Thermodynamic Analysis of the Binding of a Hepatoprotectant Drug, Thioctic Acid, by β-Cyclodextrin

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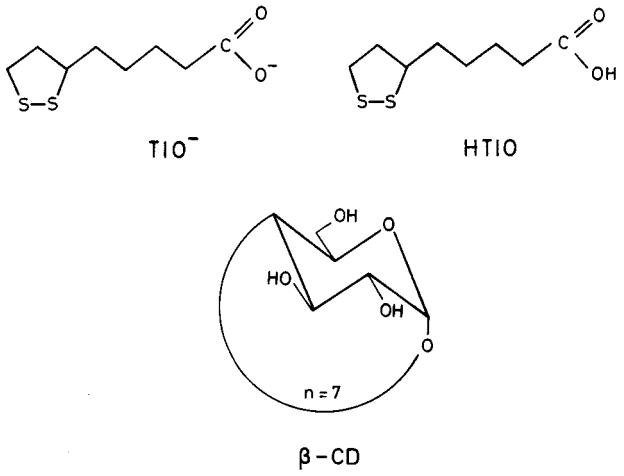
Abstract □ Spectroscopic and thermodynamic studies of the binding of a hepatoprotectant drug, thioctic acid, by β-cyclodextrin (β-CD) have been carried out using UV–vis and pH potentiometric measurements. The UV–vis spectra and the pH of the aqueous solutions of the drug were measured (i) as a function of total drug concentration in the absence of cyclodextrin, and (ii) as a function of cyclodextrin concentration at constant drug concentration. The spectroscopic study was done at pH = 7 and 25 °C, while the potentiometric study was performed at several temperatures ranging from 15 to 40 °C. From the spectroscopic data, the molar absorption coefficient, ϵ, for the pure drug in aqueous media and the stoichiometry of the inclusion complex with β-CD were determined. The dissociation constant, K_a, of the pure drug (which is a weak acid), and the association constants of the complexes formed by β-cyclodextrin and both the nonionized (HTIO) and ionized (TIO^-) forms of the drug, have been simultaneously determined at several temperatures from the pH data, without the necessity of working with buffered solutions. The nonionic forms are complexed by the β-CD with higher affinity than their ionic counterparts. From the dependency of the association constants on temperature (van't Hoff analysis), the inclusion complexes formed by HTIO or TIO^- and the β-CD were found to be enthalpy driven, with a favorable enthalpic term dominant over an unfavorable entropic term. Both contributions were found to show a possible dependence with temperature ($\Delta C_p^0 \neq 0$). This pattern may reveal the contribution of van der Waals interactions, hydrophobic effect, and solvent reorganization as the main driving forces promoting the complexation.

Introduction

The use of cyclodextrins as a new family of pharmaceutical excipients and drug carriers[1–6] has become an increasingly successful method to improve the general bioavailability of drugs. Particularly, it is known[7–10] that CD formulations of some drugs show a better tolerance since they cause fewer adverse side effects, this effect being more acute as the duration of the treatment increases. The parent cyclodextrins (CD's) are well-known nontoxic macrocyclic sugars, with doughnut-shaped structure, consisting of α(1→4) joined glucopyranose units. As a result of their hydrophobic inner surface, CD's are the most important simple organic compounds, capable of forming noncovalently bonded inclusion complexes with a variety of drug molecules in aqueous solution.

Thioctic acid, the oxidized form of α-lipoic acid (see Scheme 1), is hepatoprotectant drug used in the treatment of liver disease and as an antidote to poisonous mushrooms. It is expected that the formulation of this drug as a CD:



Scheme 1

Drug inclusion complex may help to avoid the undesirable side effects which the administration of the drug alone may provoke, thus improving its therapeutic usefulness. Structural information, such as the stoichiometry and geometry of the complex, and thermodynamic information, such as the association constant ($K_{CD:DRUG}$) and the change on the enthalpy ($\Delta H°$), entropy ($\Delta S°$), and heat capacity ($\Delta C_p°$) of binding, are necessary to draw a complete picture of the driving forces governing the CD−drug interaction. It is generally accepted[11–15] that a balance between van der Waals contacts and hydrophobic and solvent effects is mainly responsible for the overall stability of the complex.

Our group has examined the encapsulation of antiinflammatory and analgesic agents[14–18] by a series of β-cyclodextrins. These drug molecules and thioctic acid have a common characteristic: the presence of an acidic functional group. Thus, in aqueous solution both the ionized and the nonionized forms of the drug can be encapsulated by the cyclodextrin. There are two possible methods for studying the associations of these species with the CD: (i) follow the change in a physicochemical property (i.e., solubility, absorbance, fluorescence intensity, etc.) as a function of cyclodextrin concentration, but fixing the pH of the aqueous drug solution under conditions where only one of the two species is present; or (ii) directly monitor the pH of the aqueous unbuffered solution of the drug as the CD is added. In this work, we study the encapsulation of thioctic acid by β-cyclodextrin[19] in aqueous media by both methods: examination of the buffered drug solution (pH = 7) by UV−vis spectroscopy, and determination of pH of the unbuffered drug solutions in the absence and in the presence of β-CD by potentiometry. The spectroscopic study was performed at 25 °C, while the potentiometric experiments were carried out at temperatures from 15 °C to well above the temperature of human body (40 °C). We expect that the results of this work will show the strength of the potentiometric

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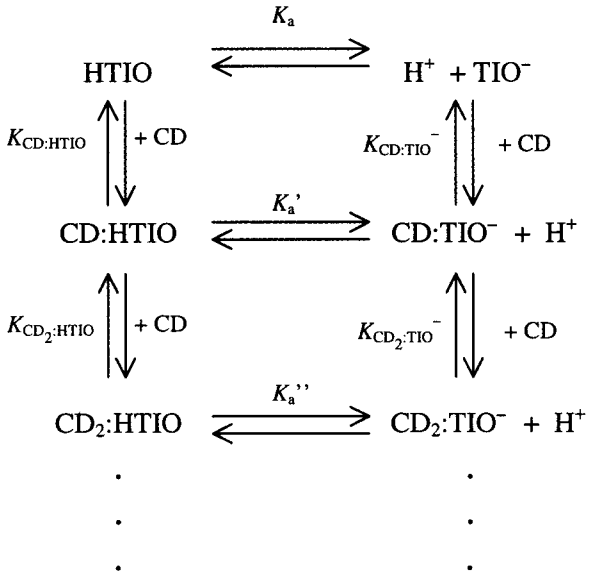

Scheme 2

method for characterizing the inclusion complexes formed by CD and the nonionized and ionized forms of an acid/base pair.

Experimental Section

Materials—DL-1,2-Dithiolane-3-pentanoic acid, named as DL-6,8-thioctic acid, and β-cyclodextrin, with purities higher than 99%, were purchased from Sigma and used without further purification. β-Cyclodextrin has been found through a thermogravimetric analysis to consist of 11% of mass water content, which was considered in the calculations of solute concentrations. All the solutions were prepared with distilled and deionized water (taken from a Millipore Super-Q System, with a conductivity lower than 18 $\mu\Omega^{-1}$ cm^{-1}). The initial solutions were brought to homogeneity by sonicating them for 3 h in an ultrasonic bath. The buffer at pH = 7, used in the UV−vis experiment, was a standard Metrohm NaH_2PO_4/Na_2HPO_4 buffer solution.

UV−vis Measurements—The UV−vis spectra of aqueous buffered solutions (pH = 7) of thioctic acid, in the absence and in the presence of β-CD, were recorded at 25 °C with a Varian Cary 5G double beam UV−vis−NIR spectrophotometer from 260 to 500 nm with 1 nm intervals. The equipment was connected to a PC Pentium via a IEEE-Bus interface. Data acquisition and analysis of UV−vis spectra were performed with the software supported by the manufacturer. Two 10 mm, stoppered, rectangular silica UV cells (sample and reference cells) were placed in a stirred cuvette holder, whose temperature was kept constant at 25.00 ± 0.05 °C with a recirculating water bath. The scan rate was selected in all the cases as 300 nm/min. On the experiments with the drug−water binary systems, the thioctic acid concentration was varied from 0.0 to 5.8 mM, while for the CD−drug−water ternary systems, the thioctic acid concentration was kept constant at 4 mM, and β-CD concentration was varied from 0.0 to 4.5 mM, both in the sample and in the reference cells.

pH Potentiometric Measurements—Potentiometric data were collected with a Metrohm 713 Ion Meter (sensitivity of ± 0.001 units), using a combined glass electrode containing 3 M KCl as reference electrolyte solution. The adjustment of both the asymmetry potential and the Nernst slope of the combined glass electrode was made by daily calibration of the electrode with three Metrohm buffer solutions of pH = 1, 4, and 7, at each working temperature. The equipment and the fully computerized experimental procedure used on the pH determination were described previously.[14] The accuracy on the molarity of the solutions is better than 0.1%, and the temperature is held constant within ±0.001 K. The statistical average of 250 pH measurements for each concentration allow us to improve the reproducibility of the pH data by up to 60%, with respect to that reported by the manufacturer. In these experimental conditions, the pH measurements were made (i) as a function of thioctic acid concentration, in the absence of β-CD; and (ii) as a function of cyclodextrin concentration, keeping the drug concentration at 1 mM. The cyclodextrin concentration ranges were chosen to cover at least 80% of the saturation curve in order to guarantee a proper binding constant determination.[20]

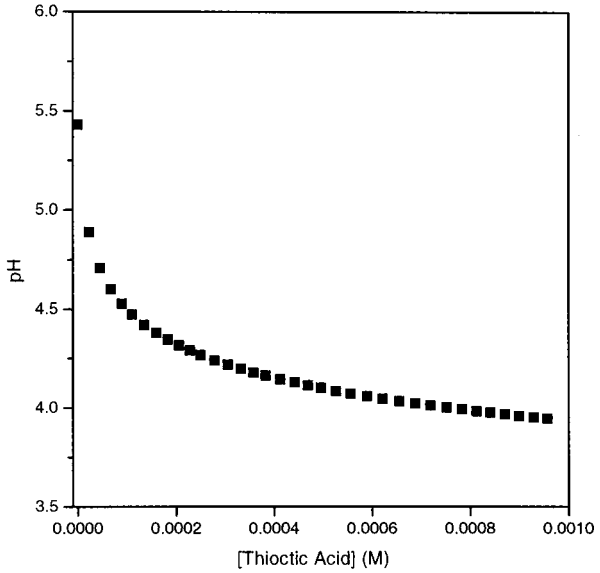

Figure 1—Plot of pH vs total thioctic acid concentration in the absence of β-CD, at 25 °C.

Results and Discussion

An aqueous solution of thioctic acid in the presence of cyclodextrin may include the equilibria shown in Scheme 2, where K_a is the dissociation constant of HTIO, and $K_{CD:HTIO}$, $K_{CD_2:HTIO}$, $K_{CD:TIO^-}$ and $K_{CD_2:TIO^-}$ are the association constants of the 1:1 and 2:1 inclusion complexes formed by the β-CD and the acid and base forms of thioctic acid, respectively. From geometrical considerations and also from previous studies[2−5] of similar systems, 1:2, 2:2, and/or even CD:drug complexes with higher stoichiometries are not expected.

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

$$K_a = (a_{H^+}a_{TIO^-})/(a_{HTIO}) \quad (1)$$

$$K_{CD:HTIO} = (a_{CD:HTIO})/(a_{CD}a_{HTIO}) \quad (2)$$

$$K_{CD:TIO^-} = (a_{CD:TIO^-})/(a_{CD}a_{TIO^-}) \quad (3)$$

$$K_{CD_2:HTIO} = (a_{CD_2:HTIO})/(a_{CD:HTIO}\, a_{CD}) \quad (4)$$

$$K_{CD_2:TIO^-} = (a_{CD_2:TIO^-})/(a_{CD:TIO^-}\, a_{CD}) \quad (5)$$

The constants K_a' and K_a'' are related to the others through simple expressions. The study reported in this work focuses on the determination of these equilibrium constants.

Thioctic Acid−Water System—It is of crucial importance, when studying the association of an acid/base pair with CD's, to measure the pK_a of the acid in order to know at which pH the presence of one of the two possible species in solution is negligible. For that purpose, the pH of the aqueous solutions of thioctic acid was measured as a function of drug concentration at 25 °C (Figure 1). From a nonlinear regression analysis (NLR)[14] of these pH data, which works with eq 1, the value of K_a for thioctic acid is 1.51×10^{-5} at 25 °C (pK_a = 4.82). Thus, in agreement with

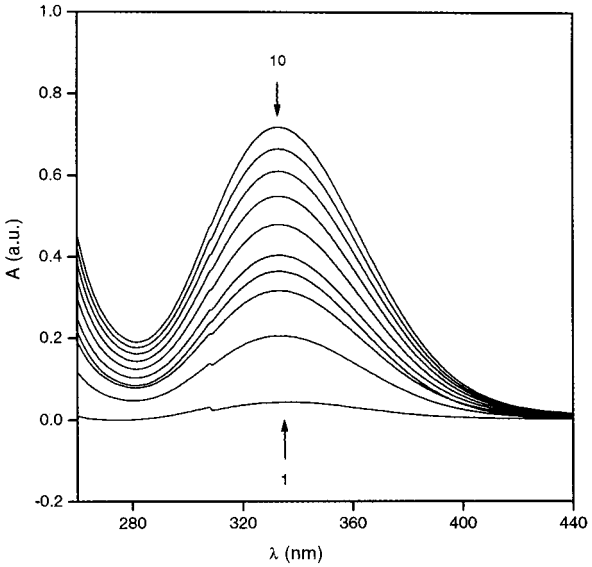

Figure 2—UV–vis spectra of aqueous solutions of thioctic acid at different concentrations, in the absence of cyclodextrin, at 25 °C and pH = 7: **1**, 0.39 mM; **2**, 1.23 mM; **3**, 2.23 mM; **4**, 2.90 mM; **5**, 3.03 mM; **6**, 3.70 mM; **7**, 4.30 mM; **8**, 4.84 mM; **9**, 5.32 mM; **10**, 5.76 mM.

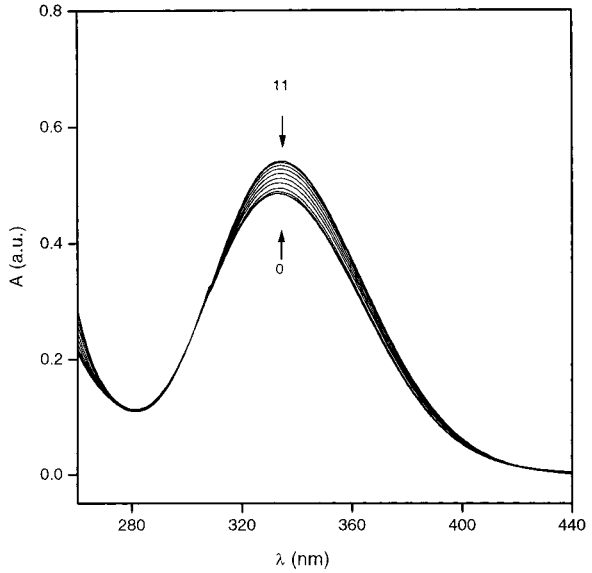

Figure 3—UV–vis spectra of an aqueous solution of thioctic acid at constant concentration (4 mM), 25 °C and pH = 7, in the absence and presence of different concentrations of β-CD: **0**, 0.00 mM; **1**, 0.12 mM; **2**, 0.35 mM; **3**, 0.77 mM; **4**, 1.34 mM; **5**, 1.94 mM; **6**, 2.52 mM; **7**, 3.10 mM; **8**, 3.62 mM; **9**, 4.10 mM; **10**, 4.53 mM.

what we have previously demonstrated,[17] the contribution of the nonprotonated (TIO^-) and protonated (HTIO) species of thioctic acid is negligible at only at pH $\leq$ 2.8 and pH $\geq$ 6.8, respectively. With this conclusion in mind, the UV−vis spectra of aqueous solutions of thioctic acid, buffered at pH = 7, were recorded at 25 °C for different drug concentrations, ranging from 0.0 to 5.8 mM (Figure 2). It can be observed that the spectra show a peak centered at λ_{max} = 333 nm, with absorbance values increasing with drug concentration, following a typical Lambert−Beer behavior. From the linear regression of the A vs [drug] values, the molar absorption coefficient, ϵ, was determined at different wavelengths. At λ_{max} = 333 nm, ϵ = 125 M^{-1} cm^{-1} (fit correlation = 0.99997), in concordance with literature values.[21,22] The experiment at pH = 1, where the nonionized form of the drug (HTIO) is the unique species in solution, was not performed since the absorbance values recorded at λ_{max} were extremely low (A < 0.1 within the whole concentration range).

Thioctic Acid-β-CD-Water System—*UV−vis Results*—Figure 3 shows the UV−vis spectra of a buffered solution of thioctic acid (pH = 7) at a constant concentration of 4.0 mM and 25 °C, at various β-CD concentrations ranging from 0.0 to 4.5 mM. As can be seen in the figure, the spectra show a peak centered at λ_{max} = 333 nm, with two isosbestic points at 300 and 420 nm, which indicate a 1:1 stoichiometry of the β-CD:TIO^- inclusion complex.[23] Although the spectroscopic characteristics of a system are known to be affected by the pH of the media, the geometry of the complex is not expected to change. Thus, once the 1:1 stoichiometry was found at pH = 7, it was assumed at pH = 1 as well. The addition of β-CD results in an increase in the absorbance intensity of the peak at 333 nm, but does not cause a shift in λ_{max}. However, the maximum change in A (ΔA_{max} = 0.06) was so small that the association constant $K_{CD:TIO^-}$ could not be determined accurately by fitting the experimental data to the well-known UV binding isotherm.[23] This feature, often found for other CD:substrate systems,[24,25] reveals that UV spectroscopy is not sensitive enough to study the β-CD−thioctic system, or any other system where the presence of cyclodextrin does not result in a clear change in the spectroscopic properties of the drug.

pH Results—Since H^+ is one of the species involved in the equilibria shown in Scheme 2, variation of pH of an aqueous drug solution, at constant concentration, as a function of β-CD concentration can be used as an indication of the shift in the equilibria, depending on the magnitude of the association constants of eqs 1−5. Figure 4 shows the plot of the ΔpH (= pH−pH_0) values, taken as the difference between the pH of the CD−drug−water system and that of the initial drug−water system (pH_0), as a function of β-CD concentration at different temperatures ranging from 15 to 40 °C. Equations 1−5, together with the following charge and mass balances for the CD and the drug:

$$[H^+] = [TIO^-] + [CD{:}TIO^-] + [CD_2{:}TIO^-] + [OH^-] \quad (6)$$

$$[drug]_{total} = [HTIO] + [TIO^-] + [CD{:}HTIO] + [CD{:}TIO^-] + [CD_2{:}HTIO] + [CD_2{:}TIO^-] \quad (7)$$

$$[CD]_{total} = [CD] + [CD{:}HTIO] + [CD{:}TIO^-] + 2\,[CD_2{:}HTIO] + 2\,[CD_2{:}TIO^-] \quad (8)$$

permit us to obtain the equilibrium constants from the pH (= $-\log a_{H^+}$) experimental data. The fitting procedure is a NLR (nonlinear regression) method based on a Newton−Raphson and a Marquardt algorithm, widely explained elsewhere.[14] The activities in eqs 1−5 are related to the concentrations shown in eqs 6−8 through the activity coefficients, determined with the extended Debye−Hückel theory. The coefficients of the fit are the equilibrium constants we are interested in, whose values for thioctic acid are reported in Table 1 at various temperatures. The data in Table 1 lead us to some general conclusions: (i) The dissociation constant, K_a, of thioctic acid is (1.5 ± 0.1) × 10^{-5}, in excellent agreement with the value obtained from the experiment done with the thioctic−water binary system, thus confirming the appropriateness of the model. It can be observed as well that K_a does not depend on the temperature within the range of temperatures studied herein. (ii) None of the equilibrium constants of Scheme 2 are fixed to zero during the fitting procedure. The inclusion complexes formed by β-CD and HTIO or TIO^- show 1:1 stoichiometries, in agreement with UV−vis results, since $K_{CD_2:HTIO}$ and $K_{CD_2:TIO^-}$ have been found to be negligible and

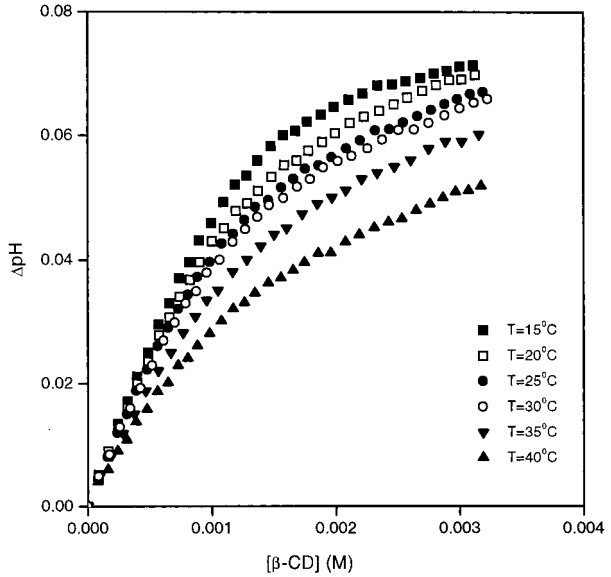

Figure 4—Plot of ΔpH vs β-CD concentration for aqueous solutions of thioctic acid at constant concentration (1 mM), at different temperatures ranging from 15 to 40 °C.

Table 1—Values of the Dissociation Constant K_a of Thioctic Acid, the Association Constants of the 1:1 Inclusion Complexes Formed by β-Cyclodextrin and the Acid and Base Forms of This Drug, $K_{CD:HTIO}$ and $K_{CD:TIO^-}$, Respectively, and the Saturation Degree Covered, f, as a Function of Temperature

T, °C	$10^5 K_a$	$K_{CD:HTIO}$, M^{-1}	$K_{CD:TIO^-}$, M^{-1}	$K_{CD:HTIO}/K_{CD:TIO^-}$	f range, %
15	1.5 ± 0.1	5790 ± 300	4060 ± 200	1.43	20–80
20	1.6 ± 0.1	3640 ± 150	2480 ± 150	1.47	18–79
25	1.6 ± 0.1	3410 ± 140	2390 ± 150	1.43	18–78
30	1.6 ± 0.1	2560 ± 130	1850 ± 100	1.40	21–80
35	1.4 ± 0.1	1650 ± 100	1140 ± 70	1.45	19–80
40	1.5 ± 0.1	1050 ± 90	740 ± 50	1.42	21–75

always below the uncertainty of the fits. This 1:1 stoichiometry is usually found for a wide variety of drug molecules upon binding β-CD or β-CD derivatives.[2–8] (iii) The affinity of β-CD by the protonated form ($K_{CD:HTIO}$) of the drug is around 1.4 times higher at all temperatures than that of the nonprotonated form ($K_{CD:TIO^-}$). This behavior, previously reported[14–17,26–29] for other carboxylic derivatives, has been attributed to either the random character of the inclusion,[27] or the repulsive interaction between the negative charge on the ionic species and the negatively charged end of the CD dipole,[29] or the differences in the entropic balance upon binding related to the structure-breaking character of the carboxylate anions.[14] However, it is worth noting that the ratio $K_{CD:HTIO}/K_{CD:TIO^-}$ found for this system is lower than that obtained for other CD:drug systems previously studied by us,[14–17] thus justifying the lower ΔpH observed for the system reported here in, compared with that observed for the other systems. This ratio implies that the therapeutic effects of β-CD as an excipient for thioctic acid are not much affected by the pH of the medium, the % of free drug being similar either in an acid medium, for example in the stomach, or in other biological media, with higher pH. Anyway, the values of $K_{CD:HTIO}$ and $K_{CD:TIO^-}$ at body temperature fall in the range of 1700−1000 M^{-1}, which can be considered optimum values regarding the use of CD's as effective drug carriers. The confirmation of all these estimations in vivo, although out of the scope of this work, would be welcomed.

It can be observed as well in Table 1 that as the temperature increases, the affinity of the cyclodextrin for

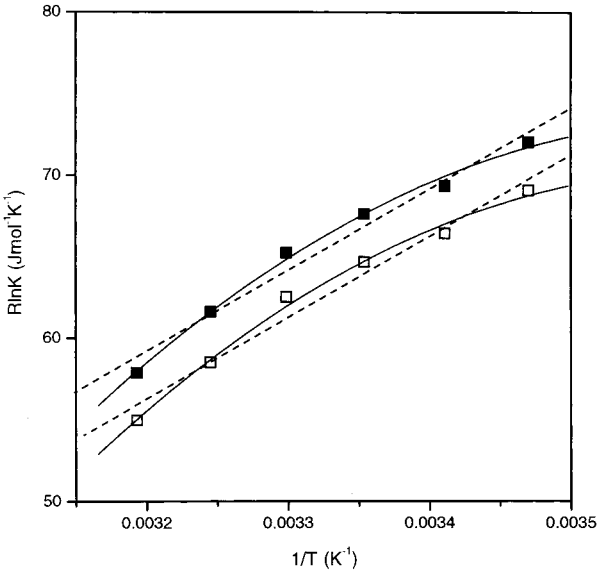

Figure 5—van't Hoff plots for the associations of β-CD with HTIO and TIO^-. Dash lines for linear fits and solid lines for nonlinear fits of eq 11.

both ionized and nonionized forms of the drug decreases. Figure 5 shows the van't Hoff plots of both association processes. A linear relationship of the R ln K vs $1/T$ data indicates the independence of $\Delta H°$ and $\Delta S°$ on T ($\Delta C_p^o \sim 0$), while the absence of such linear behavior reveals that $\Delta H°$ and $\Delta S°$ are temperature dependent, pointing to an association process with $\Delta C_p^o \neq 0$. If we assume that ΔC_p^o is temperature independent and that the dependence on temperature for ΔH and ΔS can be expressed by,

$$\Delta H = \Delta H° + \Delta C_p^o\,(T - 298.15) \quad (9)$$

$$\Delta S = \Delta S° + \Delta C_p^o \ln(T/298.15) \quad (10)$$

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

$$R \ln K = -[\Delta H° + (T - 298.15)\Delta C_p^o]/T + \Delta C_p^o \ln(T/298.15) + \Delta S^o \quad (11)$$

Equation 11 explains either the linearity or the curvature of the plots in Figure 5. When $\Delta C_p^o = 0$, eq 11 is simplified to the well-known linear relation (R ln $K = -\Delta H°/T + \Delta S°$), where $\Delta H°$ and $\Delta S°$ can be estimated from the slope and intercept of the fit, respectively. On the contrary, when $\Delta C_p^o \neq 0$, $\Delta H°$, $\Delta S°$, and ΔC_p^o can be determined by using a nonlinear regression of the experimental K values at various temperatures. We have performed both the linear and the nonlinear van't Hoff analysis of the experimental data, and both are drawn on the van't Hoff plots of Figure 5. Table 2 reports the results obtained for $\Delta H°$ and $\Delta S°$ in both cases, and for ΔC_p^o in the case of the nonlinear fit. Although the uncertainty in these results is high (up to 30%) in both the linear and the nonlinear fits, as usual for van't Hoff analysis, it can be observed in the table that both HTIO and TIO^- species bind to β-CD with a favorable enthalpic term ($\Delta H° < 0$) and an unfavorable entropic term ($\Delta S° < 0$). Both processes are exothermic and enthalpy driven ($|\Delta H°| > T\,|\Delta S°|$), as usually found[11–15,31–33] for associations between small guest molecules and an apolar cavity in water. A combination of hydrophobic effect ($\Delta H° \sim 0$; $\Delta S° > 0$), van der Waals forces

Table 2—Values of $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ for the Association of HTIO and TIO^- with β-CD, Obtained with Eq 11 in Both the Linear and Nonlinear Forms

system		$\Delta H°$, kJ mol^{-1}	$\Delta S°$, J mol^{-1} K^{-1}	$\Delta C_p°$, J mol^{-1} K^{-1}	std dev
β-CD + HTIO	linear	–50	–99	—	0.93
	nonlinear	–45[a]	–84[a]	–1980[a]	0.35
β-CD + TIO^-	linear	–50	–102	—	0.98
	nonlinear	–45[a]	–87[a]	–2000[a]	0.41

[a] Obtained at a reference temperature of 298.15 K.

($\Delta H° < 0$; $\Delta S° < 0$), and solvent reorganization could account for such a thermodynamic pattern. It appears that 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 β-CD and HTIO energetically more favorable than that of β-CD and TIO^-. Furthermore, although the use of the nonlinear form of the van't Hoff equation may be risky, given the uncertainty on K values, it is clear that the van't Hoff plots of Figure 5 are not straight lines, as indicated by the standard deviations of the fits. Thus, we would conclude that, within the uncertainty of the fits, a dependence of $\Delta H°$ and $\Delta S°$ on T, i.e., $\Delta C_p° \neq 0$, is an appropriate conclusion. From our data, we may say that $\Delta C_p°$ is negative, as usually found for the inclusion of apolar solutes by cyclodextrins[12,15,31] and cyclophanes,[11] and for carbohydrate association with lectins[33] in aqueous solution. Particularly, $\Delta C_p°$ values of around -2000 J mol^{-1} K^{-1} are typical of biological associations and recognition processes where hydrogen bonds and/or polar groups are present.[31−33] Assigning a value to $\Delta C_p°$, however, given the high error associated with this magnitude, would be just a speculation. Direct calorimetric results would be necessary to confirm these conclusions.

It has been demonstrated that the pH potentiometric technique, together with the model previously reported by us to determine association constants,[14] show clear advantages over the UV−vis technique for the examination of CD's complexing with ionizable guests. The advantages of this method include the following: (i) one pH−potentiometric experiment at each temperature, without buffering the solution, gives a complete thermodynamic description of the system, i.e., dissociation constant of the drug, association constants of both ionic and nonionic species of the drug with the CD, and the stoichiometry of the complex; (ii) the characterization of the CD:HTIO and CD:TIO^- complexes, not available through UV−vis measurements, may be studied from the pH−potentiometric experiments; (iii) the potentiometric technique accurately allows for the study of CD−drug systems for which the experimental properties of the drug in aqueous solution (such as the absorbance or the pH) are not very much affected by the presence or the absence of the CD. The only requirement of this method is that the association constants of the complexes formed by the CD and the nonionized and ionized forms of the guest must be neither very similar, nor very different. In the first case, the experimental ΔpH would be quite small, while in the second case the uncertainty in the highest binding constant would mask the value of the lowest binding constant.

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