Potentiometric Study of the Encapsulation of Ketoprophen by Hydroxypropyl-β-cyclodextrin. Temperature, Solvent, and Salt Effects

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The molecular encapsulation of ketoprophen (KET), a nonsteroidal antiinflammatory drug of the family of the arylpropionic acids, by the hydroxypropyl-β-cyclodextrin (HPBCD) has been studied from pH potentiometric measurements. For that purpose, a highly accurate and fully computerized potentiometric technique is presented, together with a model developed by us to obtain simultaneously, from pH data, the dissociation constant K_a of the pure drug (which is a weak acid), and the association constants of the inclusion complexes formed by the cyclodextrin and both the nonionized (HKET) and ionized (KET^-) forms of the drug, without the necessity of working with buffered solutions. The physicochemical characterization of the system HPBCD + KET has been carried out in three sections: (i) the pH of drugs solutions has been measured as a function of cyclodextrin concentration at different temperatures, ranging from 15 to 40 °C. From the dependency of the association constants with temperature (van't Hoff analysis), the inclusion complexes formed by HKET or KET^- and the HPBCD were found to be enthalpy driven, with a favorable enthalpic term dominant over an unfavorable entropic term. This pattern could be revealing the contribution of van der Waals interactions, hydrophobic effect, and solvent reorganization as the main driven forces promoting the interaction. (ii) Solvent effects have been evaluated by studying the influence of the presence of different constant amounts of a series of alcohols (methanol, ethanol, propanol, and butanol) on the CD−drug interaction (CD = cyclodextrin), at 25 °C. A clear influence of the solvent polarity on the affinity of binding has been found, in the sense that, as long as the medium becomes more apolar, the interaction between the drug and the cyclodextrin is weakened. A phenomenological *limit association curve* is proposed to define the limit conditions to have association. (iii) From the characterization of the HPBCD + KET system in the presence of constant amounts of 2:1 electrolytes, such as $CaCl_2$, K_2SO_4, and $ZnCl_2$ at 25 °C, neither an influence of the ionic strength of the medium nor the possible mediation of divalent cations (Ca^{2+}, calcium effect) on the CD−drug interaction have been found.

Introduction

Modern biomedical research leads to revealing insights into the molecular activity of many therapeutic molecules. An interesting activity at the organ or cellular level, however, is not sufficient to turn a molecule into an usable drug. As important as its intrinsic activity is the ability of the molecule to reach its target site in a live body. Problems such as a limited solubility or stability can make impossible to transpose interesting in vitro properties of an experimental compound to an in vivo situation. In many cases, the approach used to overcome such problems degrades the efficacy, safety, or comfort of the drug concerned. Various physicochemical methods have been used to improve aqueous solubility of poorly soluble drugs and to increase the shelf life of unstable drugs. In the recent years, molecular encapsulation has been successfully used in many technological fields.[1−8] Particularly, pharmaceutical industry have made use of it to improve the bioavailabilities of drugs, to protect them from decomposition, to convert liquids to free-flowing power, and to mask unfavorable odors and tastes.[9−11]

Cyclodextrins form a whole new family of pharmaceutical excipients. The most common cyclodextrins (α-, β-, and γ-CD, (CD = cyclodextrin)) are, respectively, composed of 6, 7 or 8 (1 → 4) α- linked D-glucose units. The result is a doughnut-shaped structure with a hydrophilic outer surface and a lipophilic cavity, where poorly water-soluble molecules can shelter their most hydrophobic parts.[12−14] It is well-known that cyclodextrins form noncovalent inclusion complexes with a wide variety of lipophilic drug molecules by taking up a whole molecule, or some part of it into the cavity,[15−17] with the stability of the complex depending on different contributions, such as van der Waals interactions, hydrophobic effect, solvent reorganization, hydrogen bonding, key−lock principle, etc.[18] The complexation usually alters the physicochemical properties of the drug molecule,[19−22] and, frequently, changes the characteristics of a drug dramatically. However, an intrinsic problem has prevented CDs from becoming general drug delivery systems; β-CD, dimensionwise the most interesting cyclodextrin for drug complexation, is markedly less soluble than either α- or γ-CD. This low solubility in water (1.85 g/100 mL at 25 °C) generally limits its use to oral applications. In this sense, β-CD derivatives such as 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) or hydroxypropyl-β-cyclodextrin (HPBCD), with a higher solubility, are becoming widely used in pharmacological formulations.[23]

Nonsteroidal antiinflammatory drugs (NSAIDS) are widely used for the short-term relief of pain, and inflammation disorders. Although these drugs are highly effective in these indications, they also have certain drawbacks. NSAIDS with a fast onset of action also have a short elimination half-life, necessitating multiple daily dosages with the increasing risk of developing drug-related adverse effects, while NSAIDS with a long duration of activity generally have a delayed onset of

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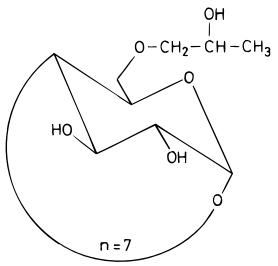
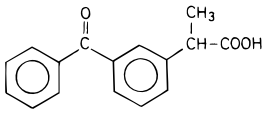
SCHEME 1



Hydroxypropyl-β-Cyclodextrin (HPBCD)



Ketoprophen (KET)

action. Another troubling aspect of NSAID therapy is its propensity to produce gastrointestinal adverse effects. The main aim of NSAID complexation with CDs is thus to take a long-acting agent and provide it with a faster onset of activity and, possibly, to improve its gastrointestinal tolerability.[10−11]

During the last 2 decades, there can be found in the literature a great number of papers involved in the study of the pharmaceutical applications of these CD−drug complexes (see refs 19−23 and references therein), but very few[24,25] are directly focused on a physicochemical characterization of these microencapsulates based on the stoichiometry and association constant determination, as well as on the analysis of the effect of different factors, such as temperature, solvent polarity, ionic strength, presence of divalent cations, etc. The study of the influence of temperature in the affinity of the binding gives an interesting information regarding the driven forces governing the process. Moreover, it is well-known that solvent effects[26−29] may play an important role in many molecular recognition phenomena. Thus, water is the best solvent for increasing the affinity between apolar binding partners, being that this effect is extremely important for supporting all functions of life. It is then interesting to have thermodynamic information about the influence of the solvent polarity on the free energy of the interaction. It has been also found[30,31] that the presence of an electrolyte and, particularly, that of salts containing divalent cations (such as Ca^{2+}) may affect the overall stability of the recognition process, due either to the contribution of favorable electrostatic interactions when either the substrate or the receptor, or both, are charged species or to the mediation of Ca^{2+} in the interaction (calcium effect).

This work consists of a physicochemical characterization of the recognition phenomenon of a NSAID drug of the family of the arylpropionic acids, such as ketoprophen (KET), by the hydroxypropyl-β-cyclodextrin (HPBCD) (see Scheme 1). These carboxylated species offer significant advantages over the antiinflammatory agents related to the acetylsalicylic acid (aspirin), since they are more potent and have a lower incidence of gastrointestinal irritation. Since KET is a weak acid, its dissociation must be considered when the encapsulation by the CD cavity occurs. To carry out this physicochemical study, we have set up a fully computerized technique to measure pH and/or the electromotive force (emf) in liquid solutions, which has the advantage of a very low uncertainty in the experimental data due to the almost no participation of human hands during the measuring process. Besides, we have developed a model, based on that of Gelb and co-workers,[32] to obtain simultaneously, from pH potentiometric measurements, the dissociation constant of pure KET, and the association constants of the inclusion complexes formed by both HKET and KET^- (where HKET and KET^-, respectively, represent the nonionized and ionized forms of the drug) and HPBCD, without the necessity of working with buffered solutions. This fact implies a clear advantage of the method because not only facilitates the experimental procedure and the K determination, but also improves the accuracy of the results. The study has been divided in three parts: (i) the pH of drug solutions has been measured as a function of cyclodextrin concentration at different temperatures, ranging from 15 to 40 °C, in order to obtain information about the driven forces involved in the complexation process through the corresponding van't Hoff plots. (ii) The influence of solvent polarity has been evaluated by characterizing the HPBCD + KET system in the presence of different amounts of a series of alcohols, such as methanol, ethanol, propanol, and butanol, at 25 °C). (iii) The effect of the ionic strength, and particularly the role of divalent cations in the molecular recognition of the drug by the CD, has been analyzed by characterizing the system in the presence of different amounts of several electrolytes, such as $CaCl_2$, $ZnCl_2$, and K_2SO_4, at 25 °C. From these results, we expect to have insight into the different factors which govern the encapsulation of a NSAID drug, such as KET, into the cyclodextrin cavity.

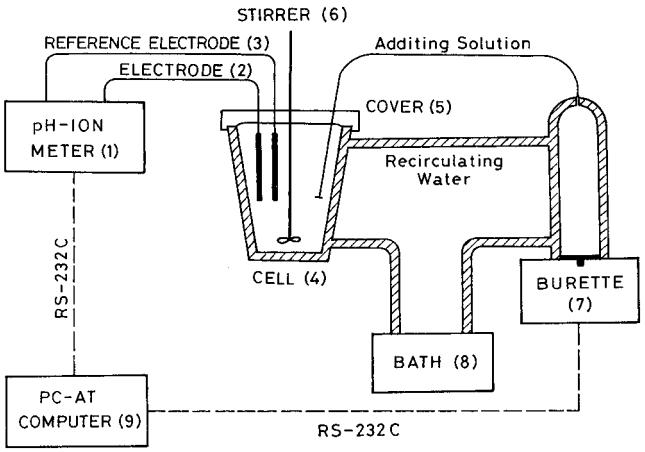


Figure 1. Block diagram of the equipment for the measurement of the emf in liquid mixtures: (1) Metrohm 713 pH-Ion meter, (2) and (3) Metrohm combined glass electrode, (4) Metrohm measurement cell, (5) Metrohm cover, (6) Metrohm polypropylene stirrer, (7) Metrohm 665 Dosimat digital burette, (8) thermostated bath of own design, (9) Olivetti PCS-286 computer.

Experimental Section

Materials. Ketoprophen (KET) was from Sigma, while hydroxypropyl-β-cyclodextrin (HPBCD), containing an average of 0.4 hydroxypropyl groups/glucopyranose unit, was from Janssen Biotech. All the alcohols, methanol (MeOH), ethanol (EtOH), propanol (PrOH), and butanol (BuOH), the hydrochloric acid, HCl, and the salts, $CaCl_2 \cdot 2H_2O$, K_2SO_4, and $ZnCl_2$, were from Merck. All of them have a purity greater than 99% (except HCl, with a 37% purity), and were used without further purification. HPBCD has been found (from a TG analysis) to consist of 3.4% water content by mass, which was considered to calculate solute concentrations. Bidistilled water was purified by using a Super Q Millipore System and was also degassed prior to the preparation of the solutions. In order to assure the homogeneity of the initial solutions, they were sonicated for 30 min in an ultrasonic bath.

pH Potentiometric Measurements. Figure 1 shows a block diagram of the potentiometric technique. A Metrohm 713 pH-Ion meter (1), with 0.1 mV or 0.001 pH units of resolution for voltage and pH measurement, respectively, together with a Metrohm combined glass electrode, (2, 3) filled with KCl 3 M solution, were used. The sample is placed in a measuring cell (4) with a cover (5) to avoid the evaporation of the solution. A Metrohm digital burette (7), with a resolution of 0.002 mL, was filled with the liquid that was to be added to the measuring cell

in the successive additions. A polypropylene stirrer (6), activated by an external motor which rounds at constant velocity, guarantees the homogeneity of the mixing process. The measuring cell and the burette cylinder are thermostated with recirculating water from the thermostated bath (8) through a Lauda pump. The bath temperature, which is held constant within ±0.5 mK, is read by a Kaynos Testoterm quartz digital thermometer. A PC computer (9) communicates with the pH-ion meter and with the burette through two standard RS-232C serial ports.

The adjustment of both the asymmetry potential and the Nernst slope of the combined glass electrode used in this work was made by calibrating the electrode with three Metrohm buffer solutions of pH = 4, 7, and 9. The Nernstian behavior of the electrode was verified by measuring the pH of a solution of increasing HCl concentration.

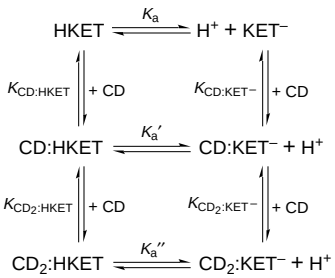
The preparation of the solutions and its placement into both the measurement cell and the burette are the unique experimental processes to be manually done by the experimenter. The molarity of the solutions both in the cell and in the burette, together with the initial volume in the cell, are requested by the program as initial data. The acquisition and averaging of the pH or emf measurements, the determination of the concentration from the added volumes, the calculation and storage of the results, and the successive additions of the solution in the burette are controlled automatically by the computer through a Quick Basic program made by us, easily adaptable to any other potentiometer or digital burette. The quantity of liquid to be successively added from the burette is automatically selected by the program, depending on its concentration, the initial volume in the measuring cell and the quantities previously added. In the experiments shown here in, the initial volume of the sample has been chosen to be around 21 mL, and the volume added from the burette around 20 mL (which corresponds to the volume of the burette cylinder). Working in the above mentioned experimental conditions, we made a total of 36 measurements in each experiment.

The fairly good temperature control of the solution, together with the statistical averaging process of 250 measurements for each pH data, allow us to increase the accuracy of the experimental data up to a 30% (±0.003 mV for the voltage and ±0.0003 for the pH), with respect to that specified by the manufacturers of the equipment. Moreover, the accuracy in the initial volume of the solution in the cell and in the added volume from the burette make possible to know the composition of the mixture with an accuracy better than 1×10^{-5} M.

Results and Discussion

Binding Constants Determination. Since KET is a weak acid, both nonionized (HKET) and ionized (KET^-) species are present in the solution. As a consequence, when the CD is added, both CD−HKET and CD−KET^- inclusion complexes are formed, following the equilibria shown in Scheme 2.

SCHEME 2



where K_a is the dissociation constant of KET, and $K_{CD-HKET}$, $K_{CD2-HKET}$, K_{CD-KET^-}, and $K_{CD2-KET^-}$ are the association constants of the 1:1 and 2:1 inclusion complexes formed by the HPBCD and the acid and base forms of KET, respectively. From geometrical considerations and also from previous studies[24,25] of similar systems, 1:2, 2:2, and/or even CD−drug complexes with higher stoichiometries are not expected to be found.

The equilibrium constants can be expressed as a function of the activities of the species, as follows:

$$K_a = (a_{H^+}\, a_{KET^-})/(a_{HKET}) \quad (1)$$

$$K_{CD-HKET} = (a_{CD-HKET})/(a_{CD}\, a_{HKET}) \quad (2)$$

$$K_{CD-KET^-} = (a_{CD-KET^-})/(a_{CD}\, a_{KET^-}) \quad (3)$$

$$K_{CD2-HKET} = (a_{CD2-HKET})/(a_{CD-HKET}\, a_{CD}) \quad (4)$$

$$K_{CD2-KET^-} = (a_{CD2:KET^-})/(a_{CD-KET^-}\, a_{CD}) \quad (5)$$

The constants K_a' and K_a'' are related to the other ones through simple expressions. Equations 1−5, and those for the activity coefficients using the extended Debye−Hückel theory, together with the corresponding mass and charge balances, permit us to obtain the equilibrium constants from the experimental pH data with a nonlinear regression method based on a Marquardt algorithm. The program to carry on the numerical analysis was written by us in TURBO C. Several models can be found in the literature which are involved as well in the determination of binding constants from pH measurements.[32−34] Particularly, the model we present in this work, although is initially based on that of Gelb and co-workers,[32] introduce several improvements. In fact, Gelb's procedure consists of fitting the pH data as a function of known but variable portions of solid cyclodextrin added to an equimolecular solution of the acid and conjugated base species of the substrate. In our method, the total concentration of the substrate is kept constant as well, while the CD concentration is varying by the successive addition of a CD solution. Thus, the corrections[32] applied to the solution volume and to the pH measurements when the solid CD is added through auxiliary experiments are avoided. Other differences must be noted: (i) although the ion size parameters a_i of the extended Debye Hückel theory for H^+ and OH^- are taken from the tables in the literature[35,36] in both models, the a_i for free KET^- in our model has been calculated from conductivity measurements. Anyway, we have verified that, given the low concentrations used in the experiments, the effect of these parameters in the final K values is almost negligible. (ii) None of the equilibrium constants, which are obtained as fitting parameters, are fixed to zero. The mathematical procedure gives negligible K values (within the experimental error and the uncertainty of the fit) for those associations which do not occur. For example, in most of the studied complexation processes with cyclodextrins we have found only 1:1 inclusion complexes; thus, our model would obtain values around zero for K_{CD2-S} (S is the substrate), while Gelb's model fixed them to zero. The accuracy and physical meaning of the fitting parameters increases when they are allowed to vary freely in the numerical analysis; (iii) our method allows us either to obtain the dissociation constant of the acid/base conjugated pair (K_a) as a fit parameter, or to fix it when its value is well-known. The comparison of the results obtained in both cases is another control of the suitability of the method.

The model proposed by us in this work has been used to study the complexation of KET by HPBCD. The three parts of the study, i.e., the influence of temperature, the solvent

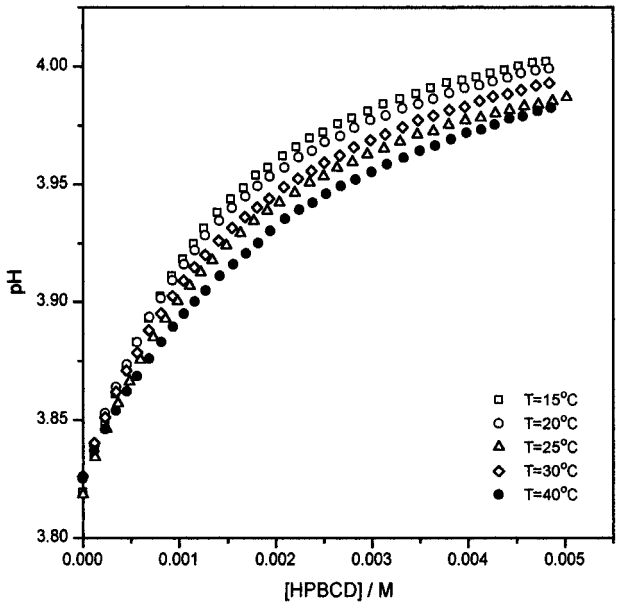


Figure 2. Plot of pH vs [HPBCD] for aqueous solutions where [KET] is kept constant at 5×10^{-4} M at different temperatures ranging from 15 to 40 °C.

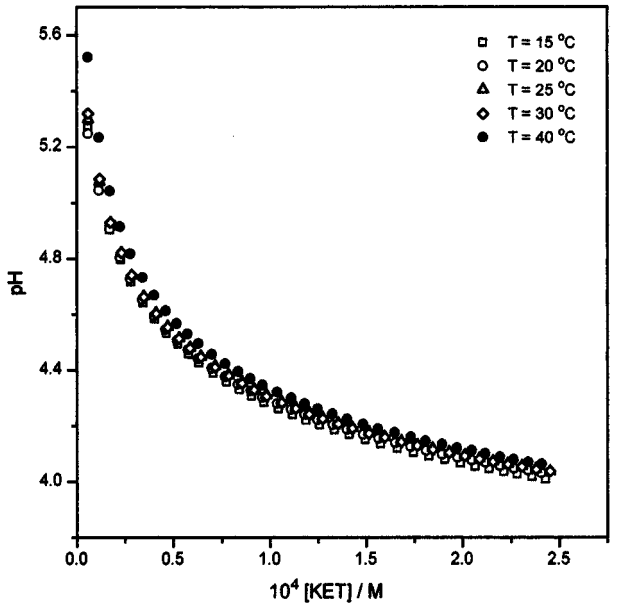


Figure 3. Plot of pH vs [KET] in the absence of HPBCD at different temperatures ranging from 15 to 40 °C.

TABLE 1: Values of the Dissociation Constant K_a of Ketoprophen (KET) and the Association Constants of the 1:1 Inclusion Complexes Formed by Hydroxypropyl-β-cyclodextrin and the Acid and Base Forms of KET, $K_{CD-HKET}$, and K_{CD-KET^-}, Respectively, as a Function of Temperature

T (°C)	$10^5 K_a$	$K_{CD-HKET}$ (M^{-1})	K_{CD-KET^-} (M^{-1})	$K_{CD-HKET}/K_{CD-KET^-}$
15	7.4 ± 0.7	1940 ± 200	580 ± 60	3.3
20	6.8 ± 0.7	1610 ± 160	500 ± 50	3.2
25	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
30	6.2 ± 0.6	1300 ± 130	400 ± 40	3.2
40	5.2 ± 0.5	930 ± 90	275 ± 30	3.4

effects, and the influence of the ionic strength, and, possibly, a calcium effect in the CD−drug interaction, will be discussed in the following sections.

Temperature Dependence. Figure 2 shows the curves of pH as a function of HPBCD concentration, with total [KET] kept constant at 5×10^{-4} M, at various temperatures ranging from 15 to 40 °C. When a HPBCD solution is added to the drug solution, all the equilibria of Scheme 2 must be considered, since both HKET and KET^- species, belonging from the dissociation equilibria of KET, can be encapsulated by the cyclodextrin following 1:1 and may be 2:1 (CD−drug) stoichiometries. The pH values are indicative of the shifting of the equilibria as long as CD is added, depending on the magnitude of the association constants involved. These equilibrium constants have been obtained following the model proposed in this work, and they are reported in Table 1. From this table, we can conclude that (i) the dissociation of KET slightly decreases with the increasing temperature, revealing the exothermic character of the dissociation process. These results (which are not included in Table 1) have been found to be in agreement with the K_a values obtained in other experiments, where the pH of the medium was been measured as a function of KET concentration in the absence of cyclodextrin and at the same temperatures (the experimental data are shown in Figure 3). (ii) HKET and KET^- form 1:1 inclusion complexes with HPBCD. Since $K_{CD2-HKET}$ and $K_{CD2-KET}-$ have been found to be negligible and always below the uncertainty of the fit, they are not reported in the table. (iii) At all the temperatures, the binding affinity of the acid form of KET (HKET) by the HPBCD ($K_{CD-HKET}$) is around 3 times higher than that of the ionized form KET^- ($K_{CD-KET}-$). The tighter binding of neutral acid forms with respect to their ionic partners has been previously reported in the literature for other carboxylic derivatives.[32,37,38] It is believed that, when the substrate is a carboxylic acid derivative, it penetrates the cavity by the wider ring, carboxylic group end first, i.e., the ionizable group is included into the CD cavity when the encapsulation occurs. Some authors[37] attribute this pattern to the fact that carboxylate species, although they may experience some charge delocalization, penetrate the cavity more randomly than their neutral partners and without the concurrence of any additional dispersion interaction. Moreover, it is believed[37] that these carboxylated species would not be completely stripped of water of solution when they are encapsulated by the cyclodextrins, thus lowering the insertion interaction. Whatever the possible causes could be, either the random character of the inclusion or the energy balance of transferring species from the bulk (with a high dielectric constant) to the CD cavity (of low dielectric constant), the fact is that the system studied in this work behaves consistently with previous findings. The origin of this behavior will probably better understood with the values obtained for the entropy and enthalpy changes (ΔS and ΔH) for the inclusion process. (iv) It can be observed as well in the table that as long as the temperature increases, the affinity of the cyclodextrin for both ionized and nonionized forms of ketoprophen decreases. Figure 4 shows the van't Hoff plots of both association processes. Although van't Hoff analysis assume that ΔC_p is zero, which is not always true for this kind of processes,[39−41] they are useful to have at least a qualitative information about the enthalpic or entropic character of the process. From the linear fit of van't Hoff plots of Figure 4, enthalpy (ΔH) and entropy (ΔS) changes have been determined for the association of both HKET and KET^- with HPBCD. The results are $\Delta H = (-5 \pm 1)$ kcal mol^{-1} and $\Delta H = (-5 \pm 2)$ kcal mol^{-1} for HPBCD−HKET and HPBCD−KET^- inclusion complexes, respectively, while $\Delta S = (-3 \pm 2)$ cal mol^{-1} K^{-1} and $\Delta S = (-5 \pm 4)$ cal mol^{-1} K^{-1}, respectively, as well for the mentioned complexes. Although the uncertainty in these results is high, as usual for van't Hoff analysis, it can be observed that both HKET and KET^- species bind to HPBCD with a favorable enthalpic term ($\Delta H < 0$) and an unfavorable entropic term (ΔS

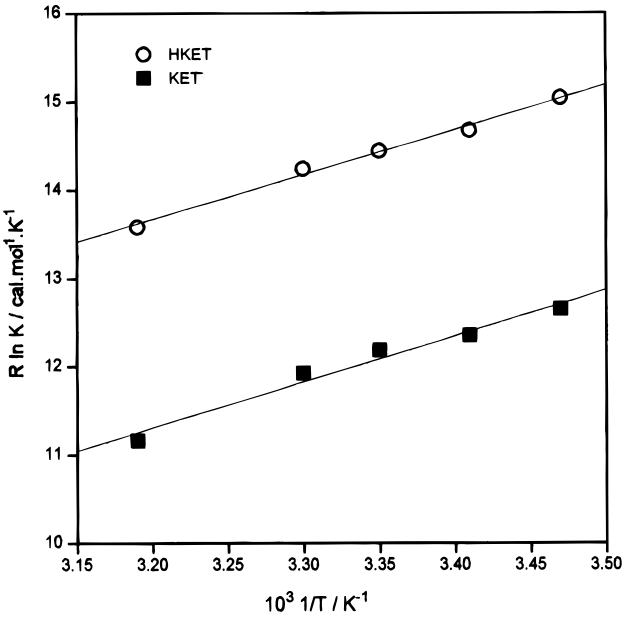


Figure 4. van't Hoff plots for the associations of HPBCD with HKET and KET^-.

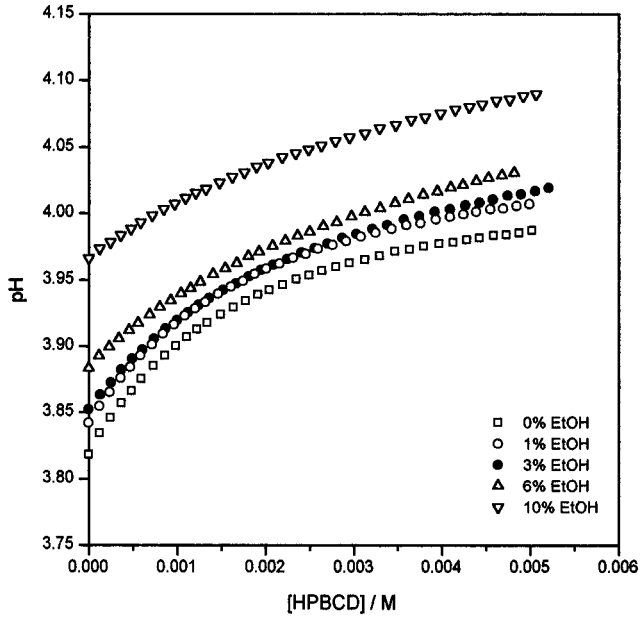


Figure 5. Plot of pH vs [HPBCD] for aqueous solutions where [KET] is kept constant at 5×10^{-4} M in the presence of various constant concentrations of ethanol (EtOH) at 25 °C.

< 0). Both processes are exothermic and enthalpy driven ($|\Delta H| > T\,|\Delta S|$), as usually found[27,41−44] for associations between small guest molecules and an apolar cavity in water. However, this is not what is to be expected for a typical hydrophobically driven process, where positive change in entropy and close to zero enthalpy change are observed.[17] A combination of hydrophobic effect ($\Delta H \sim 0$ and $\Delta S > 0$), van der Waals forces ($\Delta H < 0$ and $\Delta S < 0$) and solvent reorganization could account for the thermodynamic parameters of HKET and KET^- binding to HPBCD. Within the great uncertainty surrounding these parameters, it can be concluded that the enthalpic term is similar in both associations, while the entropic term is basically more unfavorable for the association of HPBCD with KET^- than for that one with HKET. Thus, the responsible of the higher affinity of HKET by the cyclodextrin could be the difference between the entropic terms, and it could be related to the water-structure-breaking character of carboxylate anions. These anions can be classified within the group of hydrophilic ions, which tend to orient the water molecules radially with respect to their electrostatic field breaking the initial water structure.[45] When the KET^- species is encapsulated into the CD cavity, it is expected that most of the solvation water molecules are transferred to the bulk, increasing the overall order of the system and decreasing the entropic balance. However, in the case of HKET, although the encapsulation process is as well entropically not favorable, the global balance is less unfavorable than in the case of KET^- encapsulation, since HKET itself alters the water structure in a lesser extent. It looks like the contribution of van der Waals interactions and hydrophobic effect could be similar in both inclusion processes, while the contribution of solvent reorganization could be the factor which makes the binding of HPBCD and HKET energetically more favorable than that of HPBCD and KET^-.

Solvent Effects. Several studies can be found in the literature regarding medium effects [26−28,46] and in particular the role of water[47−50] in binding processes, but very few quantitative correlations of association constants to solvent properties exist due to the absence of suitable general parameters describing solvophobicity. With the aim of evaluating the effect of the medium in the binding characteristics of KET and HPBCD, the association constants $K_{CD-HKET}$ and K_{CD-KET^-} as well as the dissociation constant K_a of pure KET have been determined at 25 °C in the presence of several constant percent (w/w) of methanol, ethanol, propanol, and butanol. For that purpose, the pH of aqueous alcoholic solutions, where the total KET concentration is kept constant at 5×10^{-4} M, are measured as a function of HPBCD concentration. Figure 5 shows, as an example, the pH vs [HPBCD] plot of the system KET + HPBCD in the presence of 0, 1, 3, 6, and 10% (w/w) of ethanol. As long as the cyclodextrin is added, both HPBCD−HKET and HPBCD−KET^- are formed, and the equilibria of Scheme 2 are shifted following Le Chatelier's principle. As a result of this competition, the pH of the medium changes, and these changes are used to study all the processes involved. Table 2

TABLE 2: Values of the Dissociation Constant K_a of Ketoprophen (KET) and the Association Constants of the 1:1 Inclusion Complexes Formed by Hydroxypropyl-β-cyclodextrin and the Acid and/or Base Forms of KET, $K_{CD-HKET}$, and K_{CD-KET^-}, Respectively, in the Presence of Different Amounts of a Series of Alcohols at 25 °C

% alcohol (w/w)	$10^5 K_a$	$K_{CD-HKET}$ (M^{-1})	K_{CD-KET^-} (M^{-1})	$K_{CD-HKET}/K_{CD-KET^-}$
		KET + HPBCD + MeOH		
0.00	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
0.98	5.6 ± 0.6	1280 ± 130	400 ± 40	3.2
3.01	5.5 ± 0.5	1130 ± 110	350 ± 35	3.2
6.05	5.1 ± 0.5	750 ± 75	210 ± 20	3.6
10.11	3.7 ± 0.4	670 ± 70	210 ± 20	3.2
		KET + HPBCD + EtOH		
0.00	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
0.99	5.6 ± 0.6	1190 ± 120	370 ± 40	3.2
3.01	5.6 ± 0.6	840 ± 80	230 ± 20	3.7
5.92	4.7 ± 0.5	550 ± 60	140 ± 14	4.0
10.02	3.6 ± 0.4	420 ± 40	120 ± 13	3.4
		KET + HPBCD + PrOH		
0.00	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
0.99	5.6 ± 0.6	840 ± 80	260 ± 30	3.2
1.53	5.5 ± 0.6	570 ± 60	160 ± 20	3.5
3.02	4.6 ± 0.5	450 ± 40	130 ± 13	3.3
5.85	4.5 ± 0.5	195 ± 20	45 ± 5	3.3
		KET + HPBCD + BuOH		
0.00	6.3 ± 0.6	1430 ± 140	460 ± 50	3.1
0.25	5.7 ± 0.6	880 ± 90	280 ± 30	3.2
0.50	5.5 ± 0.6	600 ± 60	170 ± 17	3.4
1.00	5.9 ± 0.6	440 ± 40	125 ± 13	3.6
2.99	4.8 ± 0.5	80 ± 8		

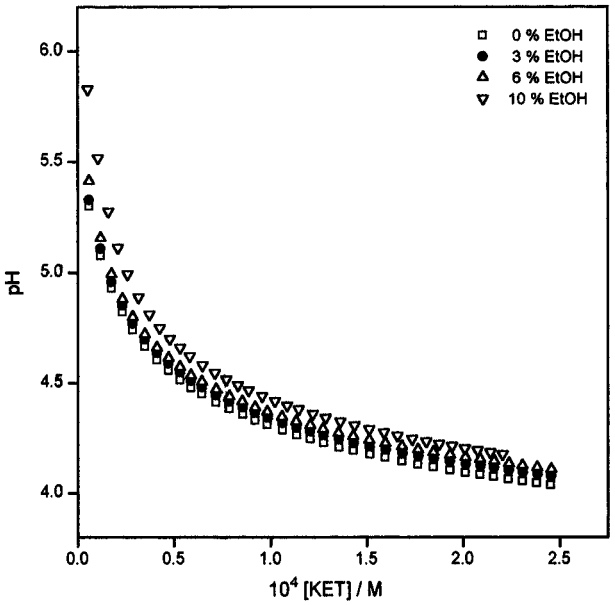


Figure 6. Plot of pH vs [KET] in the absence of HPBCD but in the presence of various constant concentrations of ethanol (EtOH) at 25 °C.

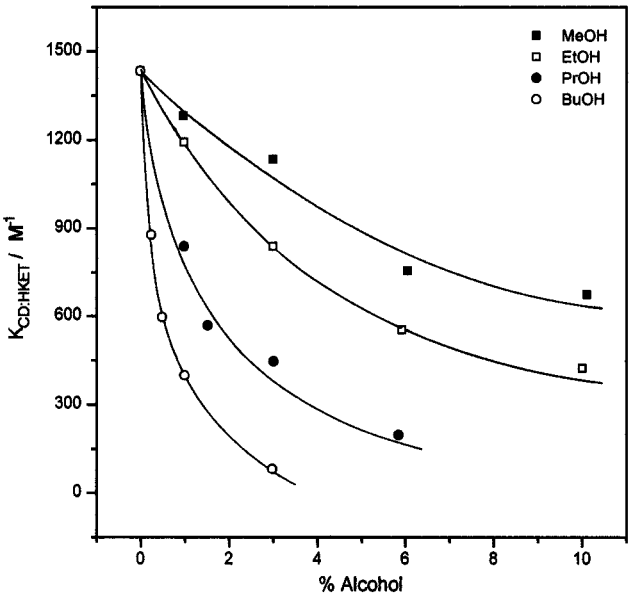


Figure 7. Values of the association constant obtained for the inclusion complex HPBCD: HKET ($K_{CD-HKET}$) in the presence of different alcohols as a function of the percentage (w/w) of alcohol at 25 °C. The solid lines are a guide to the eye.

reports the determined constants $K_{CD-HKET}$, K_{CD-KET^-}, and K_a values, together with their corresponding uncertainties for all the systems. Again, $K_{CD2-HKET}$ and $K_{CD2-KET^-}$ are not included in the table because they have been found to be negligible and always below the uncertainty of the fit. As previously mentioned, the K_a of pure KET is also determined by measuring the pH changes of an aqueous alcoholic solution as a function of KET concentration, in the absence of cyclodextrin. As an example, Figure 6 shows the results obtained in the presence of the same percentage of ethanol of the previous experiment. The K_a values thus obtained, which are not included in the table, are in very good agreement, within the uncertainty of the numerical procedure, with those obtained from the fit of pH vs [HPBCD] data.

When the effect of the medium on the encapsulation process of a substrate by a cyclodextrin is studied, it is necessary to check that any of the species of the medium compete with the substrate in the inclusion. Thus, the only encapsulation analyzed will be that of the substrate, and all the solvent effects observed will be due just to the change of the medium characteristics (polarity, hydrophobicity, etc). In this work, since the medium is an hidroalcoholic solution, it is important to consider the possible encapsulation of the alcohol molecule by the cyclodextrin, in competition with the CD−drug complex formation. However, the $K_{CD:Alcohol}$ values reported by Matsui et al.[51] and more recently by Tee et al.,[52] although alcohol chain length dependent, are always lower than 1.5×10^{-2} M^{-1} for the binding of HPBCD to methanol, ethanol, propanol, and butanol. Thus, the association of HPBCD with all the alcohols used in this work, with K values 3 orders of magnitude lower than the uncertainty of the $K_{CD-drug}$ values here determined, can be clearly neglected. As a consequence, all the effects observed in the presence of alcohols can be attributed only to a change on the solvophobic characteristics of the medium, which obviously affects the affinity of an apolar drug on binding the cyclodextrin.

The values of $K_{CD-HKET}$ and K_{CD-KET^-} reported in Table 2 are plotted as a function of the percentage (w/w) of alcohol in Figures 7 and 8, respectively, for all the alcohols used. As can be seen, either in Table 2 or in Figures 7 and 8, the association of both the acid HKET and the base KET^- forms of KET with

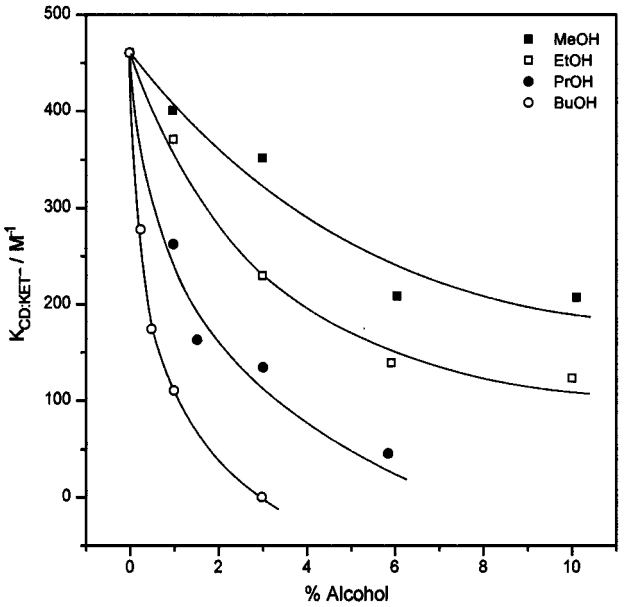


Figure 8. Values of the association constant obtained for the inclusion complex HPBCD−KET^- (K_{CD-KET^-}) in the presence of different alcohols as a function of the percentage (w/w) of alcohol at 25 °C. The solid lines are a guide to the eye.

the HPBCD decreases as long as the hydrophobicity of the medium increases, due either to the increase in the percentage of alcohol for a given alcohol, or to the lengthening of the alcohol chain for a given percentage of alcohol. This fact reveals a clear contribution of the hydrophobic effect as a driven force of both complexation processes. Furthermore, it can be also observed in Figure 7 and 8 that the decrease in the binding affinity, which is not linear with the percentage of alcohol, results in basically the same percentage for both associations with respect to the affinity found in the absence of alcohol. It can be also noted that the decrease in association constants is sharper as the number of C atoms of the alcohol chain increases.

The intersection of X-axis with the lines resulting from the representation of $K_{CD-HKET}$ and K_{CD-KET^-} as a function of n_c for a given alcohol would represent the length of alcohol chain necessary to cancel the association in these conditions. These

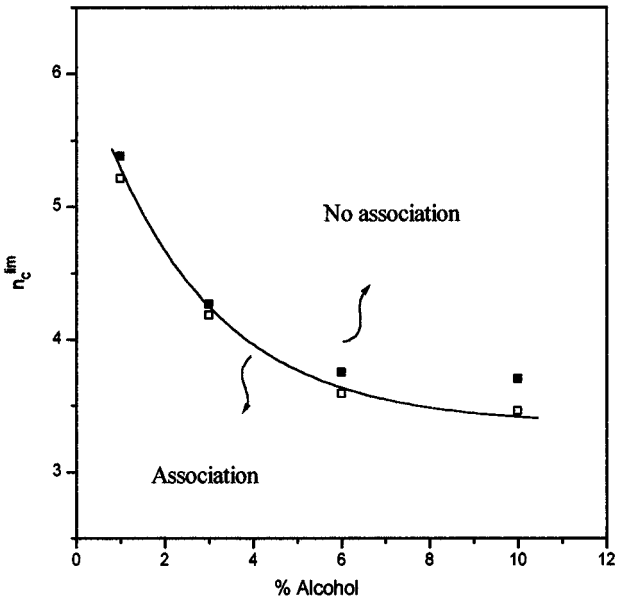


Figure 9. Limit association curve (vs percentage w/w alcohol) for the associations of HPBCD with HKET (solid symbols) and KET$^-$(open symbols).

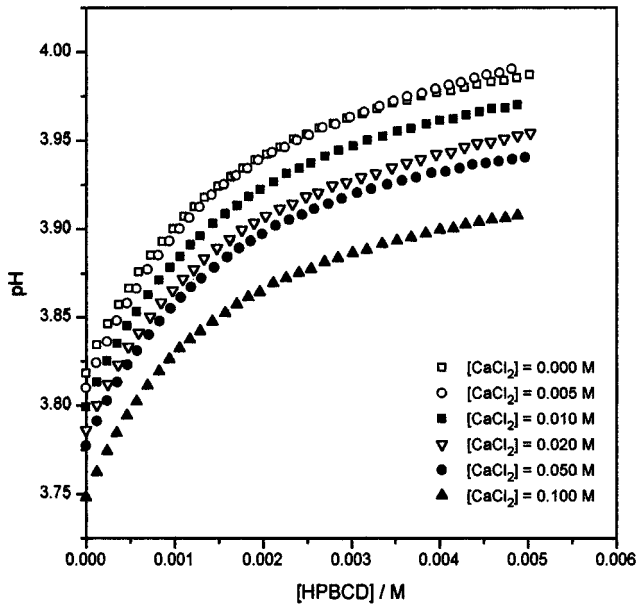


Figure 10. Plot of pH vs [HPBCD] for aqueous solutions where [KET] is kept constant at 5×10^{-4} M in the presence of various constant concentrations of $CaCl_2$ at 25 °C.

limiting n_c values, n_c^{lim}, could give us information about the necessary conditions to have association with the cyclodextrin, regarding the hydrophobicity of the medium in terms of either the length or amount of alcohol. Figure 9 shows a plot of the values as a function of the percentage of alcohol for both CD−HKET and CD−KET$^-$ complexes. The resulting curve, that we have called the *limit association curve*, represents an empirical correlation of the association conditions to parameters related to the apolar character of the medium, such as length and amount of alcohol. For those experimental conditions falling above the limit association curve, no association will occur, while the opposite is true for those ones falling below the curve, as it is indicated in the figure. The same curve is obtained for both complexes within the uncertainty of the K values. The points corresponding to a 10% of alcohol have been less considered to draw the curve because they are affected by a higher error, since at this percentage of alcohol only two experiments (those with methanol and ethanol) have been done due to the alcohol−water miscibility limit. This figure summarizes all the information previously presented; for the shorter alcohols, such as methanol and ethanol, it is necessary to add a high percentage of alcohol to cancel the association, while for the longer ones, such as pentanol or even longer alcohols of the series, with a 1% of alcohol the association will not occur.

It is interesting to emphasize that the ratio $K_{CD-HKET}/K_{CD-KET^-}$ remains basically constant at 3.5 ± 0.5 (Table 2), independently of the length and amount of the added alcohol. This feature is even more interesting when one realizes that the same ratio was found when the association of both acid and base forms of KET and HPBCD were studied as a function of temperature (Table 1). It strengthens the idea about the important role of the hydrophobic effect in the binding of the apolar drug molecules to cyclodextrins.

There can be found in the literature other phenomenological models to evaluate solvent effects on complexation processes.[26,29] Abraham et al.,[53] assuming a linear relation between the logarithm of the association constant K and the percent volume of organic cosolvent, proposed a general equation to correlates the association constant and the solvophobicity parameter S_p. This linear relationship has been found for our results of log $K_{CD-HKET}$ and log K_{CD-KET^-} as a function of percent (v/v) of alcohol, in agreement with the model proposed by Abraham et al.

Finally, it is noteworthy in Table 2 that the dissociation constant of KET (K_a) decreases as well with the apolar character of the medium. Thus, for a given alcohol, K_a decreases as the amount of alcohol increases, and for a given amount of alcohol the same behavior is found as long as the number of carbon atoms in the alcohol chain increases. It could be concluded, then, that the hydrophobicity of the medium affects as well the dissociation of an acid/base-conjugated pair. Since all equilibria of Scheme 2 are coupled, this effect is obviously reflected in the complexation of both acid and base forms with CD, as has been previously demonstrated.

Salt Effects. It is known that the participation of divalent cations (Ca^{2+} and Mg^{2+}) in ternary complexes are common binding motifs in different biological interactions.[30,31] With the aim of analyzing a possible calcium effect in the recognition of KET by HPBCD, the pH of an aqueous solution, where the total KET concentration is kept constant at 5×10^{-4} M, is measured as a function of HPBCD concentration in the presence of different constant $CaCl_2$ concentrations at 25 °C. In order to evaluate if the possible effect of the addition of $CaCl_2$ in the CD−drug interaction is due either to a Ca^{2+} participation or just to the change in the ionic strength, the experiment has been repeated in the presence of different amounts of other 2:1 electrolytes, such as $ZnCl_2$ and K_2SO_4, at 25 °C as well.

Figure 10 shows, as an example, the pH vs [HPBCD] plot of a drug solution ([KET] = 5×10^{-4} M) in the presence of several constant [$CaCl_2$]. The salt concentration have been chosen in the range where the extended Debye−Hückel theory is suitable to be applied.

From these pH data, the values of $K_{CD-HKET}$ and K_{CD-KET^-}, as well as the K_a of the pure KET, have been also determined with our model, while considering the change in the ionic strength with the addition of these electrolytes. $K_{CD2-HKET}$ and $K_{CD2-KET^-}$ have been found again to be negligible and always below the uncertainty of the fit. Table 3 reports these equilibrium constants for all the systems KET + CD + Salt, together with their corresponding uncertainties. The values of $K_{CD-HKET}$ and K_{CD-KET^-} are close to those for the KET + HPBCD system in the absence of salt, independently either of

TABLE 3: Values of the Association Constants of the 1:1 Inclusion Complexes Formed by Hydroxypropyl-β-cyclodextrin and the Acid and/or Base Forms of KET, $K_{CD-HKET}$, and K_{CD-KET^-}, Respectively, in the Presence of Different Amounts of 2:1 Electrolytes at 25 °C

[electrolyte] (M)	$K_{CD-HKET}$ (M^{-1})	K_{CD-KET^-} (M^{-1})	$K_{CD-HKET}/K_{CD-KET^-}$
	KET + HPBCD + $CaCl_2$		
0.000	1430 ± 140	460 ± 50	3.1
0.005	1450 ± 150	420 ± 40	3.5
0.010	1430 ± 140	430 ± 40	3.3
0.020	1390 ± 140	440 ± 40	3.1
0.050	1550 ± 160	520 ± 50	3.0
0.100	1570 ± 160	550 ± 60	2.9
	KET + HPBCD + $ZnCl_2$		
0.000	1430 ± 140	460 ± 50	3.1
0.005	1410 ± 140	420 ± 40	3.4
0.010	1600 ± 160	460 ± 50	3.4
0.020	1530 ± 150	390 ± 40	4.0
	KET + HPBCD + K_2SO_4		
0.000	1430 ± 140	460 ± 50	3.1
0.005	1560 ± 160	560 ± 60	2.9
0.010	1530 ± 150	550 ± 60	2.8
0.020	1450 ± 130	490 ± 50	3.0
0.050	1440 ± 130	540 ± 50	2.7

the electrolyte or its concentration. None of the equilibrium constants determined seem to be affected neither by the presence of Ca^{2+} cation or any other electrolyte, nor by the change in the ionic strength of the medium. This pattern reveals the no contribution of electrostatic interactions as a driven force promoting the binding by salting-in or salting-out effect[26] in the HPBCD + KET system, as it is expected for uncharged host, or guest, or both. But it also manifests that a calcium effect mediating the interaction between the cyclodextrin and the drug is not present in this system.

Conclusions

This paper constitutes a systematic physicochemical characterization of the molecular recognition of a drug by an artificial receptor, such as a cyclodextrin. We present in this work a highly accurate and fully computerized potentiometric technique to measure emf or pH in liquid mixtures, and a model, based on that of Gelb and co-workers, to obtain simultaneously the dissociation constant of a weak acid substrate and the association constants of the inclusion complexes formed by the cyclodextrin and both the ionized and nonionized forms of the substrate.

Particularly, the results found for the system HPBCD and KET (a NSAID drug with an ionizable carboxylic group in its molecule) call for some remarks: (i) both HKET and KET^- species form inclusion complexes with HPBCD with 1:1 stoichiometries. Neither 2:1 nor 1:2, let alone even higher stoichiometries, have been determined; (ii) the carboxylic form of KET always binds to HPBCD with a higher affinity (around three times higher) than its carboxylate partner does. This behavior has been found at all the temperatures and in the presence or in the absence of any cosolvent and/or electrolyte. (iii) The associations of HPBCD with HKET and/or KET^- have been found to be exothermic- and enthalpic-driven processes, with favorable enthalpic terms being dominant over unfavorable entropic terms. These results could point to the contribution of van der Waals interactions, hydrophobic effect, and solvent reorganization, as the main driven forces governing the interaction between the CD and the drug. (iv) As could be expected for an association process where the hydrophobic effect plays an important role, the increase of the apolar character of the medium results in a clear decrease on the affinity of binding. Thus, the lower the dielectric constant of the medium is, due either to an increase of the amount of organic cosolvent, or to a lengthening of its apolar chain, the less promoted the inclusion of the apolar part of the drug into the CD cavity results. A phenomenological limit association curve has been proposed to define the limit conditions to expect association between HPBCD and both ionized and nonionized forms of KET. (v) From the study in the presence of 2:1 electrolytes, it could be concluded that neither a contribution of electrostatic interactions nor a calcium effect mediating the interaction are relevant in this system.

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