (β_1) were calculated using the 2277-131 Digitam software, the operation of which has already been described previously [6].

2.5. ^{1}H NMR analysis

^{1}H NMR spectra were recorded on a Bruker Avance III 400 MHz with auto-sampler (Bruker BioSpin GmbH, Rheinstetten, Germany). After dissolving the drug and the respective cyclodextrin in heavy water (molar ratio 1:1), the spectra were recorded at 25 °C and the signal generated from the water (impurity of D_2O) was used as the reference (4.640 ppm). A modification of a method previously described [8] was used for the characterization of the NMR spectra.

3. Results and discussion

3.1. Solubility studies

To identify the stoichiometry of the complex between IBP and β-CD derivatives, solubility studies were performed in isotonic and isohydric phosphate buffer (73 mM) at 25 °C [15]. Results reported in Fig. 2 evidence that for all β-CD derivatives, apparent solubilities of ibuprofen increase with increasing amounts of CD. Starting from a common solubility of 5 mg/ml of the drug in solution (solubility of IBP in isotonic phosphate buffer at pH 7.4) the increase is proportional to the amount of the respective CD derivative added. The solubilities achieved are between 7 and 8 mg/ml, where HP-β-CD with a molar degree of substi-

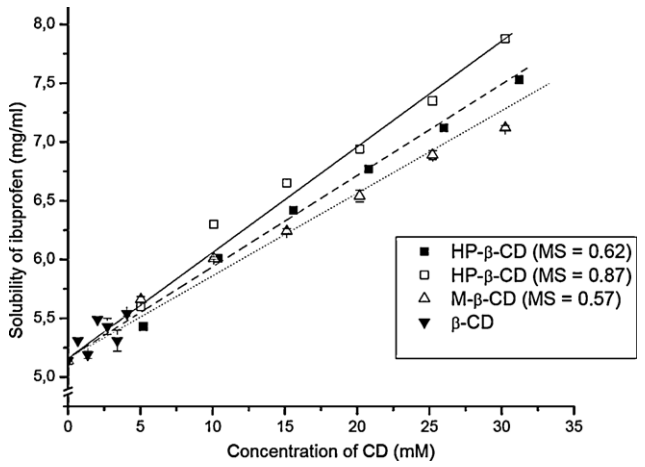

Fig. 2. Solubility profiles of ibuprofen in the presence of β-CD, two types of HP-β-CDs, and M-β-CD; mean and std. dev. ($n = 3$) for β-CD and M-β-CD.

tution of 0.87 (HP-β-CD 0.87) yields the highest solubility of IBP (8 mg/ml), followed by HP-β-CD with a substitution per mole of 0.62 (HP-β-CD 0.62). Methyl-β-CD (M-β-CD) has the lowest solubilization capacity, reaching a solubility of 7 mg/ml. However, non-substituted β-CD (β-CD) seems not to improve IBP solubility due to its own low solubility. For all β-CD derivatives, a linear dependence within the studied concentration range of ibuprofen and CD was found. In accordance with the literature [3,4,10] these results suggested a 1:1 stoichiometry of the complexes between ibuprofen and all the respective β-CD derivatives. This is of importance for the characterization of the thermodynamic parameters with isothermal titration calorimetry. The constants of equilibrium calculated from the solubility study were found to be between approx. 3000 M^{-1}(for the M-β-CD) and 6000 M^{-1} (HP-β-CD with MS of 0.87).

3.2. Isothermal titration calorimetry studies

Calorimetric investigations of the complexation process between β-CD derivatives and IBP were performed at 25 °C in isotonic phosphate buffer at pH 7.4. The results show that each type of β-CD derivative has its characteristic value of enthalpy of interaction, and a specific equilibrium constant of complexation with IBP (Table 1). β-CD and M-β-CD show the highest values of enthalpy of complexation, -14.6 ± 0.7 kJ/mol and -11.4 ± 0.3 kJ/mol, respectively. The value of enthalpy of complexation obtained for the M-β-CD used in this work (MS = 0.57) differs considerably from a literature value; Mura et al. [4] reported an enthalpy value of −16.8 kJ/mol for the complexation of IBP with M-β-CD (MS = 1.8). This indicates that the molar degree of substitution may influence the complexation processes. For the two HP-β-CDs the complexation process is less exothermic and the values of enthalpy in this case range between -9.4 ± 0.4 kJ/mol for the derivative with MS of 0.87 and -10.4 ± 0.4 kJ/mol for the HP-β-CD with lower degree of substitution. These values are in the same range as the findings of Perlovich et al. [6] (-11.0 ± 0.5 kJ/mol), with small variances probably related to the different buffer compositions. Moreover, the mass equilibrium constant was of the same order of magnitude as in previous works [6]. The highest value of mass equilibrium constant was found for the complexation between M-β-CD and IBP ($9100 \pm 1500\ M^{-1}$) and consequently this complex should be the most stable under the present experimental conditions. The complex formed with HP-β-CD (higher degree of substitution) is less stable according to the mass equilibrium constant ($2100 \pm 300\ M^{-1}$). This is also in accordance with earlier reported values of Junquera et al. [7]; again small differences probably related to different experimental approaches were noted. The entropy of the reaction is positive in all cases, but to a different extent. Both the absolute values of enthalpy and the entropy effects are in the same rank order: highly substituted HP-β-CD < low substituted HP-β-CD < M-β-CD. In this order, both the enthalpy and the entropy effects increase the stability of the respective complexes. However, the β-CD (unsubstituted) which has the highest enthalpy term, shows the lowest entropy (+7.4 kJ/mol), which keeps the Gibbs free energy and therefore the mass equilibrium constant at a medium level, a typical example of entropy–enthalpy compensation. Different hypotheses can be discussed for the explanation of these results, based on the structure of the complexes. Previous studies using X-ray powder diffraction combined with differential scanning calorimetry (DSC) [12] have clarified that the benzyl-isobutyl-section of the ibuprofen molecule is incorporated in the cyclodextrin cavity due to lipophilic interactions. More recently [14], thermodynamic parameters and structures of IBP/CD with CDs of different numbers of glucose units (σ-CD, β-CD and γ-CD) have been studied. The enthalpy value for β-CD and ibuprofen (−14 kJ/mol) and constant of equilibrium (10,000 M^{-1}) found in [14] are comparable with the present results. The constants of equilibrium found by ITC are within the same range as those extrapolated from the solubility studies. Although the solubility-derived constants are quite close to each other, it should be mentioned that their rank order is inverted in comparison to those found by ITC. This discrepancy may firstly be related to the different experimental conditions in the experiments: all the ITC experiments were performed in diluted solutions whereas the solubility studies necessitate saturated conditions. Secondly, the accuracy of the solubility studies may be limited due to other factors, e.g., formation of drug molecule dimers or non-inclusion complexes between drug molecules and cyclodextrins [16]. Therefore it is the authors' opinion that the constants of equilibrium detected by ITC are more reliable in comparison to those detected by solubility studies.

The complex formed between non-substituted β-CD and IBP is the one with the highest enthalpy of formation probably due to strong interaction of the isobutyl-benzyl part of IBP in the cavity of the CD. Furthermore, the presence of substitution groups with high sterical impediment at the entrance of the CD (as in the case of the two hydroxy-propyl and the methyl-derivatives) reduces the affinity of the lipophilic part of the IBP for the CD cavity, in the order of their (assumed) degree of steric hindrance: no substituent < methyl < hydroxy-propyl (0.62) < hydroxy-propyl (0.87). The difference in enthalpy between the two types of HP-β-CDs of 1 kJ/mol indicates that the higher the degree of substitution, the less intensive is the interaction, probably also due to steric hindrance.

Table 1
Mean ± std. dev. ($n = 3$) of the thermodynamic parameters for the complexation of IBP with β-CD, two types of HP-β-CDs, and M-β-CD from ITC experiments.

Type of CD	MS	K (M^{-1})	ΔH (kJ/mol)	$T\Delta S$ (kJ/mol)	ΔG (kJ/mol)
HP-β-CD	0.87	2100 ± 300	−9.4 ± 0.4	9.5	−19.0
HP-β-CD	0.62	4900 ± 900	−10.4 ± 0.4	10.7	−21.1
M-β-CD	0.57	9100 ± 1500	−11.4 ± 0.3	11.2	−22.6
β-CD	0	7000 ± 700	−14.6 ± 0.7	7.3	−21.9

Table 2
^{1}H chemical shifts of ibuprofen (*italic*) and CD protons in the inclusion complexes with β-CD (**2a**) and derivatives (**2b**, **2c**, and **2d**). The induced shift ($\Delta\delta$) was defined as the differences between the chemical shifts in the presence or absence of the other respective reactant ($\delta_{complex} - \delta_{free}$).

Proton (H^x)	Multiplicity	δ_{free} (ppm)	$\delta_{complex}$ (ppm)	$\Delta\delta$ ($\delta_{complex} - \delta_{free}$) (ppm)
(a) β-Cyclodextrin				
H^1	Doublet	4.9149	4.9111	**−0.0038**
H^2	Doublet	3.4935	3.4898	**−0.0037**
H^3	Triplet	3.8111	3.8073	**−0.0038**
H^4	Triplet	3.4289	3.4258	**−0.0031**
H^5	Triplet	3.6999	3.6954	**−0.0045**
H^6	Multiplet	3.7233	3.7191	**−0.0042**
H^a	Doublet	0.7122	0.745	**0.0328**
H^b	Doublet	1.229	1.2371	**0.0081**
H^c	–	1.66	–	–
H^d	Doublet	2.3179	2.3067	**−0.0112**
H^e	Multiplet	3.35	–	–
H^f	Doublet	7.0563	6.8708	**−0.1855**
H^g	Doublet	7.1096	7.0882	**−0.0214**
(b) Hydroxypropyl-β-cyclodextrin (ms = 0.62)				
H^1	Singlet	4.9197	4.917	**−0.0027**
H^{1*}	Singlet	5.0712	5.0706	**−0.0006**
H^2	Doublet	3.4876	3.4838	**−0.0038**
H^3	Triplet	3.8078	3.8059	**−0.0019**
H^4	Triplet	3.4357	3.4323	**−0.0034**
H^5	Triplet	–	–	–
H^6	Multiplet	3.7185	3.7155	**−0.003**
H^{7a}	–	–	–	–
H^8	Doublet	0.9932	0.9907	**−0.0025**
H^9	–	–	–	–
H^a	Doublet	0.7122	0.7794	**0.0672**
H^b	Doublet	1.229	1.2508	**0.0218**
H^c	–	1.66	–	–
H^d	Doublet	2.3179	2.3224	**0.0045**
H^e	Multiplet	3.35	–	–
H^f	Doublet	7.0563	6.8701	**−0.1862**
H^g	Doublet	7.1096	7.0761	**−0.0335**
(c) Hydroxypropyl-β-cyclodextrin (ms = 0.87)				
H^1	Singlet	4.9248	4.9218	**−0.003**
H^{1*}	Singlet	5.0951	5.0918	**−0.0033**
H^2	Doublet	3.4721	3.4689	**−0.0032**
H^3	Triplet	3.8069	3.8045	**−0.0024**
H^4	Triplet	3.4323	3.4304	**−0.0019**
H^5	Triplet	–	–	–
H^6	Multiplet	3.7159	3.7124	**−0.0035**
H^{7a}	–	–	–	–
H^8	Doublet	0.9932	0.9904	**−0.0028**
H^9	–	–	–	–
H^a	Doublet	0.7122	0.7907	**0.0785**
H^b	Doublet	1.229	1.2501	**0.0211**
H^c	–	1.66	–	–
H^d	Doublet	2.3179	2.327	**0.0091**
H^e	Multiplet	3.35	–	–
H^f	Doublet	7.0563	6.8772	**−0.1791**
H^g	Doublet	7.1096	7.0824	**−0.0272**
(d) Methyl-β-cyclodextrin (ms = 0.57)				
H^1	Singlet	4.9174	4.9125	**−0.0049**
H^{1*}	Multiplet	5.1078	5.1035	**−0.0043**
H^2	Doublet	3.5005	3.4958	**−0.0047**
H^3	Triplet	3.808	3.8037	**−0.0043**
H^4	Triplet	3.43	3.4262	**−0.0038**
H^5	Triplet	–	–	–
H^6	Multiplet	3.7155	3.7103	**−0.0052**
H^{7b}	Singlet	3.4062	3.4013	**−0.0049**
H^a	Doublet	0.7122	0.7572	**0.045**
H^b	Doublet	1.229	1.2152	**−0.0138**
H^c	–	1.66	–	–
H^d	Doublet	2.3179	2.31	**−0.0079**
H^e	Multiplet	3.35	–	–
H^f	Doublet	7.0563	6.8359	**−0.2204**
H^g	Doublet	7.1096	7.0292	**−0.0804**

3.3. ^{1}H NMR analysis

It is interesting to study the structure of these complexes in more detail in order to evaluate the above-discussed hypothesis. Nuclear magnetic resonance experiments were performed to investigate the interaction between IBP and each of the β-CD types on a molecular level. Spectra of all single materials (β-CD, the two types of HP-β-CD, M-β-CD and IBP) were recorded as references. In addi-

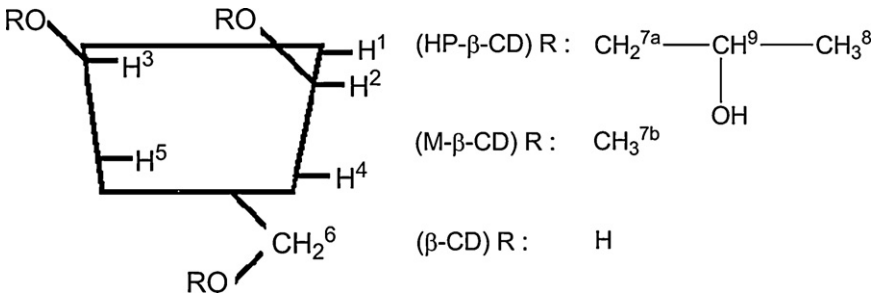

Fig. 3. Schematic representation of the β-CD and its derivatives.

tion, the spectra of the respective complexes of the CD derivatives and IBP were also recorded. The nomenclature and the numbering of the protons of both IBP and the different CDs are shown in Figs. 3 and 4. The induced shift in the resonance position, $\Delta\delta$, was defined as the differences between the chemical shifts in the presence or absence of the other respective reactant. Results are reported in Table 2. Each complex between IBP and each type of CD derivative shows a specific chemical shift for each of the protons, indicating differences in the three dimensional arrangements of the IBP molecule in the cavity of the respective CD derivative. For the native β-CD, the shifting for the ibuprofen as well as the glucose ring protons are comparable with the ones reported in previous works, e.g., Xing et al. [14]. In general, the protons of the benzyl-isobutyl-group of IBP (H^a, H^d) and the benzyl protons (H^f and H^g) show the highest absolute value of shifting for all the complexes, confirming that the lipophilic part of the drug molecule was incorporated in the cyclodextrin cavity. Furthermore, it is observed that some of the induced shifts are positive while the majority is negative. Although definite justification of the sign of the shift results appears difficult, it is certainly connected to the high sensitivity of the protons (especially benzyl protons due to the circulating π electrons) to the local electrical density of their surroundings.

In particular, the shifts identified for the proton H^a in the interaction of IBP with the CDs are interesting. They can be arranged in the following order: standard-β-CD < M-β-CD and HP-β-CD (0.62) < HP-β-CD (0.87) (0.00328 < 0.045 and 0.0672 < 0.0785 ppm, respectively). The same rank order is found for the neighbouring proton (H^d). These results show firstly, that in each complex the methyl-isobutyl part of IBP is involved in the CD cavity in a different way. Secondly, it should be noted that this rank of order is the same as for the enthalpy of the interaction between the IBP molecule and the respective CD (Table 1). Interestingly, the signal of the proton of the methyl group at the chiral carbon of ibuprofen (H^b) is more shifted in the complexes formed with the HP-β-CDs (0.0218 ppm and 0.0211 ppm, respectively) compared to β-CD and M-β-CD (0.0081 and −0.0138 ppm, respectively). This indicates that this proton of IBP being situated near the entrance of the CD is heavily affected by larger CD substituents. The induced shift connected to proton H^3 of the CD is one of the most meaningful because this proton is placed inside the cavity (Fig. 3). For this proton, the signal is shifted twice as much for M-β-CD and standard β-CD (0.0038 and 0.0043 ppm, respectively) compared to the two HP-β-CDs (0.0024 and 0.0019 ppm, respectively). This is probably due to higher energies of interaction between the less sterically hindered CDs and IBP. The results can be summarized as follows: (a) the larger the substituent, the weaker the interaction (NMR) and the less enthalpy of the interaction (ITC); (b) the less entropy gain, the less stable is the complex. However, if there is no substituent present, the enthalpy of the interaction is larger than expected, but there is also enthalpy–entropy compensation.

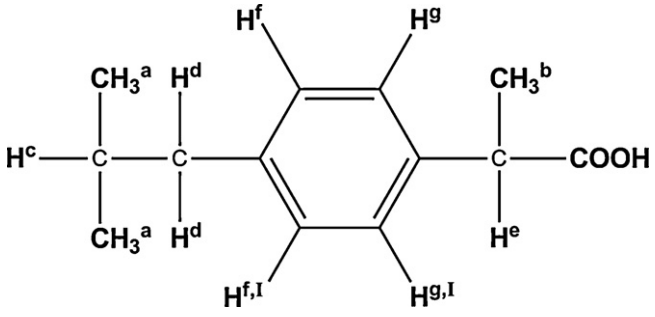

Fig. 4. Schematic representation of ibuprofen.

4. Conclusion

The NMR studies evidence that the thermodynamic differences measured by ITC for the interaction of IBP with CD derivatives are due to the specific three-dimensional conformation of each complex. The substituents bound to the glucose rings play an important role in the interaction between the respective CD derivative and the IBP molecule: large hydrophilic substituents (e.g., hydroxy-propyl-groups) at the entrance of the CD cavity enhance the solubility of the CD itself, but at the same time, they reduce the depth that IBP can reach into the cavity. The thermodynamic consequence of the weaker interaction between the drug molecule and CD is a lower enthalpy of the complexation. Furthermore, NMR analysis confirms that the benzyl-isobutyl-part of IBP can be incorporated more deeply in the cavity if no or only small substituents are bound to the CD, which is in agreement with the higher energy measured in the ITC experiments for the incorporation of the drug molecule into methyl- and standard β-CD.

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