Binding Studies of Ions with Cyclodextrins Using Ion-Selective Electrodes

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Abstract □ The use of ion-selective electrodes (ISEs) to study the binding of various organic ions to α- and β-cyclodextrins (CDs) is described. The ISEs respond selectively, directly, and continuously to the activity of the free ion of interest in solution. Binding parameters (intrinsic binding constants and number of binding sites) are calculated with a computer program which performs a nonlinear fit of the Scatchard model to the experimental data (electrode potential versus total ion concentration) of a titration of a CD solution with the ion of interest. Electrodes selective to chloramine-T, naproxenate, *p*-nitrophenolate, and chlorpromazine ions were used as models. One binding site was found for all cases, with binding constants varying from 0.67×10^3 M^{-1} (naproxenate to βCD) to 1.27×10^4 M^{-1} (chlorpromazine to βCD) at 25 °C and a CD concentration of 0.010 M. Precision for the estimations of the binding parameters was 2.0–7.5% within run and 8–10% between run (n = 3). Nonspecific binding was observed for CAT:βCD and CPM:βCD interactions. The effects of pH, temperature, CD concentration, and ionic strength on the binding are also presented. The overall binding phenomenon is discussed in light of the estimates of the thermodynamic parameters in relation to the size of the guest molecule, the possible mechanism(s) involved, and the forces participating in the interaction of ions with CDs.

Cyclodextrins (CDs), or cycloamyloses, are cyclic oligosaccharides consisting of six to twelve D-glucose units connected at the 1 and 4 carbon atoms. They are known to form inclusion complexes in aqueous solution and solid state with many types of molecules. The complexation has been attributed to nonspecific van der Waals forces, hydrogen bonding, hydrophobic interactions, and, in some cases, to strain-energy alleviation.[1–3] However, the cavity size of the toroidally shaped CDs in relation to the detailed structural conformation and size of the guest molecule are parameters of prime importance for the formation of a guest:CD complex.[1–3] These characteristics make the interaction of CDs with small ligands a model system for understanding the features of the general host:guest binding phenomenon and, in particular, protein binding. For both phenomena (i.e., CDs binding and protein binding), the complex formation presupposes that both the host and the guest molecule possess favorable structural conformations.

As a result of their ability to form host:guest complexes, CDs have found numerous applications. The catalytic ability of host:guest complexes and their use as model enzymes have been studied,[2–5] and their industrial potential[3,6] and analytical applications[3,7–11] have been reported.

Recently, increased attention has been given to the binding of CDs with drug molecules, as complexation alters various physicochemical properties of the guest drug molecule. Among the properties that can be modified are stability, solubility, dissolution rate, membrane permeability, and chemical reactivity. This capability for changing the physicochemical behavior of the guest molecule through complexation has considerable pharmaceutical potential. Stability, solubility, and dissolution rate usually increase by complexation.[12–15] Improvement of the rate of absorption and enhancement of bioavailability have also been achieved.[15–18]

The binding of CDs with several guests has been studied with a variety of techniques such as spectrophotometry,[19–22] fluorescence enhancement,[22–24] calorimetric titration,[25–27] circular dichroism,[28,29] potentiometry[22,26,27,30–33] (using pH measurements), and solubility techniques.[28,34]

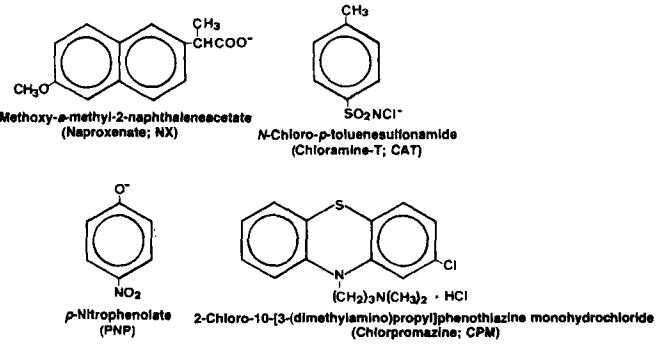
Ion-selective electrodes (ISEs) are electrochemical transducers that respond selectively, directly, and continuously to the activity of the free ion of interest in solution. The electromotive force of the electrochemical cell (E_{cell}), consisting of an ISE for ion i and a reference electrode, is described by the Nernst equation:

$$E_{cell} = E_{const} + \frac{2.303RT}{zF} \log a_i \qquad (1)$$

where E_{const} is a constant term, R the ideal gas constant, T the absolute temperature, F the Faraday constant, z the charge of the ion (i), and a_i is the activity of the ion (i). When the ionic strength of the solution is kept constant, or at low concentrations ($C < 10^{-2}$ M), the concentration values (C) can be used instead of the activity in eq 1.

Due to their inherent advantages (i.e., sufficient selectivity and sensitivity, wide analytical range of the analyte concentration, insensitivity to optical interferences, low cost, fast response, and simplicity in assembly), ISEs have found many applications in the fields of pharmaceutical[35–40] and clinical analysis.[41,42] Recently, the application of ISEs to albumin: drug binding studies has been described.[43] The potential use of ISEs in these studies is due to their ability to directly measure the concentration of the free ion in the presence of albumin and the bound drug.

In this paper, ISEs are used as sensors for the study of CDs binding with four ions (see structures). The selection of these ion models was based on the diversity of their physicochemical properties (e.g., size, charge, solubility). Electrodes of the



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liquid-ion exchanger type, selective to chloramine-T ion (CAT), naproxenate (NX), and *p*-nitrophenolate (PNP), and a PVC membrane ISE for chlorpromazine (CPM), were used. The binding and associated parameters (binding constants and number of potential binding sites or binding stoichiometry) were studied as a function of temperature and pH.

Experimental Section

Reagents—All chemicals used were of analytical reagent grade, and deionized water was used in solution preparation. Pure substances of naproxen and chlorpromazine hydrochloride were obtained from local manufacturers and were used without further purification for the preparation of standard solutions of the corresponding ions (0.100–0.0300 M). Chloramine-T and *p*-nitrophenol were obtained from Merck (Darmstadt, FRG). α-Cyclodextrin, βCD, sodium tetraphenylborate (TPB), 1-isopropyl-4-nitrobenzene (*p*-nitrocumol), tetraheptylammonium bromide, and 2-nitrophenyloctyl ether (2-NPOE) were from Fluka (Buchs, Switzerland). Polyvinyl chloride (PVC) of high molecular weight (d = 1.385) was from Janssen Chimica (Beerse, Belgium).

Buffers and Ionic Strength Adjustors—The following pH buffers were used: borate (0.010 M, pH 9.0), acetate (0.050 M, pH 4.5), succinate (0.050 M, pH 5.5), and phosphate (0.050 M, pH 3.1). These solutions were prepared from the corresponding acid or acid salt and concentrated NaOH solution. Sodium sulfate solution (0.020 M) was also used as an ionic strength adjustor.

Liquid Ion Exchangers—The liquid ion exchangers for NX and CAT were their $(tetraheptylammonium)^+$ and $[(bathophenanthroline)_3Ni]^{2+}$ ion pairs in *p*-nitrocumol, respectively. The construction and characteristics of these two electrodes have been previously described.[40,44]

The PNP ISE was prepared for the first time for the goals of this study and its liquid ion exchanger (tetraheptylammonium$^+$-PNP ion pair in *p*-nitrocumol) was prepared as follows. A 0.010 M tetraheptylammonium bromide solution in *p*-nitrocumol was shaken with a suspension of silver oxide in water to exchange Br^- with OH^- according to the following reaction:

$$2R_4N^+Br^- + Ag_2O + H_2O \rightleftharpoons 2R_4N^+OH^- + 2AgBr \quad (2)$$

After centrifugation, the aqueous phase was decanted and the organic phase was separated from the solid residue of AgBr and the excess of Ag_2O. The organic phase was then shaken 10 times with a saturated aqueous solution of *p*-nitrophenol in order to convert the ion pair to the *p*-nitrophenolate form according to the following reaction:

$$R_4N^+OH^- + PhO^-H^+ \rightleftharpoons R_4N^+PhO^- + H_2O \quad (3)$$

The aqueous phase was decanted every time and the remaining organic phase was finally dried with anhydrous sodium sulfate.

The PNP electrode shows near-Nernstian response in the range 8×10^{-5}–1×10^{-1} M of *p*-nitrophenolate concentration, with slope of 58–60 mV/decade (r = 0.9998). The detection limit is 5×10^{-5} M, and the potential was found to be not dependent on pH in the range 8.3–12 (pK_a of *p*-nitrophenol is 7.5).

For CPM, its TPB salt[37] was used and 2-NPOE was selected as the organic solvent for its better plasticizing properties when trapped in a PVC membrane. For the preparation of the PVC membrane for the CPM ISE, the method introduced by Graggs et al.[45] was followed.

Internal Reference Solutions—These solutions were 0.010 M with respect to the measured ion in 0.10 M sodium chloride, saturated with silver chloride.

***α*- and *β*-Cyclodextrin Stock Solutions**—These solutions were 0.010 M with respect to the CD in the medium of choice. More dilute solutions were prepared from stock solutions by dilution in the same medium. All standard stock solutions of the ions studied were kept in amber bottles in the refrigerator and renewed every week.

Apparatus—The system used for measurements consists of an Orion model 801 digital pH/mV meter, with a readability of ±0.1 mV, connected to a radiometer strip chart recorder (REC 21) through a high sensitivity unit (REA 112) interface. All measurements were carried out in a 50-mL double-walled glass cell, thermostated at the appropriate temperature with an Edmund Buhler 7400 Tubigen type 7TH-4 water bath, with constant magnetic stirring of the solutions. For pH measurements, a Metrohm model E-350B pH-meter with a combination glass electrode was used. The fluoride and perchlorate commercially existing ISEs (Orion, Boston, MA), of solid state and liquid ion exchanger type, respectively, were also used in the course of this study.

Electrode Assembly—For the liquid ion exchanger-type electrodes, an Orion liquid membrane electrode body (series 92), with Orion polycellulose acetate membranes (92-07-04 nitrate porous membranes), was used. For the PVC-type electrode of CPM, a laboratory constructed electrode assembly, shown in Figure 1, was used. In both cases, the electrodes were used with an external Orion 90-01-00 Ag/AgCl single junction reference electrode filled with an Orion 90-00-01 internal reference solution, consisting of 1.70 M KNO_3, 0.040 M KCl, 0.060 M NaCl, and 0.037% formaldehyde as preservative. All ISEs were conditioned after their preparation, for 24 h before use, in a magnetically stirred 0.0010 M solution of the measured ion; they were also stored in this solution when not in use.

Procedures—*Calibration Curves*—A 30.00-mL volume of the medium of choice was pipetted into the measurement cell. The electrodes were immersed in it and, after the potential had stabilized, various aliquots of the standard solution of the ion of interest were added with a 100-μL Hamilton microsyringe (concentration range covered 1×10^{-6}–1×10^{-2} M). The emf values were recorded and measured after stabilization (±0.1 mV) following each addition. The potential values E were plotted against $-\log C$ to give the calibration curve using a least squares fitting program in BASIC.[43] Correction for the changes in volume after each addition was performed by the program.

Binding Experiments—Titration of CD with the ion of interest was accomplished by pipetting a 30.00-mL volume of α- or βCD solution of known concentration (0.0100–0.00100 M), in the appropriate medium, in the cell; the electrodes were then immersed in the solution. After the stabilization of the potential, small amounts of the standard solution of the measured ion were added. The emf values were recorded to check stabilization (±0.1 mV) and measured following each addition. The same procedure was used in another

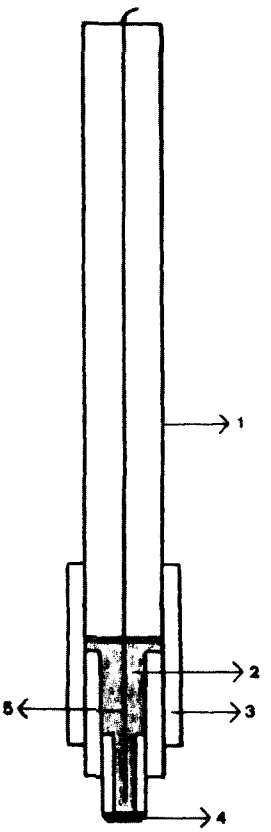

Figure 1—Electrode assembly for the PVC-type chlorpromazine ion-selective electrode: (1) body of an old glass electrode; (2) internal reference solution; (3) system of three PVC tubes; (4) PVC electroactive membrane; (5) internal reference electrode (Ag/AgCl).

binding experiment; however, to overcome the effect of dilution of the CD solution (sample solution), a mixed solution containing 0.100–0.0500 M of the measured ion and the same concentration of CD as in the sample solution (0.0100–0.00100 M) was used. In this way, the concentration of CD during the experiment was kept constant.

Data Treatment—Two different models were examined for their ability in describing the CDs:ions binding at equilibrium: the thermodynamic model[22,24,44] for 1:1 and 1:2 complex formation and the Scatchard model for one class of binding sites.[22,46] The fitting of the models to the experimental data was performed with STATGRAPHICS[47] by a nonlinear least squares fitting procedure. The use of the 1:1 thermodynamic model for the description of data resulted in r values varying from 0.824 (NX) to 0.993 (PNP). The same model for 1:2 complex formation gave higher values for r (0.961–0.9999), but negative estimates for the second association constant (K_2) were obtained for all ions studied. The Scatchard model was proven the most successful in describing the complexation equilibrium ($0.996 \leq r \leq 0.99999$) and became the model of choice for the analysis of data. In all cases, one class of binding sites (i.e., CD cavity) and linear Scatchard plots were observed when eq 4 was fitted to the experimental data:

$$B = \frac{nKF}{1 + KF} CD_t \quad \text{or} \quad T = F + \frac{nKF}{1 + KF} CD_t \qquad (4)$$

where B, F, and T are the bound, free, and total molar concentration of the measured ion, respectively, n is the number of equal binding sites on the CD molecule (representing the stoichiometry of the complex), K is the overall binding constant which describes the equilibrium $CD + n(ion) \rightleftharpoons [CD - (ion)_n]$, and CD_t is the total concentration of CD in the sample solution. For the curve fitting we preferred to use a BASIC program,[43] specifically developed for the needs of binding experiments using ISEs, which performs nonlinear least squares fitting of the Scatchard model to the experimental data. The program requires the experimental data (potential readings, E, and volumes of addition, V), the volume of the sample solution (V_o), the concentration of the standard solution of the measured ion (C_s), the concentration of the CD in the sample solution, and the number of the experimental data (number of additions). The program undertakes the calibration of the ISE and the determination of the binding parameters (n, K). The first task is performed by linear least square fitting of eq 1 while the second is done by nonlinear least square fitting of eq 4 to the experimental data (E and T values are the dependent and independent variables, respectively). Initial values for the binding parameters are required and calculated by linear least squares fitting of eq 4 to the experimental data. The numbers of the first and last experimental points for the fitting (for both calibration and binding experiments) are also required. The results can be obtained graphically and numerically.

Results and Discussion

In order to examine the effect of CDs on the performance characteristics of the electrodes selective to ions that either do not form, or only slightly form, inclusion complexes (because of their small size), the two commercially available ISEs fluoride and perchlorate were used. By calibration curves constructed in the absence (a) and presence (p) of βCD with both electrodes, the following values were obtained for the slope (S_a, S_p), the constant potential (E_a, E_p), the least linear measurable limit (L_a, L_p) of the electrode, and the correlation coefficient (r_a, r_p) of the calibration curve (i.e., the electrode characteristics): (*a*) fluoride ISE: $S_a = 58.5$ (±0.3) and $S_p = 60.8$ (±0.2) mV/dec, $E_a = -117.4$ (±1.3) and $E_p = -124.4$ (±0.6) mV, $L_a = L_p = 6 \times 10^{-6}$ M, and $r_a = 0.9997$ and $r_p = 0.9999$; (*b*) perchlorate ISE: $S_a = 53.9$ (±0.1) and $S_p = 53.9$ (±0.05) mV/dec, $E_a = -98.6$ (±0.5) and $E_p = -95.8$ (±0.1) mV, $L_a = L_p = 5 \times 10^{-6}$ M, and $r_a = r_p = 0.99999$. As shown from the results, the presence of βCD (0.010 M, 25 °C) has no effect on the electrode characteristics. The potential measurements were also free of drift, even at the very low concentrations. From this study, it is concluded that ISEs can be used successfully to monitor free ion concentrations in the presence of CD.

Preliminary experiments using several laboratory-made ISEs showed that βCD interferes with some anion-selective electrodes (picrate and salicylate). This interference (drift of the initial electrode potential), is most likely attributed to a parallel interaction of βCD with the other components of the electrode membrane (bulky counter cation and organic solvent). The fact that no interference was observed when the same organic solvent was used for the preparation of the liquid membrane of a cation-selective electrode makes the assumption of a βCD:counter cation interaction more plausible. It is interesting to note, however, that βCD did not interfere with the CPM cation.

α-Cyclodextrin (αCD) did not interfere with either the anion or the cation-selective electrodes used in this study. It seems likely that all the bulky counter ions used for the preparation of the liquid membranes do not interact with αCD, probably due to steric hindrance.

In all experiments with ISEs, the optimum pH value was used in order to assure complete ionization of the species under study, without any interference from the buffer ions. Table I shows the optimum pH used and the mean of its control, the linear concentration range, and the slope of each ISE used in this study.

Typical calibration curves (actually titration curves) in the absence and presence of αCD is shown in Figure 2. Typical Scatchard plots for the binding of *p*-nitrophenolate and chloramine-T ions with αCD and βCD, respectively, are shown in Figures 3 and 4. The linearity of the curves, as well as the theoretical Scatchard curves which were drawn based on the calculated binding parameters, confirm the reliability of the selected model and its good fitting to the experimental data. Similar plots (not shown) for the binding of the other ions studied were obtained.

Table II shows summarized results from all binding studies performed with αCD and βCD. Within-run standard deviations of the binding parameters are shown in parentheses and standard deviations of the squares of residuals (SD_{re}) are also given. The latter represent the error in the potential (E) readings (dependent variable). The between-run relative standard deviations (three runs) were 8–10% in all cases.

A brief discussion for the binding study of each of the four ions examined follows.

Naproxenate:β-Cyclodextrin (βCD)—No drift of the electrode was observed with a βCD concentration of 0.0020 M, and the initial potential stabilized to a value corresponding to a concentration of the measured ion lower than the detection limit of the electrode. The binding parameters determined at 25 °C, listed in Table II, are in good agreement with recently reported results.[34] The values of n and K show a 1:1 complex formation and a low binding affinity. To study the effect of temperature, experiments at 15, 25, and 37 °C at a constant βCD concentration (0.0020 M) in borate buffer (0.010 M, pH

Table I—Optimum Working Conditions and Characteristics of the Ion-Selective Electrodes Used[a]

ISE	pH and/or Ionic Strength Adjustor	Slope, mV/decade	Linear Response Range, M
NP	Borate buffer (0.01 M, pH 9.0)	58	1×10^{-4}–1×10^{-1}
CAT	Na_2SO_4 (0.02 M, pH 7.5 with NaOH)	56	5×10^{-5}–1×10^{-1}
PNP	Na_2SO_4 (0.020 M, pH 9.0 with NaOH)	59	8×10^{-5}–1×10^{-1}
CPM	Na_2SO_4 (0.020 M)	54	1×10^{-4}–1×10^{-2}

[a] Temperature = 25 °C.

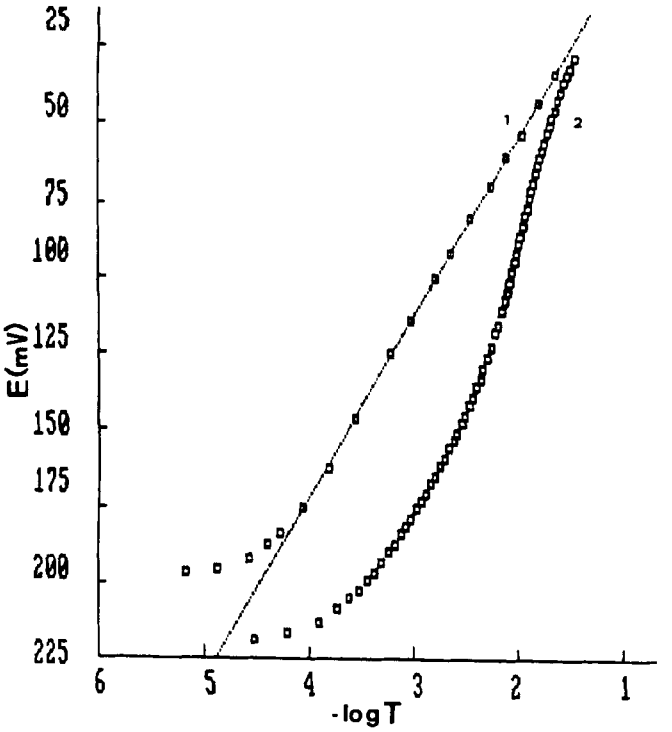

Figure 2—Plot of electromotive force values, E, versus the negative logarithm of the total concentration, $-\log T$, of *p*-nitrophenolate (**1**) in the absence of αCD (calibration curve) and (**2**) in the presence of 0.0100 M αCD (actual experiment). For a given total concentration, T, the reading on the ordinate, which corresponds to the intersection of curve (**2**) with the value of $-\log T$, represents the E value of the free ion in solution. The concentration of the free ion is read as $-\log F$ on the abscissa by coupling the E value of free ion with the calibration curve (**1**).

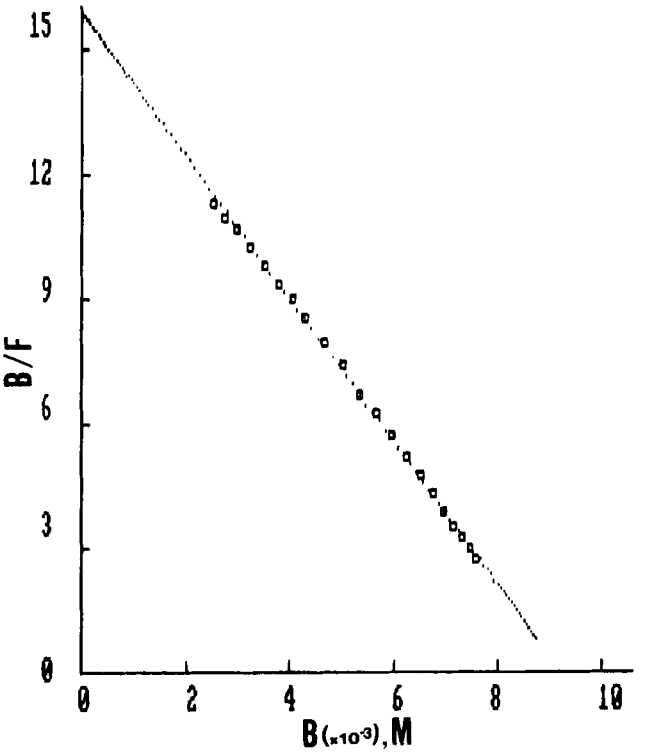

Figure 3—Scatchard plot for the binding of *p*-nitrophenolate with αCD (0.0100 M) at 25 °C. The theoretical Scatchard curve, based on the calculated binding parameters, is also shown. The concentration of αCD was kept constant during the binding experiment (see *Experimental Section*).

9.0) were carried out. The results are presented in Table III. Although the value of n remained constant in the range of temperature studied, there was a significant increase of K values with decreasing temperature. This behavior implies that the binding is an exothermal reaction. Changes in entropy and enthalpy that follow the binding of NX to βCD were calculated from eq 5:

$$\log K = \Delta S/(2.303R) - (\Delta H/(2.303R))(1/T) \quad (5)$$

where ΔS and ΔH are the changes in entropy and enthalpy, respectively, T is the absolute temperature, R is the ideal gas constant, and K is the binding constant at T. Good linearity ($r = 0.9998$) was observed by plotting $\log K$ against $1/T$ (Van't Hoff plot); the estimated values for ΔH and ΔS are quoted in Table IV.

Chloramine-T Ion:β-Cyclodextrin (βCD)—No substantial interference from βCD was noted in the studies with the CAT ISE. However, the Scatchard plot for CAT binding to βCD (Figure 4a), shows an interesting pattern. At high free concentration of CAT, a minimum value for the ordinate (B:CD_t:F) is reached rather than the usual intersection of the regression line with the x-axis. In other words, by increasing the total concentration of CAT, a proportional increase in the parameters B:CD_t and F is observed, resulting in a constant value for the ratio B:CD_t:F. This behavior has been reported[43,48,49] in protein binding studies and is explained by the accumulation of ions or molecules around the binder molecule. The corrected concentration of the bound molecule is then given by the following equation:[43,48,49]

$$B_o = \sum_{i=1}^{m} \frac{n_i K_i F}{1 + K_i F} P_t + N_s F \quad (6)$$

(The Scatchard equation is corrected by the term N_sF related to the nonspecific binding.) This means that the concentration of the nonspecifically bound molecule is proportional to its free concentration and when F tends to infinite, the term B:P_t:F tends to the value of N_s:P_t, which is constant. In this study, we suggest the same behavior for the CAT:βCD system. The corrected B_o and T_o are then given from the following equations:

$$B_o = \frac{nKF}{1 + KF} CD_t + N_s F \quad (7)$$

$$T_o = F + \frac{nKF}{1 + KF} CD_t + N_s F \quad (8)$$

The determination of the binding parameters was performed, using the same program,[43] by nonlinear least squares fitting of eq 8 to the experimental data. An initial estimate for N_s was required and was calculated by the program from the Scatchard plot (the term N_s/CD_t is the limiting value of the ordinate from where N_s and N_sF are calculated; Figure 4a). The specifically bound concentration of CAT was calculated from the following difference: $B = T_o - F - (N_sF)$. The Scatchard plot for βCD binding after this correction is shown in Figure 4b. The existence of only one class of binding sites is now obvious.

The effect of temperature on the binding parameters was

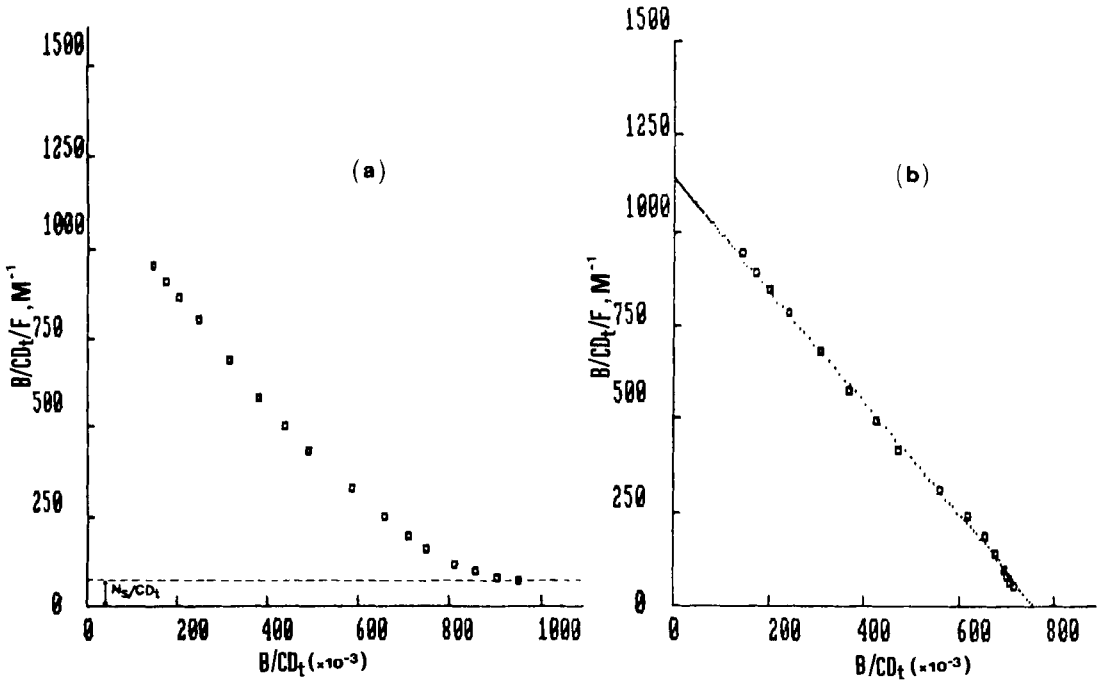

Figure 4—(**a**) Scatchard plot for the binding of chloramine-T with βCD (0.0100 M) at 25 °C. The portion of nonspecific binding is shown. (**b**) Scatchard plot for the binding of chloramine-T with βCD (0.010 M) at 25 °C after correction for the nonspecific binding as described in the text. The theoretical Scatchard curve, based on the calculated binding parameters, is also shown. The concentration of βCD was not kept constant during the binding experiment (see *Experimental Section*).

Table II—Binding Parameters for Various Ions with α- and β-Cyclodextrin

Ion	Cyclodextrin	n	$K \times 10^3$, M^{-1}	N_s[b]	SD_{re}
NX	β	0.76 (±0.02)	0.67 (±0.05)	—	0.2
	α	—	—	—	—
CAT	β	0.76 (±0.01)	1.50 (±0.03)	0.14 (±0.01)	0.3
	α	—	—	—	—
PNP	β	—	—	—	—
	α	0.921 (±0.004)	1.77 (±0.12)	—	0.3
CPM	β	0.834 (±0.001)	12.7 (±0.3)	0.22 (±0.01)	0.2
	α	0.86 (±0.02)	0.12 (±0.03)	—	0.1

[a] Temperature = 25 °C; cyclodextrin concentration (C_{CD}) = 0.0100 M (except for NX where $C_{\beta CD}$ = 0.002 M). [b] Nonspecific binding.

Table III—Temperature Effect on the Binding of β-Cyclodextrin with Naproxenate, Chloramine-T, and Chlorpromazine

Ion	Temperature, °C	n	$K \times 10^3$, M^{-1}	N_s[b]	SD_{re}
NX	5	—	—	—	—
CAT		0.771 (±0.003)	3.01 (±0.03)	—	0.2
CPM		0.864 (±0.003)	41.0 (±1.5)	0.32 (±0.02)	0.6
NX	15	0.75 (±0.04)	0.96 (±0.09)	—	0.3
CAT		0.813 (±0.003)	1.90 (±0.02)	0.024 (±0.005)	0.2
CPM		0.841 (±0.001)	23.1 (±0.2)	0.50 (±0.01)	0.3
NX	25	0.76 (±0.02)	0.67 (±0.05)	—	0.2
CAT		0.76 (±0.01)	1.50 (±0.03)	0.14 (±0.01)	0.3
CPM		0.834 (±0.001)	12.7 (±0.3)	0.22 (±0.01)	0.2
NX	37	0.84 (±0.05)	0.45 (±0.05)	—	0.2
CAT		0.713 (±0.01)	0.76 (±0.02)	0.18 (±0.01)	0.3
CPM		0.854 (±0.003)	6.70 (±0.06)	—	0.4

[a] β-Cyclodextrin concentration ($C_{\beta CD}$) = 0.01 M (except for NX, where $C_{\beta CD}$ = 0.002 M). [b] Nonspecific binding.

studied with experiments carried out at 5, 15, 25, and 37 °C. The value of K increased by decreasing the temperature, while temperature has no significant effect on the n value (Table III). Van't Hoff analysis of the data showed a good correlation (r = 0.987), and the estimates obtained for ΔH and ΔS are reported in Table IV. The N_s value showed a significant decrease by decreasing the temperature and became zero at 5 °C.

***p*-Nitrophenolate:αCD**—Binding parameters for the PNP:αCD system determined at 25 °C are given in Table II. In Table V (taken from ref 22), a comparison of our results with those obtained with other methods (references are quoted in Table V) is presented. The calculated mean for K (2392 ± 463 M^{-1}) signifies the considerable variability of the techniques employed for the study of PNP:αCD binding. However, all published papers support a 1:1 stoichiometry for

Table IV—Theoretical Structural Data and Experimental Thermodynamic Parameters for the Binding of Ions to β-Cyclodextrin

Ion	R[a]		ΔH, Kcal/mol	ΔS, cal/mol/deg	$K \times 10^3$, M^{-1}[b]
	Axial Approach	Equatorial Approach[c]			
NX	124.4	36 (24.3)	−6.27	−8.10	0.67
CAT	87.4	36 (0.0)	−7.12	−9.66	1.50
CPM	114.0	101.8 (14.0)	−9.82	−14.20	12.70
PNP[d]	109.8	49.0 (0.0)	−7.72	−11.20	1.77

[a] R is defined in the text; approximate intramolecular distances were calculated with the "Desktop Molecular Modeller" program (ref 53). [b] Estimates are quoted for comparative purposes and correspond to 25 °C. [c] Numbers in parentheses represent the percent portion of the guest molecule not inserted into the βCD cavity. [d] Interaction with αCD was considered.

Table V—Comparison of the Determined Binding Constants for *p*-Nitrophenolate (PNP) with α-Cyclodextrin (αCD) with Earlier Reported Values at 25 °C

Method	K, M^{-1}[b]	Reference
Spectrophotometry	2290	19
Potentiometry[a]	2200	33
Spectrophotometry	2500	20
Nuclear magnetic resonance	2700	20
Optical rotation	1590	20
Polarography	2439	22
Spectrophotometry	1890	51
Spectrophotometry	2720	22
Potentiometry[a]	2143	22
Potentiometry[a]	2403	31
Gel filtration	2270	22
Gel filtration	3550	52
ISE direct potentiometry	1770 ± 120	

[a] Using pH measurements. [b] The mean value of K for all methods except ISE direct potentiometry is 2392 ± 463 M^{-1}.

the system studied, which is in accord with the value of n = 0.921 (±0.004) that was experimentally determined in the present study.

The effect of temperature on the binding was also studied and the results were analyzed according to eq 5. Good correlation was observed and the estimates for ΔH and ΔS are listed in Table IV. The value of n remained constant within the range 5–37 °C, while the value of K decreased with increasing temperature (Table VI).

The effect of the ionic strength was found to be negligible. Binding experiments in Na_2SO_4 (0.020 M) and in water, at pH 9.0 (adjusted with NaOH), gave values of n = 0.921 (±0.004) and K = 1770 (±120) M^{-1}, and n = 0.972 (±0.002) and K = 1711 (±11) M^{-1}, respectively. No measurable binding of PNP with βCD was observed when studied at 25 °C using a βCD concentration of 0.010 M.

Binding of Chlorpromazine to β- and α-Cyclodextrin—No interfering effect on the response of CPM ISE was observed in the presence of either αCD or βCD (0.010 M). The very low observed binding constant for the CPM:αCD system should be attributed to the inability of the bulky CPM ion to fit into the αCD cavity. In contrast, the CPM:βCD interaction is two orders of magnitude stronger (Table II), probably because of the larger size of the βCD cavity which allows the inclusion (totally or partially) of the CPM cation.

The results for CPM:αCD and CPM:βCD binding parameters, shown in Table II, reveal a 1:1 stoichiometry for both CDs and are in good agreement with earlier reported results,[50] while nonspecific binding was observed only for the CPM:βCD interaction.

The influence of pH on the CPM:βCD interaction was studied with experiments performed at pH 3.1, 4.5, and 5.5, in 0.050 M phosphate, acetate, and succinate buffers, respectively (25 °C, $C_{\beta CD}$ = 0.010 M). Taking into account the between-run variability in the estimation of the binding parameters, statistically insignificant differences in the values of n, K, and N_s were observed within the range of pH studied (Table VII). The results are also in good agreement with those obtained from experiments in 0.020 M Na_2SO_4. Therefore, the binding proved to be not dependent on pH changes within the working pH range of the electrode. This behavior is expected to be general (for anions and cations) at pH values < 10. At pH > 10, CDs are also ionized to some extent[4,25] due to their secondary hydroxyl groups with a pK_a value of 12.

Binding experiments at different temperatures (5–37 °C) showed significant increases of K values with decreasing temperature, while the value of n remained constant (Table III). The values for ΔH and ΔS, derived from Van't Hoff analysis (r = 0.999), are presented in Table IV. The value of the N_s parameter also decreased with increasing temperature and was negligible at 37 °C. Thus, the temperature dependence of N_s follows the opposite pattern to that observed with CAT.

The effect of the concentration of βCD on the estimates of the binding parameters was also studied (concentration range covered 0.0010–0.0100 M). Considering the between-run RSD% in the estimation of the binding parameters, the differences of n and K values observed were statistically insignificant (Table VIII), indicating that the binding is not dependent on βCD concentration. On the contrary, nonspecific binding is dependent on βCD concentration (Table VIII).

Overall Consideration of Guest:CD Interactions—Although multiple substrate species were used in our study, a general consideration of the results obtained can be helpful in deriving conclusions concerning the snugness of fit of

Table VI—Effect of Temperature on *p*-Nitrophenolate: α-Cyclodextrin Binding[a]

Temperature, °C	n	$K \times 10^3$, M^{-1}	SD_{re}
5	0.974 (±0.003)	4.19 (±0.04)	0.3
15	0.950 (±0.003)	2.45 (±0.02)	0.3
25	0.921 (±0.004)	1.77 (±0.12)	0.3
37	0.989 (±0.003)	0.971 (±0.006)	0.3

[a] α-Cyclodextrin concentration = 0.01 M.

Table VII—Effect of pH on Chlorpromazine: β-Cyclodextrin Binding[a]

pH	n	$K \times 10^3$, M^{-1}	N_s	SD_{re}
3.1	0.810 (±0.002)	12.1 (±0.1)	0.13 (±0.01)	0.3
4.5	0.880 (±0.001)	10.60 (±0.05)	0.13 (±0.05)	0.2
5.5	0.860 (±0.004)	13.4 (±0.3)	0.22 (±0.02)	0.4
—[b]	0.834 (±0.001)	12.7 (±0.3)	0.22 (±0.01)	0.2

[a] Temperature = 25 °C; $C_{\beta CD}$ = 0.01 M. [b] Binding experiment in 0.02 M Na_2SO_4 as ionic strength adjustor.

Table VIII—Effect of β-Cyclodextrin Concentration on Chlorpromazine:β-Cyclodextrin Binding[a]

$C_{\beta CD}$, M	n	$K \times 10^3$, M^{-1}	N_s	SD_{re}
0.0100	0.834 (±0.001)	12.7 (±0.3)	0.22 (±0.01)	0.2
0.0075	0.841 (±0.001)	12.84 (±0.06)	0.26 (±0.01)	0.1
0.0050	0.815 (±0.002)	11.8 (±0.1)	0.097 (±0.005)	0.2
0.0025	0.781 (±0.002)	11.02 (±0.05)	—	0.1
0.0010	0.827 (±0.002)	12.14 (±0.6)	—	0.1

[a] Temperature = 25 °C.

guests to CDs. Relying on the calculated values of the thermodynamic parameters ΔH and ΔS (Table IV), the binding of the guests studied to αCD and βCD was found to be driven by an exothermic enthalpy change. This nonclassical hydrophobic behavior is possibly attributed to van der Waals interactions between the substrates and the apolar CD cavity.[26,27] This is in agreement with the fact that $-\Delta H$ values increase as the association constant (K) of the guest to βCD increases. On the other hand, the loss in free energy, reflected in the negative ΔH values, is compensated by the negative ΔS values, since a linear compensation plot (ΔH versus ΔS, r = 0.9995) was observed. This kind of compensation is usually associated with the participation of water molecules in complex formation,[1,2] implying that the guest molecule loses its hydration sphere on entering the CD cavity, while water molecules are expelled from the cavity; such a mechanism signifies the hydrophobic character of the complexation.

Guest:CD complexation presupposes that the guest is sterically free to slip into the CD cavity. Two different approaches, referred to as "axial" and "equatorial", have been suggested[24] for the mode of complexation. Taking into account the specific mechanism involved, a measure of the snugness of fit can be obtained by the ratio $R = (l_g/d_c) \times 100$, where l_g is the maximum length of the guest molecule associated with the specific mode of complexation (axial or equatorial) and d_c is the internal CD cavity diameter (5.7 Å for αCD, 7.8 Å for βCD). This ratio, with optimum values near 100, is an indicator of the relative sizes of the incoming guest and the CD cavity. A number of calculations for "R" were made assuming either the axial or equatorial approach of the incoming guest (Table IV). For NX:βCD complexation, the axial approach seems to be energetically unfavored, resulting in a too "tight" complex since strain of the CD ring is required. On the contrary, the equatorial approach permits the energetically favored apolar naphthalene moiety to slip into the βCD cavity. In this case, the low R value is expected to result in a weak complex formation; this is in accord with the small value for K determined experimentally (Table IV). For the CAT:βCD complex formation, the less favored axial approach is hindered by the positioning of the $(-SO_2NCl)^-$ group. The equatorial approach is energetically favored since the charged group of the CAT molecule can be extended towards the bulk solution. The snugness of fit is reflected in a low value of R. In the case of CPM, the axial approach is precluded by steric factors (R = 114). The strongest binding found to occur, between CPM:βCD, agree fairly well with the near-perfect match (R = 101.8) between the cavity and CPM length of the equatorial approach. Accordingly, the values of the association constant estimated for the CPM:βCD interaction, at the temperature range examined, varied from 0.7×10^4 to 4×10^4 M^{-1}, values which are comparable to those found in protein binding studies. As far as the PNP:αCD interaction is concerned, the equatorial approach has been suggested in previous reports.[20,31]

Some conclusions concerning the binding forces in relation to the structural insights can be derived from the evaluation of thermodynamic parameters. Apart from the classical hydrophobic forces, a significant contribution to the binding results from van der Waals forces[26,27] since negative values for ΔH and ΔS were found in all cases (Table IV). The more exothermic ΔH and more negative ΔS values were found for CPM:βCD interaction. Thus, van der Waals forces resulting from the snug fit of CPM into the βCD cavity (R = 101.8) seem to play an important role in the CPM:βCD interaction. For CAT and NX, the participation of van der Waals forces is anticipated to be smaller on the basis of the less negative values of ΔH and ΔS. These observations are in agreement with the snugness of fit of CAT and NX inside the βCD cavity. In fact, for both ions (CAT and NX), the fit is not snug (R = 36, Table IV).

An interesting finding of the present study is the nonspecific binding observed between CAT and βCD and CPM and βCD (Tables III, VII, VIII). This finding constitutes a valid experimental observation and is not due to artifacts since (*i*) it was verified with a great number of experiments, (*ii*) the reliability of measurements based on the ISEs at the highest concentration range is unquestionable, (*iii*) the technique employed is capable of monitoring the guest:CD interaction even if the specific binding is near to or beyond completion, and (*iv*) a very good fitting was obtained with the theoretical model implemented in protein–ligand studies[43,48,49] to explain nonspecific binding phenomena.

A detailed physical interpretation of the nonspecific binding can not be made with any certainty due to the limited data available. The accumulation of CPM and CAT ions in the neighborhood of the βCD molecule can be postulated as a reasonable explanation since the value of N_s for CPM increases with increasing βCD concentration and reaches an upper limit (N_s = 0.026 at 25 °C) when the concentration of βCD is equal to 0.0075 M (Table VIII). It is interesting to note, however, that both CAT and CPM ions appear to be entrapped entirely (CAT) or to a large extent (CPM) in the βCD cavity. This means that both ions in the bulk solution can attain spatial arrangements close to the rims of the secondary or primary hydroxyl groups of βCD, thus making possible a weak interaction of a nonspecific character. Further studies are required to elucidate whether or not the nonspecific binding is a general phenomenon encountered in the field of the CD inclusion complexes. Studies focusing on the far end of the binding isotherm should reveal the characteristics of this phenomenon.

Conclusions

The study of the binding of four ions (NX, CAT, PNP, CPM) with αCD and βCD was accomplished using the ISE potentiometric technique. Based on the Scatchard model, binding parameters were estimated for the interactions of NX, CAT, and CPM ions with βCD and of PNP and CPM ions with αCD. The continuous monitoring of the binding phenomenon, based on the rapid measurement in the CD solution without need for separation of the bound species, enabled us to observe that nonspecific binding participates in the overall binding of CAT and CPM to βCD. The determined thermodynamic parameters for all ions studied revealed the participation of both hydrophobic and van der Waals forces in the ion:CD interactions.

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Note Added in Proof

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