Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **31:** 333–349, 1998.

Spectroscopic Study of the Inclusion Complexes of Phenylbutazone and Oxyphenbutazone with Cyclodextrins in Polar Reaction Media

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(Received: 15 July 1997; in final form: 4 November 1997)

Abstract. The formation of inclusion complexes of α-, β-, hydroxypropyl-β- (HP-β-) and γ-cyclodextrins with phenylbutazone and oxyphenbutazone has been studied in aqueous buffer solution (pH 7.5 and 0.1 mol dm^{-3} NaCl), dimethylsulfoxide, and 25, 50 and 75% dimethylsulfoxide/water mixtures. These complexation reactions have been followed by UV electronic absorption spectroscopy. In addition, 1D and 2D ^{1}H NMR spectra were recorded to obtain structural information about the inclusion complexes formed in solution; 136 binding constant values were determined at five different temperatures (288, 293, 298, 303 and 310 K) from the electronic absorption data and, from these $\Delta H_{\text{binding}}$ and $\Delta S_{\text{binding}}$ values were obtained. At the studied cyclodextrin and guest concentration ranges, 1 : 1 inclusion complexes were detected. Only in three cases were 1 : 2 complexes detected, those of phenylbutazone and oxyphenbutazone with α-cyclodextrin in aqueous, and oxyphenbutazone with hydroxypropyl-β-cyclodextrin in 75% dimethylsulfoxide/water solutions.

Key words: cyclodextrins, phenylbutazone, oxyphenbutazone, inclusion complexes.

1. Introduction

α-, β- and γ-cyclodextrins (CDs) are cyclic oligosaccharides formed by six, seven or eight glucopyranic rings, respectively. Due to their amphiphilic properties and topology, the cyclodextrins can admit guest molecules, thus allowing the formation of inclusion complexes [1–5]. The modification of physical and chemical properties of the included guest molecules depends on the interaction forces and the guest orientation inside the cyclodextrin cavity [6, 7].

Dimethylsulfoxide (DMSO) is an extremely hygroscopic solvent that is miscible with water in all proportions [8]. DMSO can form hydrogen bonds with water molecules and they are stronger than the existing links among the water molecules themselves [9, 10]. We have chosen DMSO as a nonaqueous reaction medium because it can generate solvated species stabilized by induced dipole–

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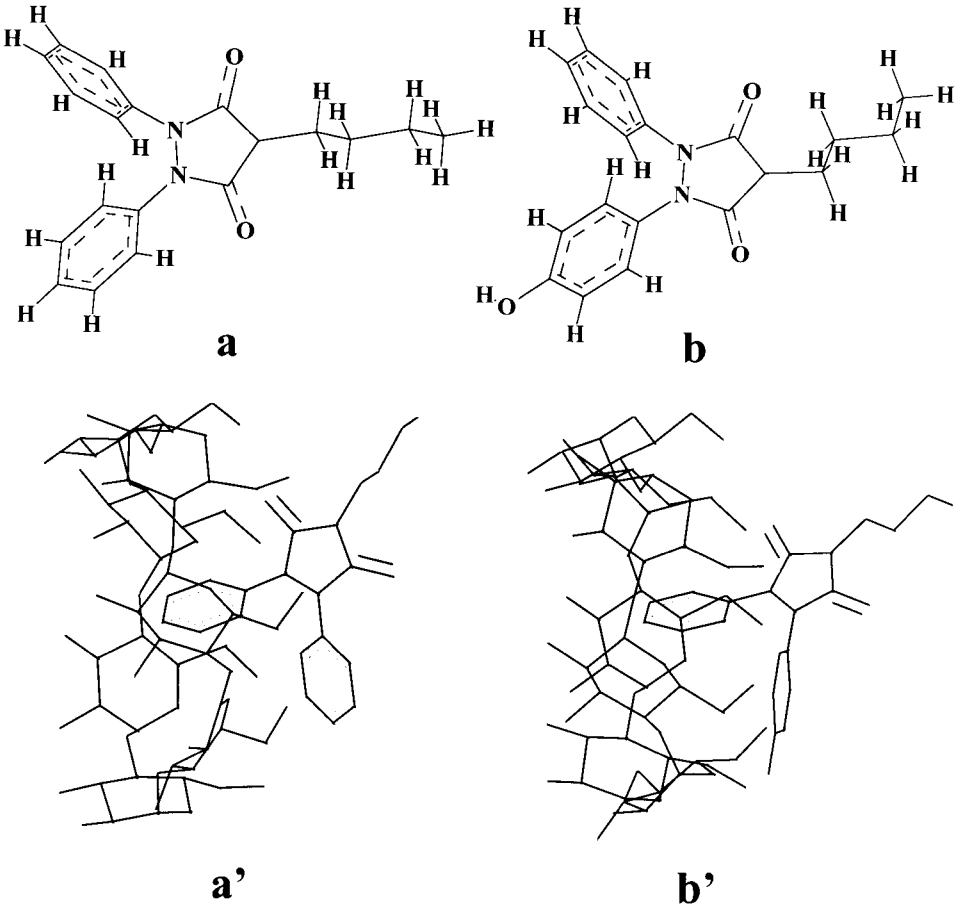

Figure 1. Structures optimized by molecular mechanics using the MM2 force field. (a) Phenylbutazone; (a') suggested structural model for [phen $\subset$ β-CD] complex in aqueous medium based on ^{1}H NMR data; (b) oxyphenbutazone; (b') suggested structural model for [oxyphen $\subset$ β-CD] complex in aqueous medium based on ^{1}H NMR data.

permanent dipole or permanent dipole–permanent dipole interactions [11]. There is a lack of published data for the formation of cyclodextrin inclusion complexes in nonaqueous polar solvents, so it is hoped that this work will be a source of such experimental data. Thermodynamic parameters for the complexation of cyclodextrins and guests in organic polar media are also almost nonexistent in the literature [12].

Phenylbutazone (phen) and oxyphenbutazone (oxyphen) are pyrazole derivatives (Figures 1a and 1b, respectively) that show anti-inflammatory action but are considered to be toxic [13–16]. Their complexation with cyclodextrins would avoid the undesirable toxic colateral effects [1, 17].

The object of this work is to obtain experimental information to help establish the nature of the complexing intermolecular forces among phen and oxyphen with CDs and solvent molecules.

2. Experimental

2.1. CHEMICALS

Phenylbutazone and oxyphenbutazone hydrate were purchased from Sigma Chemical Co. DMSO, reagent grade, was purchased from J.T. Baker S. A de C. V. The buffer components, Na_2HPO_4 and $KH_2\ PO_4$, and D_2O (99.9 at.-% D) were supplied by Aldrich Chem. Co. NaCl, R.A. was purchased from Mallinckrodt. Water was distilled in a Barnstead Thermolyne System and given a second treatment in the Easypure RF Compact Ultrapure Water System. α-, γ-, and HP-β-CD (degree of substitution = 9) were obtained as free samples from Cerestar USA Inc. β-CD was a donation from Arancia Mexico. All chemical reagents were used without any purification except for β-CD. A 2% aqueous solution of β-cyclodextrin was left to stand at room temperature a considerable time in order to promote aggregation of insoluble impurities and complexes; the solution was then filtered before recrystallization. β-Cyclodextrin was recrystallized from boiling water and then it was rinsed several times with ethanol, acetone and cold water [18].

2.2. PREPARATION OF THE COMPLEXES

Prior to the inclusion complex study, we prepared a series of phen and oxyphen solutions in the calculated concentration range in the different solution media to generate a calibration curve. The calibration curves were used to determine the absorbance of guests at 10^{-5} mol dm^{-3}. In all solvents the absorbance of the cyclodextrin solutions in the concentration range studied (10^{-3}–10^{-2} mol dm^{-3}) was also known. Inclusion compounds were prepared by direct dissolution. The 10^{-5} mol dm^{-3} guest solutions were employed to dissolve increasing concentrations of cyclodextrin. In this way the inclusion compound solutions were prepared. The buffer solution, pH 7.5, consists of Na_2HPO_4/KH_2PO_4 (0.07895 mol dm^{-3}) with constant ionic strength of 0.1 mol dm^{-3} NaCl.

The absorption spectra of each solution series were measured 24 h after their preparation using a 1 cm pathlength cell. Each solution was measured several times. Thus, the resulting absorption data are average values, to minimize the measurement errors. The same general procedure was followed for the formation of inclusion complexes in the other reaction media.

2.3. MEASUREMENTS

The UV-vis electronic absorption spectra were determined using a Hewlett Packard 8452A Diode Array Spectrophotometer. The temperature was kept constant by

a Peltier Hewlett Packard 89090A system. In all measurements in the different solution media the solvent itself was used as baseline.

^{1}H NMR experiments were carried out in unbuffered D_2O solutions. 1D ^{1}H NMR spectra were collected on a 300 MHz Varian Unity Plus spectrometer using a frequency of 299.95 MHz, with a 45° pulse (6.7 μs), spectral width of 3229.5 Hz, 3.002 s of acquisition time and 298 K. The number of transients acquired (32 to 128) was dependent on sample sensitivity. Nuclear Overhauser Effect (NOESY) data were collected with the same 300 MHz Varian Unity Plus spectrometer, with a broad band switchable probe. Spectral width was 2731.5 Hz in both dimensions, with an acquisition time of 0.187 s.

3. Data Processing

For 1 : 1 complexes, with general formula [guest $\subset$ CD], the mathematical expression used to compute the binding constants was the inverse Benesi–Hildebrand Equation [19].

$$\Delta Y = \frac{K_{11} P Y_0 X}{1 + (K_{11} X)}. \tag{1}$$

For 1 : 2 complexes, with general formula [guest $\subset$ $(CD)_2$], the equation used to determine the binding constant values was Equation (2),

$$\Delta Y = \frac{K_{12} P Y_0 X^2}{1 + (K_{12} X^2)} \tag{2}$$

where ΔY = [(total absorbance of the complex) $-$ (absorbance of the CD for the corresponding concentration) $-$ (absorbance of the initial guest)]; Y_0 = absorbance of the guest in the absence of CD; K_{11} = binding constants for [guest $\subset$ CD] complexes; K_{12} = binding constants for [guest $\subset$ $(CD)_2$] complexes; $P = (\epsilon_{Ic} - \epsilon_g)/\epsilon_g$, where ϵ_{Ic} is the molar absortivity for the complex formed, and ϵ_g is the molar absortivity for the guest; X = CD concentration. $[CD]_{total} = [CD]_{initial}$, which is a good approximation when $[CD]_{total} \ggg [G]_{total}$.

The errors were estimated by treating a single data set. The computer program provides the binding constant values with the standard deviations from the fitting data.

It is important to point out that very weak interactions have been reported between α-CD and DMSO (0.41 $\pm$ 0.04 in 10% DMSO/water and 0.37 $\pm$ 0.04 in 20% DMSO/water) [20] and also between β-CD and DMSO (0.0018 $mmol^{-1}$ dm^3) [21]. For this reason, the DMSO–CD and DMSO/water mixtures–CD interactions were neglected in Equations (1) and (2).

The values of $\Delta H_{binding}$ and $\Delta S_{binding}$ were computed from the temperature dependence of the equilibrium constant, at constant pressure [22].

$$\ln K = \Delta S/R - \Delta H/RT. \tag{3}$$

Table I. Binding constants of phen and α-CD complexes in different reaction media.

	Solvent			
T/K	Buffer pH 7.5	25% DMSO	75% DMSO	DMSO
	$K_{1:2}$/(mol^{-1} dm^3)^2	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3
288	1600 ± 100	7000 ± 2000	[a]	1080 ± 60
293	2100 ± 500	4500 ± 800	[a]	580 ± 40
298	4000 ± 600	6100 ± 900	300 ± 100	540 ± 80
303	6000 ± 1000	5200 ± 900	370 ± 50	400 ± 70
310	8000 ± 1000	4700 ± 800	900 ± 400	320 ± 30

[a] It was not possible to determine the binding constants.

Table II. Binding constants of [phen $\subset$ β-CD] complexes in different reaction media.

	Solvent				
T/K	Buffer pH 7.5	25% DMSO	50% DMSO	75% DMSO	DMSO
	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3
288	3100 ± 700	[a]	41200 ± 200	360 ± 50	3700 ± 900
293	8000 ± 5000	[a]	1200 ± 200	230 ± 20	3000 ± 1000
298	7000 ± 2000	4900 ± 600	1200 ± 300	100 ± 2	1400 ± 300
303	6000 ± 1000	4000 ±1000	1200 ± 200	50 ± 2	100 ± 1
310	4900 ± 800	3200 ± 500	1200 ± 200	[a]	450 ± 50

[a] It was not possible to determine the binding constants.

The number of species in solution was obtained by the triangulation method [23]. The Microcal Origin Program (Version 4.00 from *Microcal Software, Inc.*) was used to process electronic absorption data and compute the binding constants. HyperChem (Molecular Modeling System 4.5 from *Hypercube, Inc.*) was used to optimize the geometry of the structures, with molecular mechanics using an MM2 force field.

4. Results and Discussion

4.1. Electronic absorption data

The binding constants for the 1 : 1 and 1 : 2 complexes for phen and oxyphen with all the cyclodextrins were calculated using Equations (1) and (2), respectively, from the collected electronic absorption spectral data in different reaction media at five temperatures. The 136 binding constant values are displayed for phen in Tables I to IV and for oxyphen in Tables V to VIII, listed according to the type of cyclodextrin.

Table III. Binding constants of [phen $\subset$ γ-CD] complexes in different reaction media.

	Solvent			
T/K	Buffer pH 7.5 $K_{1:1}$/mol^{-1} dm^3	25% DMSO $K_{1:1}$/mol^{-1} dm^3	75% DMSO $K_{1:1}$/mol^{-1} dm^3	DMSO $K_{1:1}$/mol^{-1} dm^3
288	[a]	7100 ± 900	7400± 400	590 ± 80
293	[a]	3700 ± 600	11000 ± 2000	400 ± 100
298	1900 ± 400	3600 ± 500	15000 ± 3000	230 ± 60
303	1400 ± 300	4000 ± 1000	14000 ± 2000	120 ± 30
310	1100 ± 300	2440 ± 10	[a]	[a]

[a] It was not possible to determine the binding constants.

Table IV. Binding constants of [phen $\subset$ HP-β-CD] complexes in different reaction media.

	Solvent		
T/K	50% DMSO $K_{1:1}$/mol^{-1} dm^3	75% DMSO $K_{1:1}$/mol^{-1} dm^3	DMSO $K_{1:1}$/mol^{-1} dm^3
288	2200 ± 200	140 ± 10	1480 ± 40
293	1900 ± 200	88 ± 5	1000 ± 300
298	1800 ± 200	61 ± 5	700 ± 100
303	1619 ± 6	52 ± 3	50 ± 6
310	1400 ± 200	38 ± 2	200 ± 40

Table V. Binding constants of oxyphen and α-CD complexes in different reaction media.

	Solvent				
T/K	Buffer pH 7.5 $K_{1:2}$/(mol^{-1} dm^3)^2	25% DMSO $K_{1:1}$/mol^{-1} dm^3	50% DMSO $K_{1:1}$/mol^{-1} dm^3	75% DMSO $K_{1:1}$/mol^{-1} dm^3	DMSO $K_{1:1}$/mol^{-1} dm^3
288	4200 ± 200	510 ± 70	560 ± 30	370 ± 40	700 ± 100
293	1110 ± 40	1000 ± 300	900 ± 100	510 ± 60	6300 ± 200
298	950 ± 70	510 ± 60	1200 ± 100	600 ± 100	3300 ± 400
303	400 ± 20	700 ± 200	1200 ± 100	700 ± 100	4000 ± 2000
310	110 ± 7	700 ± 100	1600 ± 200	470 ± 60	1800 ± 400

Table VI. Binding constants of [oxyphen $\subset$ β-CD] complexes in different reaction media.

	Solvent		
T/K	Buffer pH 7.5	25% DMSO	50% DMSO
	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3
288	1800 ± 300	10.0 ± 0.4	220 ± 10
293	1200 ± 100	26 ± 1	240 ± 20
298	1000 ± 20	58 ± 3	260 ± 20
303	970 ± 80	61 ± 3	300 ± 30
310	600 ± 100	55 ± 3	250 ± 30

Table VII. Binding constants of [oxyphen $\subset$ γ-CD] complexes in different reaction media.

	Solvent	
T/K	50% DMSO	DMSO
	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3
288	1100 ± 20	1650 ± 60
293	240 ± 30	2000 ± 500
298	320 ± 40	1300 ± 100
303	490 ± 90	1300 ± 80
310	500 ± 100	1070 ± 80

Table VIII. Binding constants of oxyphen and HP-β-CD complexes in different reaction media.

	Solvent		
T/K	Buffer pH 7.5	50% DMSO	75% DMSO
	$K_{1:1}$/mol^{-1} dm^3	$K_{1:1}$/mol^{-1} dm^3	$K_{1:2}$/(mol^{-1} dm^3)^2
288	700 ± 100	900 ± 100	23000 ± 3000
293	600 ± 40	1000 ± 200	11000 ± 1000
298	600 ± 100	800 ± 100	11000 ± 2000
303	530 ± 80	1200 ± 200	17000 ± 3000
310	460 ± 60	1100 ± 300	9000 ± 1000

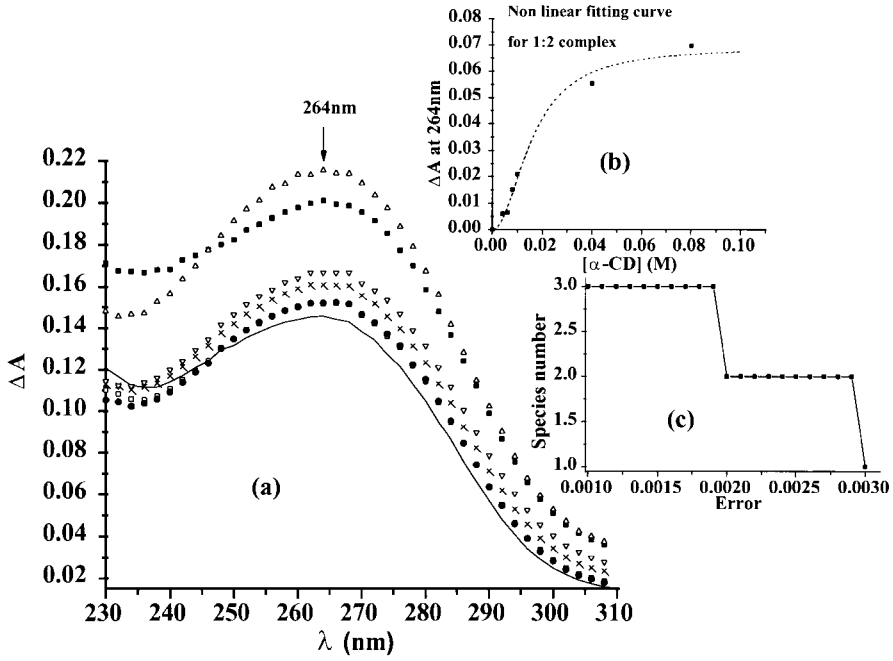

Figure 2. (a) Absorption spectra of resulting complexes between phen and α-CD in buffer solution at 298 K. [phen] = 1×10^{-5} mol dm^{-3}; [α-CD] = (—) 0; (□) 4×10^{-3} mol dm^{-3}; (●) 6×10^{-3} mol dm^{-3}; (×) 8×10^{-2} mol dm^{-3}; (▽) 1×1^{-2} mol dm^{-3}; (■) 4×10^{-2} mol dm^{-3}; (△) 8×10^{-3} mol dm^{-3}. Insert (b) typical nonlinear fitting curve from Equation (2) for the same complex. Insert (c) species number in solution, see text.

Figure 2a shows absorption spectral changes for the [phen $\subset$ α-$(CD)_2$] system in buffer solution as a function of α-CD concentration. The insert (b) in Figure 2 shows the typical nonlinear fitting curve corresponding to Equation (2) in agreement with [phen $\subset$ α-$(CD)_2$] complex formation, and the insert (c) shows a graphical treatment of the matrix which provides evidence for the existence of several species in solution [23]. For equilibria involving three or more species in solution, it is usually necessary to use computational methods to determine the rank of the matrix [24, 25]. For that purpose the triangulation [23] method was used. The rank of the matrix is given by the number of non-zero elements on the diagonal. If a range of error matrix values is used, the variation of the rank found with error provides an insight into the reliance one can place on the number of species [23].

In Figure 2, insert (c), we found a 1 : 2 complex, and the three species in solution are probably the 1 : 1 and 1 : 2 complexes and free α-CD. Presumably the free guest concentration is so small that it can be neglected, as suggested by Ueno [26] and Pendergast and coworkers [27]. On increasing the uncertainty values, the number of species in solution decreases. The same happens for 1 : 2 complexes concerning the number of species in solution, [oxyphen $\subset$ α-$(CD)_2$] in buffer solution and [oxyphen $\subset$ HP-β-$(CD)_2$] in 75% DMSO. Again the number of species in solution

decreases on increasing the uncertainty values, but always corresponds to 1 : 2 complexes. In the cases of 1 : 1 complexes, they display two species in solution, apparently the 1 : 1 complex itself and free CD. As we said before, the free guest concentration can be neglected.

There is a similar behaviour for the majority of the phen systems, Tables I–IV, where binding constant values decrease when the temperature increases. This is because of the increased molecular mobility with temperature, so that the inclusion complex population decreases. In other words, increasing the temperature may provoke complex dissociation [28]. Some inclusion complexes with oxyphen show the same general tendency (Tables V–VIII).

On the other hand, for the other systems studied, where binding constant values increase with the temperature, there is an increased molecular mobility; however, in these cases the effect can be attributed to the mobility of guest and solvent molecules [29]. The solvophobic effect in these systems is large, therefore phen and oxyphen will form very stable complexes with the CDs. This provokes a stronger interaction between the guest and CD molecules than between the guest and the solvent and so the binding constant values will increase with temperature.

In general, for the same solution medium, the binding constant values are higher for the CDs which have the smaller internal diameter, e.g., α- and HP-β-CD. It is probable that the benzene rings fit better into these two CDs than in the remaining cyclodextrins. The HP-β-CD behaviour is similar to that shown by α-CD. It is possible to assume that these two CDs have very similar internal diameters as a consequence of the steric hindrance of the hydroxypropyl chains. These chains can be thought of as the beginning of the cavity section of the HP-β-cyclodextrin [30, 31].

Table VIII shows the particular case of [oxyphen $\subset$ (HP-β-CD)$_2$] in 75% DMSO, where a 1 : 2 complex is formed. Of all the systems studied, there are only three cases in which 1 : 2 complexes are detected, two of them with α-CD, in buffer solution, for both guest molecules, and the other is the abovementioned 1 : 2 oxyphen complex. As stated above, in view of the small size of α-CD, it is easy to avoid steric hindrance with other α-CD molecules. The formation of a 1 : 2 inclusion complex between oxyphen and HP-β-CD is possible considering that the size of HP-β-CD is similar to that of α-CD. It is known that hydrogen bonds greatly stabilize inclusion complexes with CDs [32]. In the case of the oxyphen, HP-β-CD and 75% DMSO system, it is possible that the complex [oxyphen $\subset$ (HP-β-CD)$_2$] is stabilized by the formation of hydrogen bonds, probably among its hydroxypropyl O—H groups and the oxyphen oxygen atom. When there is an expansion of the hydrophobic region of cyclodextrin, e.g. HP-β-CD, an increase of the guest binding has been found [33]. In the other two cases of 1 : 2 complexes with α-CD, this α-cyclodextrin does not present hydrocarbon chains capable of strongly stabilizing the complex. Here there is a low probability for hydrogen bonds to act as a guideforce.

From the electronic absorption data it is possible to observe that the oxyphen binding constants show smaller error values than those of phen. Both structures differ only in the hydroxyl group, so it is likely that there is a better stabilization by the oxyphen hydroxyl group either with CDs or with solution media.

There are some reports about CD complexes with polar and nonpolar molecules [1–5]. The differences provide an illustration of several factors involved in measurements of these complexes, such as the release of water molecules from the interior of CDs, relief of CD strain energy, depth of inclusion, solvent effect and the extent of the contacting surface between host and guest. Published binding constants are fraught with error, as communicated by Nozaki [34], Frankewich [35], Hacket et al. [36] and others [37–39]. All errors are due to the various factors that affect this complexation process and to the association–dissociation dynamic equilibrium that characterizes these complexes.

Phen and oxyphen show large accessible surface areas and this factor helps to increase the solvophobic effect, and therefore the binding constant values increase.

We obtained binding constant values from four cyclodextrins with a slightly polar molecule, phen, and with a more polar, molecule, oxyphen. The method used to obtain binding constants yields values with a narrow standard deviation range from the the program used, and although slightly more complicated than the straight-line fitting procedure, it has been successfully used here.

4.2. ^{1}H NMR EXPERIMENTS

^{1}H NMR experiments were carried out to obtain structural information about inclusion complexes in solution. The results of phen and oxyphen with β-CD in D_2O (Figures 3 and 4, respectively) are shown with 1D and NOESY spectra scanned at 300 MHz. As shown in Figure 3, the observed $\Delta\delta$ value for β-CD (0.13 ppm for 5-H) confirms the formation of an inclusion complex with phen [40–42].

The 5-H is located in the β-CD internal cavity close to the narrow section. The signals that correspond to the external protons are significantly shifted and there is a clear characteristic of interaction with them in the NOESY spectrum. This suggests that there are interactions among the guest molecule and internal and external sections of the β-CD. The bands corresponding to 3-H are intact, unchanged with respect to those shown by β-CD alone in D_2O. The 3-H is near the wide side. In addition to this, there is a strong interaction signal between 6-H (placed next to the narrow cavity) and the aromatic section (6.95 –7.25 ppm). All this means that the inclusion process occurs via the narrow cavity. From the study of these interactions we can deduce the possible inclusion complex structure in aqueous solution (Figure 1a'). Only one benzene ring is included in the cavity while the other part of phen is in contact with the external section of the β-CD and with the solvent. Because of the β-CD internal diameter ($\approx$ 7.8 Å) and the orientation and distance between the two benzene rings (see Figure 1a), it is improbable that a

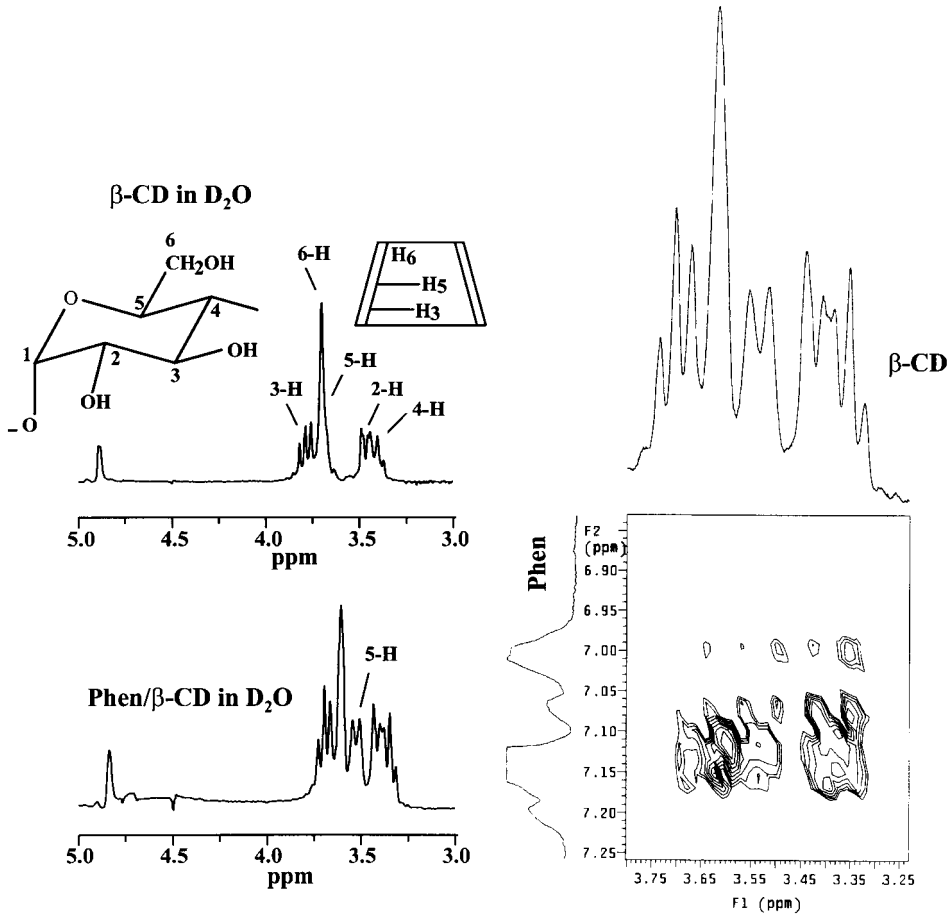

Figure 3. ^{1}H NMR and NOESY spectrum of the [phen $\subset$ β-CD] complex in D_2O at 298 K.

1 : 2 complex can exist, [phen $\subset$ β-$(CD)_2$]. The steric hindrance would prevent its formation.

Figure 4 shows that there are similar interactions between oxyphen and β-CD in D_2O. We can observe the $\Delta\delta$ in β-CD (0.08 ppm for 5-H) that again confirms the formation of an inclusion complex with the unsubstituted benzene ring (6.9–7.3 ppm) [40–42]. The signals that correspond to 3-H are unchanged, and again the interaction signal between 6-H from β-CD and the unsubstituted benzene ring appears, hence and taking into consideration the location of 6-H and 3-H in the β-CD, it is believed that the inclusion process also takes place through the narrow cavity. The bands that correspond to external protons are lightly displaced and the shape is modified. The interaction between β-CD and the substituted benzene ring (6.3–6.9 ppm) is via the external section, as a larger interaction signal appears in the NOESY spectrum. Only the unsubstituted benzene ring is included in the cavity, the rest of the oxyphen molecule is in contact with the external section of

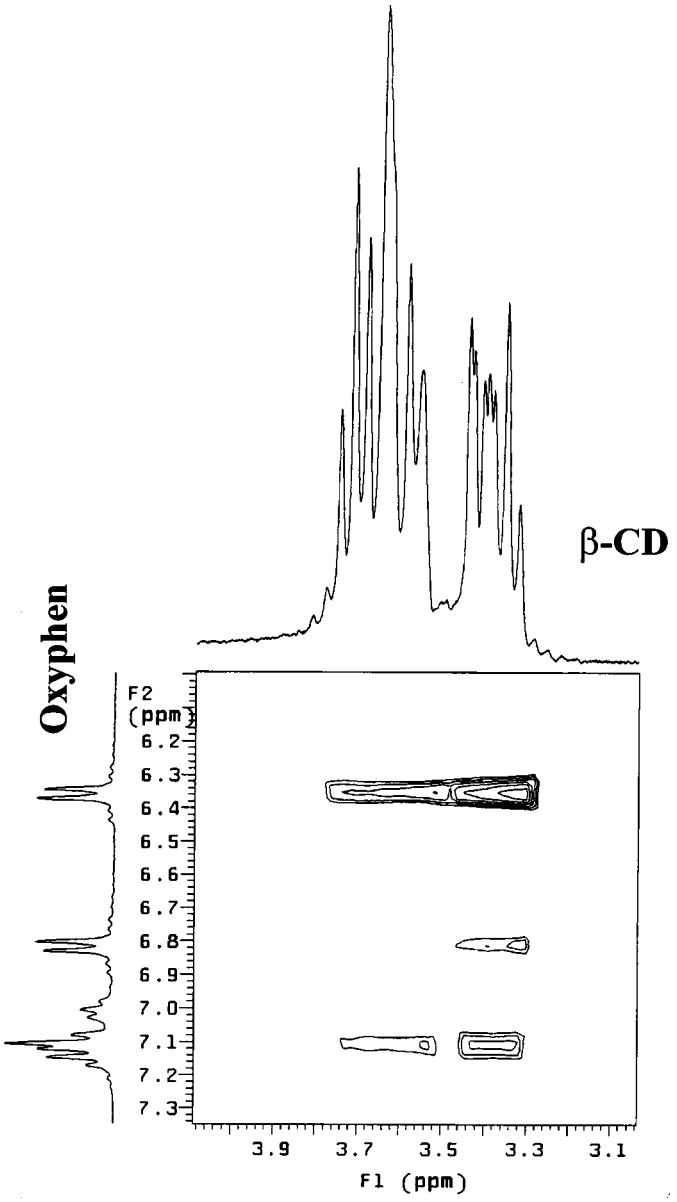

Figure 4. NOESY spectrum of the [oxyphen $\subset$ β-CD] complex in D_2O at 298 K.

β-CD and with the solvent. The possible complex structure in aqueous solution is displayed in Figure 1b'.

A comparison of Figures 3 and 4 reveals that the interaction signals in the NOESY spectrum are more intense in the case of phen than for the oxyphen. This suggests that in aqueous solution at 298 K the [phen $\subset$ β-CD] interaction is larger

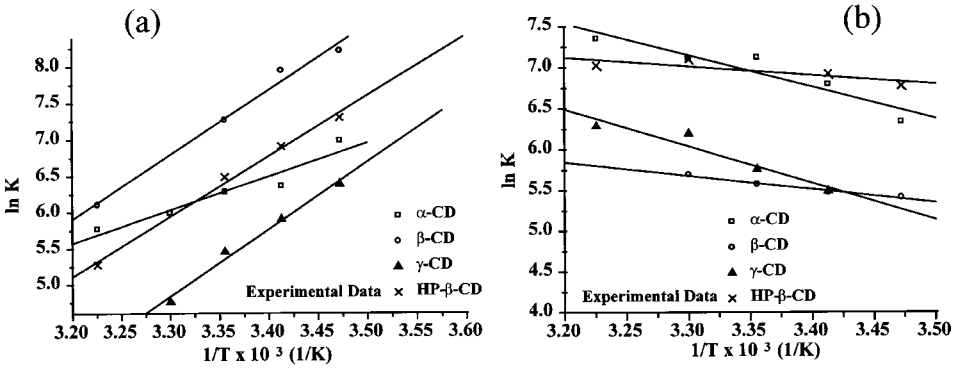

Figure 5. (a) van 't Hoff plots of phen and α-, β-, HP-β- and γ-, in DMSO solutions. Correlation coefficients of fitting lines: $\square r^2 = 0.92$, $\bigcirc r^2 = 0.99$, $\blacktriangle r^2 = 0.99$, $\times r^2 = 0.99$. (b) van 't Hoff plots of oxyphen and α-, β-, HP-β- and γ-, in 50% DMSO/water solutions. Correlation coefficients of fitting lines: $\square r^2 = 0.89$, $\bigcirc r^2 = 0.98$, $\blacktriangle r^2 = 0.92$, $\times r^2 = 0.80$.

than that the [oxyphen $\subset$ β-CD] interaction. This is in agreement with the binding constant values in buffer solution shown in Tables II and VI.

4.3. THERMODYNAMIC DATA

The $\Delta H_{\text{binding}}$ and $\Delta S_{\text{binding}}$ values for the systems formed by phen and oxyphen with cyclodextrins are presented in Table IX. They were computed using Equation (3). The footnotes in Table IX indicate the binding constant values that were not considered to calculate the thermodynamic data due to their large standard deviations. Two examples of van 't Hoff plots [43] are presented in Figure 5(a) for phen in DMSO solutions and in Figure 5(b) for oxyphen in 50% DMSO/water solutions.

The solvent has a notable role in the inclusion processes [44–46]. Among the binding forces proposed for the inclusion phenomena with cyclodextrins, only the solvophobic effect is governed by entropy, $\Delta S_{\text{binding}} > 0$, and sometimes by $\Delta H_{\text{binding}} > 0$. The effect itself is driven by an increase in entropy of the solvent molecules as a result of the exclusion of the solute molecules, a process that is accompanied by an increase in the degree of freedom of solvent molecules [43, 47, 48]. We have several cases with solvophobic effect as driving force for complexing with phen and oxyphen (Table IX).

In Figure 5(a) it is possible to observe the different behaviour in DMSO of α-CD with respect to the β-, γ- and HP-β-CDs. In Table IX we observe that $\Delta H_{\text{binding}}$ and $\Delta S_{\text{binding}}$ for [phen $\subset$ α-CD] in DMSO are less negative than those for the other CDs in the same solvent. The binding constant values for the [phen $\subset$ α-CD] complexes in DMSO are the lowest with respect to the other solution media (Table I). This means that the phen–DMSO interaction is greater than the DMSO–DMSO interaction. In the other solution media the binding constant values for [phen $\subset$ α-CD] and [phen $\subset$ γ-CD] complexes are larger than in DMSO. In these cases, this

Table IX. $\Delta H_{binding}$ and $\Delta S_{binding}$ values for the [guest $\subset$ CD_n] complexes in different reaction media; where n = 1 or 2.

Complexes	Solvent $\Delta H_{binding}$ (kJ mol^{-1}), $\Delta S_{binding}$ (kJ mol^{-1} K^{-1})									
	Buffer pH 7.5		25% DMSO		50% DMSO		75% DMSO		DMSO	
	ΔH	ΔS	ΔH	ΔS	ΔH	ΔS	ΔH	ΔS	ΔH	ΔS
[phen $\subset$ α-CD_n]	+58.9	+0.3	−14.2*	+0.03*	–	–	+71.8	+0.3	−41.4	−0.1
[phen $\subset$ β-CD_n]	+17.5*	+0.1*	−27.8	−0.02	+0.3	+0.1	−95.9	−94.9	−0.3	−70.8* **
[phen $\subset$ γ-CD_n]	−31.7	−0.04	−35.7**	−0.05**	–	–	+31.4	+0.2	−77.3	−0.2
[phen $\subset$ HP-β-CD_n]	–	–	–	–	−14.6	+0.01	−41.6	−0.1	−69.2**	−0.2**
[oxyphen $\subset$ α-CD_n]	−114.7	−0.3	−10.2	+0.1	+31.6	−0.2	+29.8	+0.2	−53.4**	−0.1**
[oxyphen $\subset$ β-CD_n]	−32.3	−0.1	+89.9	+0.3	+14.6	+0.1	–	–	–	–
[oxyphen $\subset$ γ-CD_n]	–	–	–	–	+37.2	+0.2	–	–	−14.8*	+0.01*
[oxyphen $\subset$ HP-β-CD_n]	−14.2	+0.01	–	–	+9.1	+0.1	−7.1	+0.1	–	–

* Binding constant at 293 K was not considered in calculating the thermodynamic data.
** Binding constant at 303 K was not considered in calculating the thermodynamic data.

means that the phen–solvent interactions are less intense than the solvent–solvent interactions. Here the solvophobic effect plays an important role in the inclusion process.

In the cases of β-, and HP-β-CD with phen, the presence of DMSO favours inclusion more than for α-CD and γ-CD. While the $\Delta H_{\text{binding}}$ and $\Delta S_{\text{binding}}$ values are similar and more negative, this is not characteristic of the solvophobic contribution as a guideforce [32].

In Figure 5(b) we observe that the behaviour of phen with the different CDs in 50% DMSO/water is similar for β-CD and HP-β-CD and for α-CD and γ-CD. Examining the data in Table IX, one can see that the inclusion process is largely driven by a solvophobic contribution. The presence of solution medium in the systems with α- and γ-CD is very important. The $\Delta H_{\text{binding}}$ and $\Delta S_{\text{binding}}$ values for the systems [oxyphen $\subset$ β-CD] and [oxyphen $\subset$ HP-β-CD] in 50% DMSO/water indicate that the complexation is entropically less favorable. In 50% DMSO/water the binding constant values with β-CD in 50% DMSO/water are smaller than those with HP-β-CD. Consequently, we may think of an extra association between oxyphen and the hydroxyl group of the hydroxypropyl chains from HP-β-CD, as mentioned above. Due to this association, oxyphen inclusion is obstructed and oxyphen is required to show a large surface area in contact with the solvent. This may be the reason why this system presents large binding constant values in spite of showing a small entropic behaviour.

The van 't Hoff plots of several cycloamylose–guest complexes are not curved, suggesting that the driving forces for cycloamylose–phen and cycloamylose–oxyphen complexation are not simply entropy-controlled solvophobic forces [49]. (See the r^2 values in Figure 5(b) footnote.) In general we can say that the systems with phen show a greater enthalpic contribution.

There are some examples of intermolecular association which usually yield negative enthalpy and entropy changes. It is possible to establish from the systems displayed in Table IX that the driving forces are dipole–dipole for phen and oxyphen with CDs [50]. The contacting surface between the benzene ring of guest molecules and the apolar CDs cavities is stabilized by London dispersion forces [32, 51]. Additionally, in the case of oxyphen with HP-β-CD, there probably are hydrogen bonds.

5. Conclusions

In general phen forms more stable inclusion complexes than oxyphen, except with HP-β-CD in 75% DMSO/water. The inclusion process in our work is mostly entropy-driven for oxyphen and enthalpy-driven for phen. The presence of the solvent plays a valuable role in determining the forces that govern the inclusion. It is possible to assume that in the systems driven by $\Delta S_{\text{binding}} > 0$, the solvophobic effect is the main guideforce in the formation of these inclusion complexes. In the systems driven by $\Delta H_{\text{binding}} < 0$ and $\Delta S_{\text{binding}} < 0$, the London dispersion

forces stabilize the inclusion complexes formed from dipole–dipole interaction as guideforces.

The method used for obtaining binding constants is sufficiently acceptable and dependable, since the magnitude of the errors is small.

Acknowledgements

We acknowledge helpful discussions with and suggestions by Dr. S. Castillo Blum and Prof. E. Zeller. We are grateful to DGAPA-UNAM (IN 205393) and PADEP (005322) for financial support to pursue this project and to Cerestar USA Inc. for providing the cyclodextrins. We also wish to thank J. Gracia Mora for help in drawing some of the figures and M.J. Bernad Bernad for letting us use the minimized converged cyclodextrins structures and for useful discussion.

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