Molecular Encapsulation of Flurbiprophen and/or Ibuprophen by Hydroxypropyl-*β*-cyclodextrin in Aqueous Solution. Potentiometric and Molecular Modeling Studies

E. Junquera,[†] M. Martin-Pastor,[‡] and E. Aicart*[,†,§]

Departamento de Química Física I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040-Madrid, Spain, and Química Orgánica Biológica, Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006-Madrid, Spain

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Thermodynamic and structural studies of the binding of the nonsteroidal antiinflammatory drugs, flurbiprophen and ibuprophen, to hydroxypropyl-*β*-cyclodextrin (HPBCD) have been carried out from pH potentiometric measurements, molecular mechanics, and dynamics calculations. The potentiometric study was performed by measuring the pH of aqueous solutions of the drugs as a function of cyclodextrin concentration, at several temperatures ranging from 15 to 40 °C. The dissociation constants of the drugs, as well as the binding constants of the inclusion complexes formed by HPBCD and the ionized and nonionized drugs, have been simultaneously determined at all the temperatures. The nonionic forms have shown a higher affinity to HPBCD than their ionic counterparts. A van't Hoff analysis of the determined binding constants reveals that the complexes formed by the flurbiprophen species and HPBCD are enthalpy driven, with a favorable enthalpic term and an unfavorable entropic term. Both contributions are found to be temperature independent ($\Delta C_p° = 0$). However, in the case of ibuprophen, a dependence of the thermodynamic quantities $\Delta H°$ and $\Delta S°$ with temperature has been found. Thus, these association processes change from entropy driven at $T \leq 25$ °C to enthalpy driven at $T > 25$ °C, which is due to a negative $\Delta C_p°$ value, typically found in biological associations where hydrogen-bonding interactions are present. Molecular mechanics and dynamics calculations were used to obtain structural and dynamics information of the free ligands, the receptor, and the four inclusion complexes. Flurbiprophen acid has been found to enter the HPBCD cavity leaving the carboxylic group upward, while the energetically favored inclusion of flurbiprophenate, ibuprophen acid, and ibuprophenate leaves the carboxylate or carboxylic group downward. In either case, the van der Waals contacts between the phenyl ring of the ligand and a lipophilic zone of the CD cavity, the entropy factor, and possible intermolecular hydrogen bondings are shown as the main responsible of the stability of the inclusion complexes. The estimation of the conformational stability of the free and associated ligands confirms that the carboxylic species of flurbiprophen and ibuprophen are encapsulated with a higher stability than that of their ionic counterparts, in agreement with the potentiometric results.

Introduction

The therapeutic action of a drug needs the confluence of different factors to occur. Thus, a proper intrinsic activity is not always enough to render a promising compound suitable as a drug. The drug must be not only water soluble for its adequate delivery to the cell but also somehow hydrophobic to cross the cellular membrane and reach its target site. Of the two properties, water solubility is the more elusive in the complex organic structures typically found in pharmaceutical agents. Traditional formulation systems for insoluble drugs involve the use of cosolvents, surfactants, microemulsion dosage forms, and/or pH adjustment for ionizable drugs. However, these formulations are often found to irritate the patient mucoses, causing adverse reactions and degrading the efficacy and safety of the drug concerned. Another successful method, used for increasing not only the water solubility but also the stability, the dissolution rate, and the general bioavalability of the drug, involves the use of cyclodextrins (CDs).[1−6] Many drugs are reaching the marketplace as CD formulations, and research studies exploring their applications grow exponentially.[4] The parent CDs, well-known cyclic oligosacharides obtained from the enzymatic conversion of starch, contain 6, 7, or 8 glucopyranose units joined through α(1→4) bonds and are referred to as α-, *β*-, or *γ*-CDs. Among them, *β*-CD is dimensionwise the most interesting for drug complexation, but unfortunately it is also the least soluble in water, which generally limits its use to oral applications. Hundreds of modified cyclodextrins[7] with higher solubilities have been prepared and

[†] Universidad Complutense de Madrid.
[‡] Instituto de Química Orgánica.
[§] Fax: 34-1-3944135. E-Mail: aicart@eucmax.sim.ucm.es. http://www-quifi.quim.ucm.es/qf_home/ea/ea_home.html.

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shown to have research applications, but only a few of these derivatives can be commercially used as excipients.[4] The tridimensional doughnut-shaped structure of CDs provides a cavity that is hydrophobic relative to an aqueous environment. The sequestration of hydrophobic drugs inside the cavity results in the formation of inclusion complexes with the concurrence of noncovalent interactions.[8,9] These complexes can have physical, chemical, and biological properties that are dramatically different from those of either the parent drug or cyclodextrin.[10] The driven forces for the complexation, including the requirement of the drug to "fit" into the CD cavity, have been widely studied[11] in recent years. It is known, mainly from thermodynamic studies,[12−15] that a balance between van der Waals (vdW) contacts and hydrophobic and solvent effects can be the main responsible for the overall stability of the complex, and of its enthalpy-driven character, as well.

Nonsteroidal antiinflammatory drugs (NSAIDs) are common analgesics, antipyretics and antiinflammatory agents, which inhibit the cyclooxygenase enzyme.[16] They are highly effective in these indications, but unfortunately often cause a high incidence of gastrointestinal ulcerative lesions as a result of both acute local tissue irritations and systematic inhibition of prostaglandin synthesis by the drug.[4] It is known[2,3,17] that CD formulations of some NSAIDs are more tolerable since they cause fewer gastric injuries, this effect being even more evident as long as the duration of the treatment increases.

The rational design of formulations which take advantage of cyclodextrin inclusion complexation requires a good understanding of the encapsulation equilibrium, through parameters such as the stoichiometry and the binding constant of the inclusion complex. If thermodynamic quantities for the complexation process, as well as structural information are also available, a picture of the interaction CD/drug and of the factors affecting it may be drawn. Most of the studies found in the literature regarding the CD formulation of drugs, and specifically of NSAIDs, have been carried out from a biomedical standpoint,[2,3,10,17] dealing mainly with pharmacokinetic assays, plasma level determinations, in vivo studies, etc. Few studies,[15c,18−20] however, are involved in a physicochemical characterization of the microencapsulates. We have already carried out some studies focused on the analysis of the effect of different factors, such as temperature, solvent polarity, ion strength, presence of divalent cations, and position and type of substituents on both the host and guest molecules, on the overall stability of the CD:drug complexes.[15c,21] In this connection, the microencapsulation of two NSAIDs drugs of the family of the arylpropionic acids, ibuprophen and flurbiprophen, by hydroxypropyl-β-cyclodextrin (HPBCD) (see Figure 1) has been characterized in this work, with two complementary studies. On one hand, a thermodynamic analysis has been carried out by determining the stoichiometry and the association constants for the HPBCD/drug systems, with the drug in both ionized and nonionized forms, as a function of temperature, in a range which covers from below room temperature up to well above the normal temperature of the human body. From this study, the thermodynamic quantities $\Delta G°$, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$, of crucial importance when discussing the driving forces of the process, can be obtained. These association constants have been calculated from pH potentiometric measurements of a drug solution as a function of CD concentration, by using a model recently proposed.[15c] On the other hand, structural information has also been obtained from a molecular modeling study. The ability of molecular dynamics (MD) simulations to predict structural and thermodynamics average properties of biomolecules in both solid state and solution has been widely recognized.[22] Moreover, the study of binding processes using MD simulations allows us to explore simultaneously the potential flexibility of both components, host and guest, in the bound state. The flexibility, understood as the capacity of the system to populate a certain region/s of the conformational space, may be important during the first stages of the binding to produce adaptive conformational changes in host and/or

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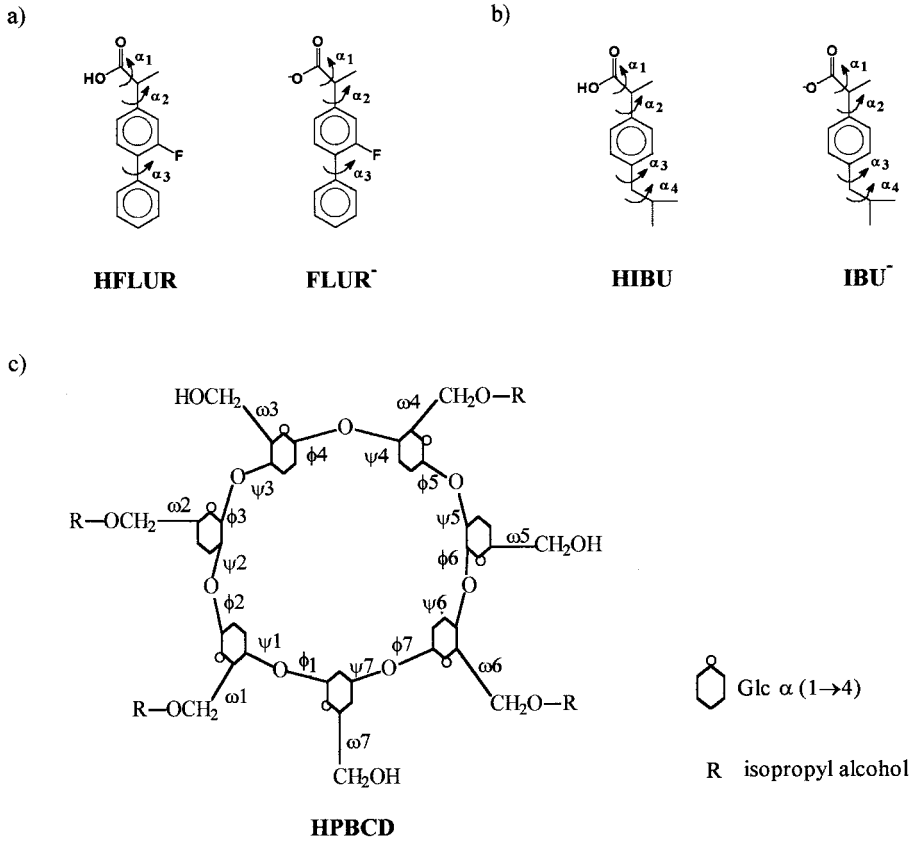

Figure 1. Schematic view of the ligand and the receptor species present in the conformational equilibria showing the torsional angles considered: (a) HFLUR and $FLUR^-$; (b) HIBU and IBU^-; (c) HPBCD.

guest in order to achieve a better self-complementarity among the surfaces of both components. The flurbiprophen and ibuprophen guests can present a high degree of translational, rotational, and conformational degrees of freedom with respect to the HPBCD host. The conventional MD simulation of these complexes would require inaccessible long simulation times in order to average over the different microstates. A more convenient strategy recently proposed[23] combines the possibilities of Monte Carlo conformational searches with MD simulations and allows for shortening of the simulation times required for host−guest systems. This strategy has been applied here to calculate reasonable geometries and to estimate thermodynamics data of the HPBCD complexes.[24]

Experimental Section

Materials. (±)-2-Fluoro-α-methyl-4-biphenylacetic acid and α-methyl-4-[2-methylpropyl]phenylacetic acid, commonly named flurbiprophen (FLUR) and ibuprophen (IBU), respectively, were purchased from Sigma. Hydroxypropyl-*β*-cyclodextrin (HPBCD), containing an average of 0.6 hydroxypropyl groups per glucopyranose unit (molecular degree of substitution, MDS = 4) was purchased from Janssen Biotech. All of them have a purity greater than 99% by mass and were used without further purification. HPBCD has been found, from a TG analysis, to consist of 2.8% mass of water content, which was considered in calculations of solute concentrations. Bidistilled water was purified by using a Super Q Millipore system and was also degassed prior to the preparation of the solutions. The homogeneity of the initial solutions was assured by sonicating them for 30 min in an ultrasonic bath.

pH Potentiometric Measurements. Potentiometric data were collected with a Metrohm 713 ion meter, using a combined glass electrode containing 3 M KCl as the reference electrolyte solution. The adjustment of both the asymmetry potential and the Nernst slope of the combined glass electrode was made by calibrating daily the electrode with three Metrohm buffer solutions of pH = 1, 4, and 7, at each working temperature. The equipment and the experimental procedure used on the pH determination were described previously.[15c,21] Mixtures were prepared volumetrically with a Metrohm 665 Dosimat digital buret with an accuracy of 0.002 mL. The buret cylinder was kept at the same temperature as that of the measuring cell. The pH ion meter and the buret were controlled from a PC computer via two RS-232C serial ports. All the steps in the measuring process and data acquisition are controlled through a Quick Basic program developed by us. The accuracy on the molarity of the solutions is better than 0.1%, the temperature is held constant within ±1 mK, and the pH data are obtained as an average of 250 measurements for each concentration, with a resolution of 0.0003. Using these experimental conditions, the pH measurements were made as a function of cyclodextrin concentration, keeping constant the concentration of the drug. The cyclodextrin concentration ranges were chosen to cover at least 80% of the saturation curve in order to guarantee a proper binding constant determination.[25]

Molecular Modeling. The study of the structure and dynamics of all the species present in the equilibrium was accomplished by molecular mechanics and dynamics calculations. The modeling of the free ligand species present in the

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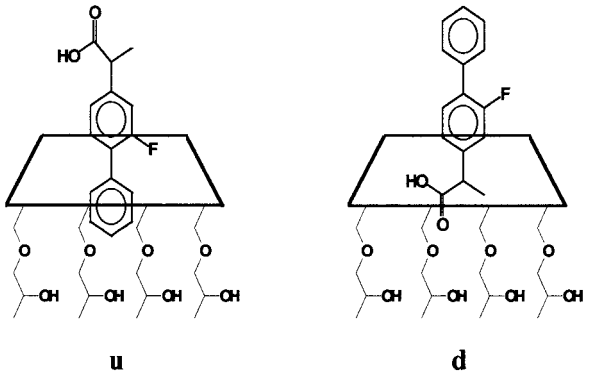


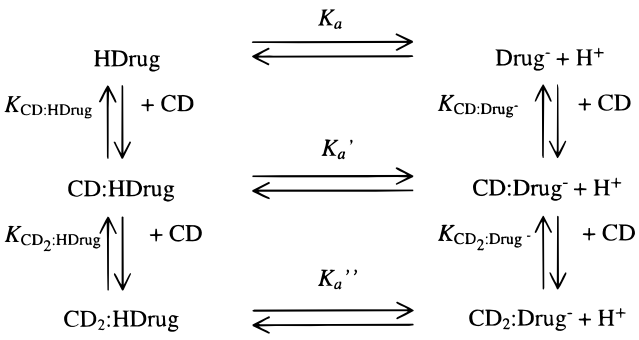
Figure 2. Schematic view of the two possible orientations of the ligand, namely *u* (up) and *d* (down), of the ligand HFLUR in the cavity of the HPBCD receptor.

equilibrium, that is, flurbiprophen (HFLUR) and ibuprophen (HIBU) acids, flurbiprophenate ($FLUR^-$) and ibuprophenate (IBU^-) anions, the free receptor HPBCD, and also the different complexes ligand:receptor were performed using mixed mode Monte Carlo/Stochastic Dynamics (MC/SD)[23] as implemented in Macromodel V4.5.[26] The previous step implies the generation of a starting minimized structure. In all the simulations the force field used was MM3,[27] with the continuous water solvation model GB/SA.[28] The temperature was maintained constant to 300 K by coupling the system to a thermal bath. The time step was 1 fs. After an initial equilibration time of 100 ps, structures were collected during the rest of the simulation time at intervals of 1 ps for subsequent analysis. The torsion angles α_1 to α_3 of HFLUR and $FLUR^-$ (Figure 1a) and α_1 to α_4 of HIBU and IBU^- (Figure 1b) were chosen for random rotation in independent simulations. For the HPBCD receptor (Figure 1c) a set of the torsions ω_1 to ω_7 and the torsions of the hydroxypropyl groups were chosen for random rotation during the simulation. For the different complexes, a set formed by those previously mentioned torsions of the ligand and those of the HPBCD were chosen for random rotation. In all cases, the total MC/SD simulation time used was sufficient to reach the stability of the system. Several structures were taken from each trajectory and subsequently minimized in order to obtain more representative conformations of the system.

The starting structures of HFLUR, $FLUR^-$, HIBU, IBU^-, and HPBCD were extensively minimized. The MC/SD simulation time after equilibration was 800 ps for HFLUR and $FLUR^-$ trajectories and 1000 ps for HIBU and IBU^-. The starting structure of the HPBCD was built from the X-ray structure of β-CD by adding an isopropylic alcohol group to the 6 position of four alternated glucoses (average MDS $\approx$ 4). The total MC/SD simulation time after the equilibration was 225 ps.

The global minima structures obtained for the free ligands HFLUR, $FLUR^-$, HIBU, and IBU^- were manually inserted into the cavity of the global minimum of the HPBCD. Two possible orientations *u* (up) and *d* (down) (Figure 2) of the ligand into the host cavity were considered and extensively minimized. These structures were used as starting structures for eight independent MC/SD simulations. During each MC/SD simulation, the ligand can be randomly translated (4 Å) and rotated (40°) around its mass center. The simulation times after equilibration for the HPBCD:HFLUR were 311 and 231 ps for each independent MC/SD simulation with the ligand inserted in the *u* and *d* disposition, respectively. The corresponding times for HPBCD:$FLUR^-$ were 951 and 549 ps, for HPBCD:IBU^- 430 and 368 ps, respectively.

Scheme 1



Analysis of the MC/SD Trajectories. To obtain the most probable conformations of the free ligand, the free HPBCD receptor, and the complexes HPBCD:ligand, the trajectories of the most relevant torsion angles were analyzed. The most populated values of each torsion were chosen as the most representative set, and the structures were extracted from the trajectory that fulfills all the possible combinations. Symmetry was taken into account in order to exclude repeated structures. The final set of structures obtained was minimized using conjugate gradient iterations with an MM3 force field with the GB/SA solvent model. The torsions of the ligands were analyzed during the trajectories. For the HPBCD receptor all the glycosidic torsion angles $\phi(H_1-C_1-O_1-C_4)$ and $\psi(H_4-C_4-O_1-C_1)$ and the ω torsion of the lateral chain $(O_5-C_5-C_6-O_6)$ were analyzed. For the complexes HPBCD:ligand, the torsion angles analyzed were those described for the HPBCD and the ligand components.

Energy and Probability Calculations. The conformational free energy ($\Delta\Delta G$) and energy ($\Delta\Delta E$) of binding were determined[29] from the average total energy and potential energy obtained in the MC/SD. These values were estimated according to

$$\Delta\Delta G = \Delta G_{\text{CD:ligand}} - (\Delta G_{\text{ligand}} + \Delta G_{\text{CD}}) \qquad (1)$$

$$\Delta\Delta E = \Delta E_{\text{CD:ligand}} - (\Delta E_{\text{ligand}} + \Delta E_{\text{CD}}) \qquad (2)$$

The single-point populations of the obtained minima were determined according to a Boltzmann distribution at 300 K after the minimization.[30] This analysis assumes that the entropy is negligible among the different minima. The validity of this last assumption was checked by analyzing the relative areas among the different minima found during the MC/SD trajectory,[31] which were indeed very similar.

Results and Discussion

Flurbiprophen and ibuprophen acids have an ionizable carboxylic group in their molecules. In aqueous solution, both the ionized and the nonionized forms of the drug are in equilibrium, and both are suitable to be included by the CD when it is also present, giving rise to the equilibria shown in Scheme 1.

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The equilibrium constants in Scheme 1 are related to the activity of the species through the well-known equations:

$$K_a = a_{H^+}a_{Drug^-}/a_{HDrug} \quad (3)$$

$$K_{CD:HDrug} = a_{CD:HDrug}/(a_{CD}a_{HDrug}) \quad (4)$$

$$K_{CD:Drug^-} = a_{CD:Drug^-}/(a_{CD}a_{Drug^-}) \quad (5)$$

$$K_{CD_2:HDrug} = a_{CD_2:HDrug}/(a_{CD}a_{CD:HDrug}) \quad (6)$$

$$K_{CD_2:Drug^-} = a_{CD_2:Drug^-}/(a_{CD}a_{CD:Drug^-}) \quad (7)$$

Since H^+ is one of the involved species, the monitoring of the pH variation of an aqueous drug solution, when drug concentration is kept constant as long as the CD is added, can be used as a way of determining the association constants of the CD_i:HDrug and CD_i:$Drug^-$ complexes ($i = 1, 2, ...$). In fact, several methods have been developed in the literature[15c,32−34] to determine the binding constants of the host−guest systems from pH potentiometric data. Particularly, we have reported a model[15c] which allows for the simultaneous determination of all these equilibrium constants by using eqs 3−7, those for the activity coefficients (using the Debye−Hückel theory) and the mass and charge balances, to fit the experimental data. The fitting process is carried out with a nonlinear regression method (NLR) based on a Marquardt algorithm. As fully described elsewhere,[15c,21] the main advantages of our model are the following. (i) Any of the equilibrium constants are fixed to zero. The numerical procedure assigns negligible values to those binding constants corresponding to association processes which do not take place or occur with negligible affinities. (ii) It permits the simultaneous determination of the dissociation constant of the guest (K_a), which constitutes a control for the suitability of the method, since these values can be compared either with literature values or with those obtained for the pure acids in the absence of cyclodextrin.

In this work, we have measured the pH of the aqueous solutions of ibuprophen and flurbiprophen in the presence of HPBCD at 15, 20, 25, 30, 35, and 40 °C. The drug concentration was kept constant; meanwhile the CD concentration was varied. Figure 3 shows, as an example, the plot of ΔpH, taken as the difference between the pH of the CD/drug/water solution and that of the initial drug/water solution, as a function of HPBCD concentration, for the system HPBCD/ibuprophen/water. The pH variation observed in the figure results from the shifting of the equilibria in Scheme 1 as the CD is added, depending on the magnitude of the binding constants. From these pH values, and those corresponding to the HPBCD/flurbiprophen/water system, the association constants of the complexes formed by HPBCD and the ionized and nonionized forms of both FLUR and IBU have been determined and the obtained values are reported in Table 1. The dissociation constants of the two acids have also been determined from the NLR fit of the experimental pH data. Their values, independent of temperature, are on average 4.5×10^{-5} and 2.3×10^{-5} for HFLU and HIBU, respectively. From the values of Table 1 we can conclude the following. (1) All the inclusion complexes studied are confirmed to be 1:1, since

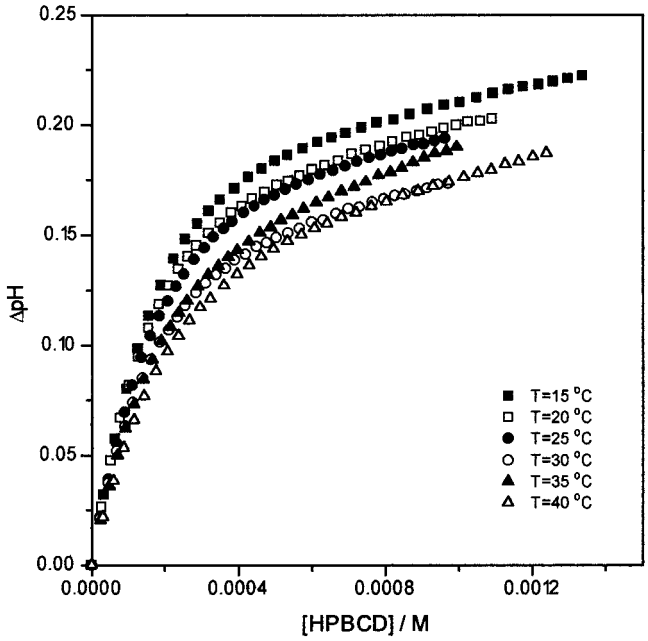

Figure 3. Plot of pH vs [HPBCD] for aqueous solutions where [ibuprophen] is kept constant at $\sim 1 \times 10^{-4}$ M, at different temperatures ranging from 15 to 40 °C.

Table 1. Values of the Association Constants of the 1:1 Inclusion Complexes Formed by HPBCD and the Acid and Base Forms of Flurbiprophen and Ibuprophen, as a Function of Temperature

T (°C)	[guest], 10^{-5} M	$K_{CD:HDrug}$ (M^{-1})	$K_{CD:Drug^-}$ (M^{-1})
	HPBCD + flurbiprophen		
15	3.99	14500 ± 1500	4000 ± 400
20	3.96	11300 ± 1100	3100 ± 300
25	4.97	9600 ± 1000	2530 ± 250
30	4.01	6800 ± 700	1750 ± 170
35	4.07	5400 ± 500	1510 ± 150
40	3.96	4500 ± 400	1180 ± 120
	HPBCD + ibuprophen		
15	10.00	16800 ± 1700	4400 ± 400
20	9.74	15400 ± 1500	4600 ± 500
25	10.06	15700 ± 1600	4700 ± 500
30	9.96	13100 ± 1300	4500 ± 500
35	10.02	10400 ± 1000	3100 ± 300
40	10.02	8700 ± 900	2600 ± 260

$K_{CD_2:HDrug}$ and $K_{CD_2:Drug^-}$ have been found to be negligible and always below the uncertainty of the fits. This 1:1 stoichiometry is usually found for small globular molecules upon binding the β-CD or β-CD derivatives.[3,4] (2) At all temperatures, the carboxylic species are encapsulated by HPBCD with a higher affinity than that of their ionized counterparts. This behavior has been previously reported[32,35−37] for other carboxylic derivatives. The lower stability of the CD:carboxylate complexes has been at-

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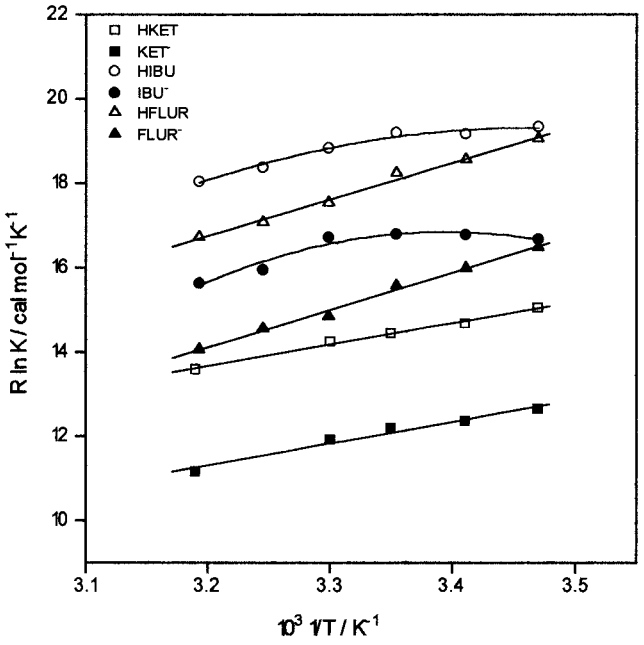

Figure 4. van't Hoff plots for the associations of HPBCD with several NSAIDs. The straight lines are fitted to $R \ln K = -\Delta H°/T + \Delta S°$. The curves are fitted to eq 10.

tributed to a number of possible factors: (i) the random character of the inclusion or the energy balance of the transferring species from the bulk to the CD cavity;[35] (ii) the repulsive interaction between the negative charge on the ionic species and the negatively charged end of the CD dipole;[37] and (iii) the differences on the entropic balance upon binding related to the structure-breaking character of the carboxylate anions.[15c] The association constants of Table 1 together with those previously found[15c] for ketoprophen acid (HKET) and ketoprophenate anion (KET^-) reveal that HPBCD binds these three NSAIDs with an affinity following the trend $K_{CD:IBU} > K_{CD:FLUR} > K_{CD:KET}$ at all the temperatures and for both the ionized and nonionized forms of the drugs. No literature values have been found for the systems studied in this work, although some results have been published[19,20] for the inclusion complexes formed by β-CD or 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) and flurbiprophen or ibuprophen. Nevertheless, these studies have been carried out only at 25 °C, and in most of the cases working with unbuffered solutions or at a pH not further enough from the pK_a of the drug to ensure a reliable binding constant value, as fully demonstrated elsewhere.[38] In fact, most of the published association constants of CD:drug complexes are apparent constants showing the contributions of the inclusions of both ionic and nonionic species. In any case, we can conclude, at least qualitatively, that for a given drug the trend in its association with β-CD and β-CD derivatives is $K_{\beta\text{-CD:HDrug}} \ll K_{DIMEB:Drug} \leq K_{HPBCD:Drug}$. This trend, also found for the association of these CDs and surfactant molecules,[39] is usually attributed to the fact that the substitution of 14 H by CH_3 groups (DIMEB) or 4 H by hydroxypropyl groups (HPBCD) lengthens the hydrophobic CD cavity, favoring the interactions CD:guest.

Table 2. Values of the Enthalpy ($\Delta H°$), Entropy ($\Delta S°$), and Heat Capacity ($\Delta C_p°$) Changes of the Binding Processes of Several Several NSAIDs by HPBCD at 25 °C

guest	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta C_p°$ (cal mol^{-1} K^{-1})
HFLUR	-9 ± 2	-11 ± 4	
$FLUR^-$	-9 ± 2	-14 ± 6	
HIBU	-4 ± 1	6 ± 4	-400 ± 150
IBU^-	-2.5 ± 0.8	9 ± 6	-700 ± 250
HKET	-5 ± 1[a]	-3 ± 2[a]	
KET^-	-5 ± 1[a]	-5 ± 4[a]	

[a] Reference 15c.

Thermodynamic Analysis. Figure 4 shows the plots of $R \ln K$ vs $1/T$ for the complexes studied in this work together with those formed by the ionized and nonionized forms of ketoprophen with HPBCD.[15c] The different behavior found for ibuprophen in comparison with flurbiprophen and ketoprophen is remarkable. Thus, a linear relationship is obtained in the latter cases, revealing the independence of $\Delta H°$ and $\Delta S°$ with $T(\Delta C_p° \sim 0)$, while in the case of ibuprophen, the absence of such linear behavior indicates that $\Delta H°$, and $\Delta S°$ are temperature dependent, pointing to an association process with $\Delta C_p° \neq 0$. If we assume that $\Delta C_p°$ is temperature independent and that the dependence on temperature for ΔH and ΔS can be expressed by

$$\Delta H = \Delta H° + \Delta C_p°(T - 298.15) \quad (8)$$

$$\Delta S = \Delta S° + \Delta C_p° \ln(T/298.15) \quad (9)$$

where 298.15 K has been taken as the reference temperature, the thermodynamic quantities $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ at 25 °C are related to $R \ln K$ through the van't Hoff equation.

$$R \ln K = -[\Delta H° + (T - 298.15)\Delta C_p°]/T + \Delta C_p° \ln(T/298.15)\, \Delta S° \quad (10)$$

When $\Delta C_p° = 0$ (flurbiprophen and ketoprophen guests), eq 10 is simplified to the well-known linear relation ($R \ln K = -\Delta H°/T + \Delta S°$). Equation 10 explains either the linearity or the curvature of the plots in Figure 4 and permits one to obtain $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ (when it differs from zero) by using a nonlinear regression of the experimental K values at various temperatures. Table 2 reports these thermodynamic quantities for the association processes studied, together with those previously found for HPBCD:HKET and HPBCD:KET^-. The data on this table call for some remarks: (a) $\Delta H°$ is negative in all cases, (b) the complexes of HPBCD with flurbiprophen and ketoprophen (in both ionic and nonionic forms) show negative $\Delta S°$ values, while in the case of ibuprophen, and according to eq 10, $\Delta S°$ is positive at $T \leq 25$ °C and negative at $T > 25$ °C and, (c) $\Delta C_p°$ is zero for CD:FLUR and CD:KET, and clearly negative for CD:IBU, for both acid and base species. Positive $\Delta C_p°$ values can result from hydrophobic interactions,[40] while $\Delta C_p° \approx 0$ may be associated with small conformational changes upon binding.[41] On the other hand, negative $\Delta C_p°$ values are usually found for the inclusion of apolar solutes by

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cyclodextrins[13a,42] and cyclophanes[43] and for carbohydrates association with lectins[44] in aqueous solution. Particularly, $\Delta C_p°$ values of around -500 cal mol^{-1} K^{-1} are typical of biological associations and recognition processes where hydrogen bonds and/or polar groups are present.[42−45] Diederich et al.,[43b,c] and more recently, Hayashi et al.,[45] have explained these negative $\Delta C_p°$ values in terms of the increase with temperature of the enthalpy difference of the solvated states of the free species with respect to that of the solvated complex. A global analysis of all this information reveals that the complexes formed by HFLUR, FLUR$^-$, HKET, or KET$^-$ with HPBCD are enthalpy driven, with favorable enthalpic terms and unfavorable entropic ones. It is worth noting that the differences on the complex stability of the carboxylic and carboxylate species is mainly due to an entropic effect, since the enthalpic term remains basically invariable with ionization. However, CD:HIBU and CD:IBU$^-$ complexes are enthalpy driven at $T > 25$ °C but entropy driven at $T \leq 25$ °C. This pattern is usually found in biological systems where the enthalpy governs the association process at high temperature while the entropy terms does at low temperatures.[46]

Molecular Mechanics and Dynamics Calculations. The maps of the trajectory of the torsion angles α_1 to α_3 during the MC/SD trajectory of the free ligand HFLUR are shown in Figure 5. The α_1 and α_2 torsions show two different populated regions, centered around 60° and −75° and 60° and −120°, respectively. α_3 is present for four different populated regions around 120°, 60°, −60°, and −120°. This allows us to consider a maximum of $2 \times 2 \times 4 = 16$ different populated conformers. Nevertheless, when accounting for the C_2 symmetry around the α_3 torsion, this number reduces to 8 conformers which were extracted from the MC/SD trajectory and subsequently minimized. After minimization, only 3 conformers were obtained in a window of energy up to 3 kcal mol^{-1} over the global minimum. A similar analysis was performed from the MC/SD trajectories of the ligands FLUR$^-$, HIBU, and IBU$^-$, thus obtaining a total of 3, 3, and 1 different minima, respectively. The torsion angles that characterize these minima can be seen in Table 3.

During the MC/SD simulation performed for the free HPBCD receptor, the glycosidic torsion angles ϕ/ω remained stable around 0° ± 25/15° ± 40, in agreement with the operativity of the exoanomeric effect[47] for α(1→4) type linkages. The ω torsions of the lateral chain of glucose remained around the alternated *gg* (−60°) or *gt* (60°) orientation during all the simulations, with only one of these torsions showing a transition from the *gt* to *gg*

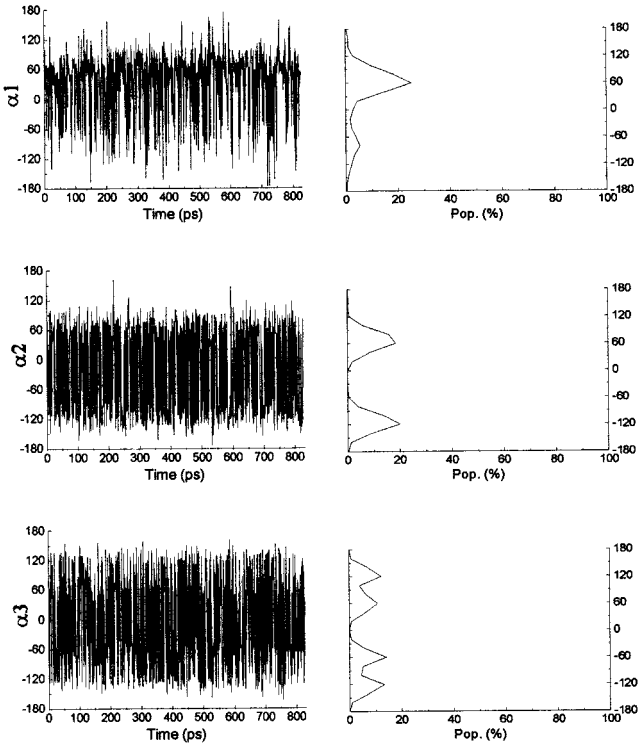

Figure 5. (Left) Trajectory of the torsion angles α_1, α_2 and α_3 in the free ligand HFLUR during the MC/SD simulation. (Right) The corresponding distribution map.

during the simulation.[48,49] The most populated different structures were extracted from the simulation and exhaustively minimized. Finally, only two low-energy minima were obtained, and both of them had very similar conformational and energetic features. The main difference between these two minima is that of the glycosidic torsions ϕ_6, ω_6, ϕ_7, and ω_7, which differ ∼25° each among the two structures. This difference affects the relative disposition of one pyranose ring of the HPBCD and, indeed, may reflect a higher degree of flexibility[50,51] around this pyranose ring.

The glycosidic torsions of the HPBCD receptor during the MC/SD trajectories of the complexes remained around the exoanomeric angles, as previously observed for the free receptor. Sometimes, a punctual transition was observed in some of the ω torsions among the *gg* and *gt* conformations of the lateral chains. A MC/SD trajectory of the ligand torsion angles (in *u* disposition) for the complex HPBCD:HFLUR is shown in Figure 6. There are four populated regions for the α_1 torsion around ca. 60°, 100°, −60°, and −100°, two for the α_2 torsion centered around 90° and −120°, and also two for the α_3 torsion around 60° and 120°. This analysis allows us to consider a maximum of $4 \times 2 \times 2 = 16$ possible energy minima for this complex. A similar analysis of the free ligands was accomplished for the MC/SD trajectory of all the complexes in the *u* and *d* dispositions. Finally, a total of 3, 3, 2, and 2 different lowest energy minima were obtained over the global minima for the complexes

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Table 3. Minima Obtained for HFLUR, FLUR$^-$, HIBU, and IBU$^-$ and Their Complexes with HPBCD

complex	min./orient.	torsion angles α_1	α_2	α_3	α_4	ΔE^a	single popln (%)
HFLUR	*A*	62.7	−118.1	−50.1		0.0	42
	B	63.9	61.3	−50.1		0.2	29
	C	63.9	61.5	−130.4		0.2	29
FLUR$^-$	*A*	105.5	64.1	130.4		0.0	35
	B	105.5	64.3	50.0		0.0	35
	C	−79.0	−116.0	50.0		0.1	30
HIBU	*A*	62.9	−118.2	77.9	60.6	0.0	38
	B	60.3	61.3	105.3	177.3	0.1	31
	C	−82.7	71.3	105.3	177.3	1.1	6
IBU$^-$	*A*	102.7	−116.9	78.0	60.9	0.0	100
HPBCD:HFLUR	*A*/*d*	72.6	−106.2	47.7		0.0	90
	B/*u*	51.3	47.1	−60.6		1.6	6
	C/*u*	60.6	48.6	131.1		1.8	4
HPBCD:FLUR$^-$	*A*/*d*	−84.8	−145.3	42.3		0.0	66
	B/*d*	131.2	−102.5	45.3		0.7	19
	C/*u*	103.8	−117.3	52.0		0.9	15
HPBCD:HIBU	*A*/*d*	97.6	87.4	117.8	−59.8	0.0	99
	B/*d*	−98.2	70.0	93.0	−65.8	2.8	1
HPBCD:IBU$^-$	*A*/*d*	131.7	−104.4	−83.0	173.6	0.0	77
	B/*d*	−78.1	−113.0	−75.2	174.1	0.7	23

[a] kcal mol^{-1}.

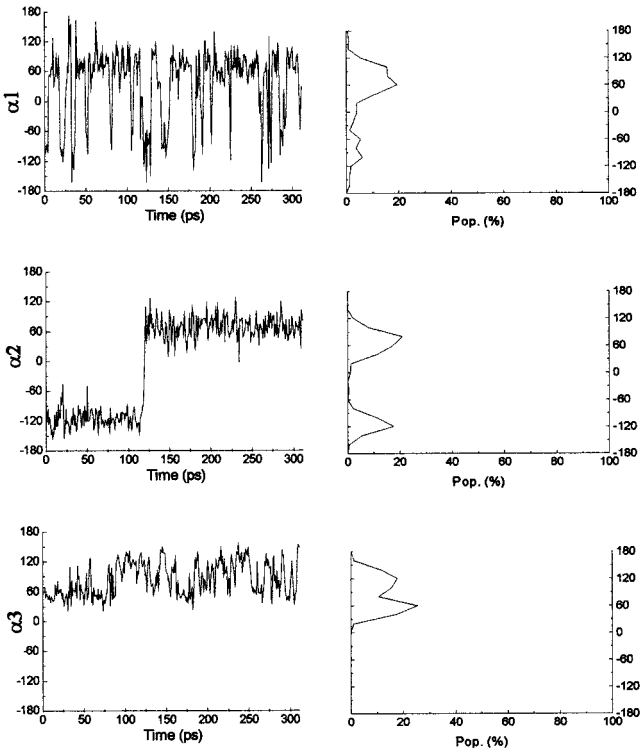

Figure 6. (Left) Trajectory of the torsion angles α_1, α_2, and α_3 in the complex HPBCD:HFLUR during the MC/SD simulation. This trajectory correspond to the ligand in the *u* disposition. (Right) The corresponding distribution map.

HPBCD:HFLUR, HPBCD:FLUR$^-$, HPBCD:HIBU, and HPBCD:IBU$^-$. The torsion angles that characterize these minima are given in Table 3. A view of the global minima of the different complexes is shown in Figures 7 and 8.

The global minimum of the HPBCD:HFLUR complex (minimum *A*/*d* in Table 3) has the ligand oriented in the *d* disposition and corresponds to a considerable population during the MC/SD trajectory. Nevertheless, this minimum has to be discarded when considering the $\Delta G_{MC/MD}$ values for the *u* and *d* dispositions in Table 4, which clearly favor *u* disposition of the ligand (91%) over

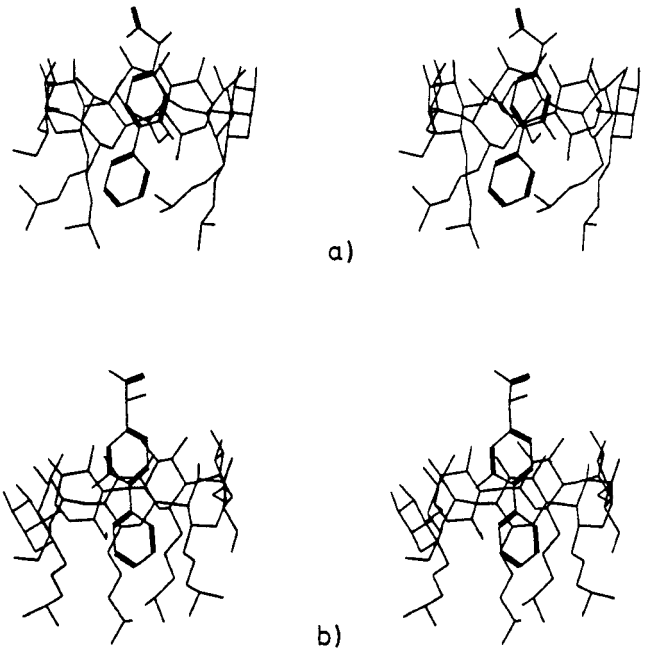

Figure 7. (a) Stereoview of the minimum *B*/*u* of HPBCD:HFLUR complex; (b) Stereoview of the minimum *C*/*u* of HPBCD:FLUR$^-$ complex. Hydrogens have been omitted for clarity.

d (9%). The other minima *B*/*u* and *C*/*u* are probably more representative of the conformation of the complex HPBCD:HFLUR. The ligand adopts a very similar conformation in these two minima (Table 3). Minimum *B*/*u* (Figure 7a) presents the four isopropylic chains of the HPBCD folded around the extreme phenyl group of the ligand. The fluorinated phenyl group of the ligand is located around the lipophilic face of the cyclodextrin ring of HPBCD. This position favors the stabilization of the complex by intermolecular van der Waals interactions between the two rings and it is in agreement with an NMR study[20c] that suggests the fluorobenzene ring is allowed into the CD cavity. Furthermore, a number of other favorable intermolecular interactions have been observed during the corresponding MC/SD of the *B*/*u* minimum. One of them is a intermolecular hydrogen

Table 4. Energy Contributions (kcal mol^{-1}) Estimated from the MC/SD Simulations for the Different Possible *u*/*d* Ligand Disposition in the Complexes

complex	$\Delta G_{MC/MD}$	single popln (%)	complex	$\Delta G_{MC/MD}$	single popln (%)
HPBCD:HFLUR			HPBCD:FLUR$^-$		
u	0.0	91	*u*	0.5	30
d	1.4	9	*d*	0.0	70
HBPCD:HIBU			HPBCD:IBU$^-$		
u	5.6	0	*u*	0.1	45
d	0.0	100	*d*	0.0	55

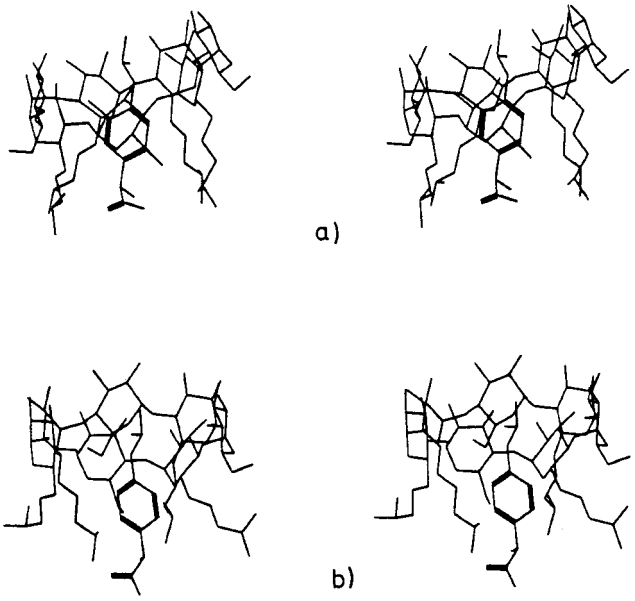

Figure 8. (a) Stereoview of the global minimum *A*/*d* of the HPBCD:HIBU complex. (b) Stereoview of the global minimum *A*/*d* of the HPBCD:IBU$^-$ complex. Hydrogens have been omittedfor clarity.

bond between the hydroxylic group of the ligand and a carbohydrate hydroxyl group of the HPBCD formed during 58% of the simulation time. The other favorable interaction is a F···HO contact between the ligand and some of the carbohydrate hydroxyl groups of HPBCD. This contact is present during 34% of the trajectory.

The three lowest minima of the HPBCD:FLUR$^-$ complex in Table 3, *A*/*d*, *B*/*d*, and *C*/*u*, are representative of this complex. A comparison of the structure of minimum *C*/*u* (Figure 7b) with the analogue minimum *B*/*u* of the HPBCD:HFLUR complex (Figure 7a) shows that the ligand FLUR$^-$ is less deeply included into the cavity of the HPBCD. Otherwise, the polar carboxylate group in all the minima is out of the cavity making contact with the solvent molecules. When the MC/SD trajectories corresponding to these HPBCD:FLUR$^-$ minima are compared with that previously commented on for HPBCD:HFLUR, a reduction of hydrogen-bonding interactions and of F···HO contacts is observed. These interactions are only present during 18% and 38% of the most populated trajectory, respectively. The minima obtained for both HPBCD:HIBU and HPBCD:IBU$^-$ complexes have the ligand preferably oriented in *d* disposition. The global minimum of the HPBCD:HIBU complex (Figure 8a) presents vdW contacts between the phenyl ring and the lipophilic face of the cyclodextrin ring of HPBCD. There is also a possible intermolecular hydrogen bond of the ligand hydroxylic group with and hydroxyl group of a lateral chain of the HPBCD receptor. This hydrogen bond is present during 27% of the MC/SD trajectory. The global minimum of HPBCD:IBU$^-$ (Figure 8b) shows the ligand less deeply included into the cavity and the carboxylate group is again in contact with the solvent. There is a reduction of the van der Waals contacts with the lipophilic surfaces, and the extension of the previously commented on intermolecular hydrogen bond is reduced to 12% of the corresponding trajectory.

Analysis of the Ligand during the MC/SD Trajectories. The analysis of the MC/SD trajectories of the ligand in the free and bound states may reveal important information relative to the recognition process. The trajectories of the torsion angles α_1 to α_3 of the free ligand HFLUR and the HPBCD:HFLUR complex are represented in Figures 5 and 6, respectively. The trajectory of HFLUR shows rapid transitions in a time scale of 1−10 ps. On the other hand, for the complex HPBCD:HFLUR the fluctuations are drastically reduced in time and space. One extreme case is that observed for α_3 torsion that describes the flip around the extreme phenyl ring. The fluctuations of α_3 around the C_2 symmetric positions 60°/−120° and −60°/120° are very frequent in the free HFLUR. Nevertheless, these transitions are no longer observed during the whole simulation of the HPBCD:HFLUR complex.

A similar analysis was performed for the different ϕ, ψ, ω torsions and the torsions of the four pendant chains of the HPBCD component of the HPBCD:HFLUR complex. The results reveal that there is a negligible difference of flexibility in the HPBCD in the bound and free states. A more quantitative estimation of the flexibility of all the complexes was accomplished by measuring the extensions of the populated regions of the map of the previously mentioned torsions of the ligand and the receptor during the MC/SD simulations. All the complexes showed a reduction of the global flexibility of the ligand molecule around 14% when the complex is formed. Otherwise, the global flexibility of the HPBCD is practically unaltered after complexation with a global reduction of ∼2% in all cases. The reduction of the flexibility of the ligand upon binding can be related with a decrease of conformational entropy, ΔS_{conf}. Nevertheless, this effect is not enough to explain the experimental ΔS results in Table 2 and contrasts with the positive ΔS values observed for the HIBU and IBU$^-$ complexes at 25 °C. A complete evaluation of the entropy would require taking into account other terms such as $\Delta S_{translational}$, $\Delta S_{rotational}$, and $\Delta S_{vibrational}$ and molecular dynamics simulations with explicit solvent molecules.

Conformational Stability of the Free and Associated Ligands. The use of molecular mechanics force fields and a continuous or explicit solvation model to emulate the water is a relatively crude approximation to estimate accurate association constants. Nevertheless, the capacity of some molecular mechanics and dynamics methods to generate an ensemble of representative

Table 5. Energy Contributions (kcal mol^{-1}) Averaged over the MC/SD Simulations for the Different Complexation Processes

transformation direction	ligand disposition	$\Delta\Delta G_{MC/MD}$	$\Delta\Delta E$
HPBCD + FLUR$^-$ →	*u*	−7.6	−10.0
HPBCD:FLUR$^-$	*d*	−7.9	−3.0
HPBCD + HFLUR →	*u*	−10.0	−14.5
HPBCD:HFLUR	*d*	−8.6	−13.6
HPBCD + HIBU →	*u*	−78.8	−52.3
HPBCD:HIBU	*d*	−84.4	−56.9
HPBCD + IBU$^-$ →	*u*	−10.4	−11.5
HPBCD:IBU$^-$	*d*	−10.5	−11.2

conformations and average energies that can serve to obtain at least a qualitative estimation of the energetic contributions for these processes has been recognized.[29,52] In a first step, the estimation of the ionization constants for the transformations of the free ligands HFLUR → FLUR$^-$ + H$^+$ and HIBU → IBU$^-$ + H$^+$ was accomplished by free energy perturbation simulations[53] using MM3 with the GB/SA water solvent model. The results indicate that the ligands are predominantly in the nonionized form, especially the HFLUR ligand, which is in qualitative agreement with the dissociation constants of both acids determined from potentiometry.

Otherwise, the average values of the potential and total energy during the MC/SD trajectory have been considered in order to estimate the $\Delta\Delta G$ and $\Delta\Delta E$ involved in the different association processes. The results shown in Table 5, represent the global contribution of the different favorable and unfavorable ligand/receptor interactions averaged during the whole simulation. The negative values obtained in Table 5 for all the complexation processes indicate that the formation of the complexes is energetically favored compared with the sum of the energies of the free ligand and receptor. This fact is in agreement with the association constants determined from the pH potentiometric data. For the HFLUR ligand, the most favorable orientation in the complex with HPBCD is *u* (Table 4), while for FLUR$^-$ both *u* and *d* orientations are possible. For HIBU the *d* disposition is most favorable to complexation, while for IBU$^-$ both the *u* and *d* dispositions have a very similar stability. The $\Delta\Delta G$ and $\Delta\Delta E$ relative values in Table 5 correctly predict the higher stabilization of the nonionized forms HIBU and HFLUR compared with the corresponding IBU$^-$ and FLUR$^-$, respectively. Also, the relatively higher stabilization of HIBU with respect to HFLUR and IBU$^-$ with respect to FLUR$^-$ is correctly predicted. Therefore, according to the analysis of MC/SD simulations and the experimental data, several intermolecular forces can drive the association process, such as vdW and hydrogen bond interactions, and in the case of flurbiprophen also the F···HO interaction. The importance of the F···HO interaction seems to be theoretically and experimentally less important than the other two, as deduced by the higher stability of the HIBU complex with respect to the HFLUR complex. The modeling also offers an explanation for the lower stability observed for the ionized complexes. In these complexes, the ligand disposes on average out of the CD cavity associated with a reduction of the possibilities to establish van der Waals and hydrogen bond interactions with the receptor.

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Supporting Information Available: pH potentiometric data and a figure showing the trajectory of the different glycosidic and ω torsions during MC/SD of HPBCD (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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