JOC Article

Inclusion Complexation of Novocaine by β-Cyclodextrin in Aqueous Solutions

Emilia Iglesias

Departamento de Química Física e E. Q. I, Facultad de Ciencias, Universidad de La Coruña, 15071-La Coruña, Spain

qfemilia@udc.es

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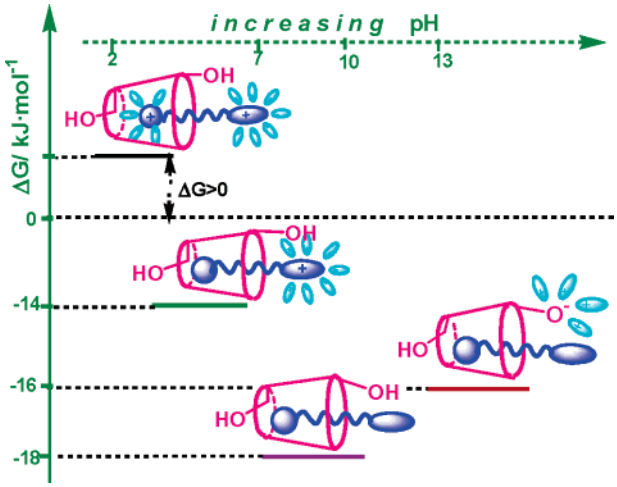

The formation of inclusion complexes between β-cyclodextrin (β-CD) and the local anesthetic 2-(diethylamino)ethyl-*p*-amino-benzoate (novocaine) in aqueous solutions under different acidity conditions, using steady-state fluorescence or UV−vis spectroscopies, electrical conductivity, or the kinetic study of both the nitrosation reaction of the primary amine group in a mild acid medium and the hydrolysis of the ester function under an alkaline medium, has been studied. The inclusion complex formation between neutral or protonated novocaine and β-CD of 1:1 stoichiometry was observed; however, the magnitude of the binding constants depends on the nature of both the guest and the host, and the higher-affinity guest−host was found under conditions when both the novocaine and the β-CD were neutral molecules.

Introduction

Cyclodextrins were one of the first molecular receptors whose ability to bind organic molecules was recognized and extensively studied by various experimental techniques.[1−3] Consequently, cyclodextrins (CDs) have pharmaceutical applications as high-performance biomaterials in drug-delivery systems due to their action of altering physical, chemical, or biological properties of the guest molecules through the formation of inclusion complexes.[4−6]

The complexation of CDs of a poorly soluble drug, the absolute majority of orally administered drugs, results in improved (accelerated, enhanced) dissolution rate, solubility, and bioavailability of drug molecules.[5,7−10] The use of natural CDs as drug carriers (a new family of pharmaceutical excipients) provides the solution to many difficulties in drug formulation,

(1) Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic Publishers: The Netherlands, 1988; Chapter 2.
(2) Connors, K. A. In *Comprehensive Supramolecular Chemistry*; Szejtli, J., Osa, T., Eds.; Pergamon: New York, 1996; Vol. 3, p 205.
(3) Rekharsky, M.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.
(4) Szejtli, J. *J. Inclusion Phenom. Macr. Chem.* **2005**, *52*, 1.
(5) Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045.
(6) Merino, C.; Junquera, E.; Jiménez-Barbero, J.; Aicart, E. *Langmuir* **2000**, *16*, 1557.
(7) Wong, J. W.; Yueu, K. H. *Int. J. Pharm.* **2001**, *227*, 177.
(8) Ozdemir, N.; Oedu, S.; Ozkan, Y. *Drug Dev. Ind. Pharm.* **2000**, *26*, 857.
(9) Woodcock, B. G.; Rietbrock, S.; Merz, P. G. *Br. J. Clin. Pharmacol.* **1993**, *35*, 546.
(10) Loftsson, T.; Leeves, N.; Bjornsdottir, B.; Duffi, L.; Masson, M. *J. Pharm. Sci.* **1999**, *88*, 1254.

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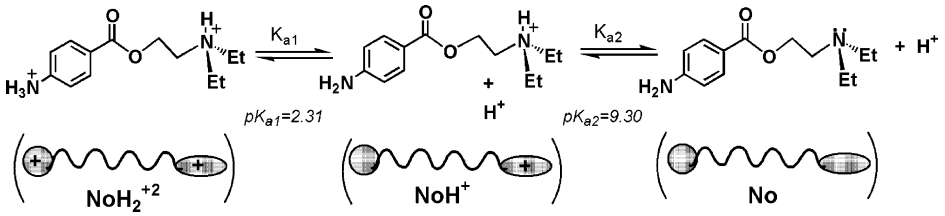
SCHEME 1. Possible Acid−Base Equilibrium of Novocaine in Aqueous Solutions



including poor bioavailability, associated to a poorly soluble drug: limited shelf life, due to the sensitivity of the drug to destructing factors, and restricted utility, when the drug is irritating to skin, membranes, tissues, and so on.[11−15]

β-Cyclodextrin and its derivates are the most interesting for drug complexation in both the solution and solid states, in which case each guest, or some part of it (commonly the less-polar part), is sequestered inside the hydrophobic CD cavity, whereas the most polar and often charged group of the guest is exposed to the bulk solvent just outside the wider opening of the cavity. A good understanding of the encapsulation equilibrium, making reference to structural information, stability, and/or reactivity of the included drug, is necessary to draw a complete picture of the CD/drug interaction.[16−19]

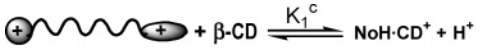
The main objective of this investigation is the analysis of the complexation by β-CD of the local anesthetic novocaine (nov) in an aqueous medium under various experimental conditions, with the aim to obtain a wide range of information about the molecular interactions that drive the complexation process. To perform the study, different methodologies have been applied, which make use of changes in spectroscopic properties of novocaine, of electrical conductance of the charged drug, and of common reactions of novocaine occurring in either aqueous acid or an alkaline medium, such as the nitrosation of the primary amine group in an acid medium or the hydrolysis of the ester in an alkaline medium. The kinetic characteristics of the two reactions in an aqueous medium have been investigated in detail.[20]

Novocaine is a tertiary amine that also contains a primary amine group bonded to a phenyl ring (Scheme 1). The pK_a values of these two basic centers are largely different (primary amine, pK_{a1} = 2.31;[20] tertiary amine, pK_{a2} = 9.30[6,21]) because no interaction effects are expected between both centers. Therefore, depending on the acidity of the solution, novocaine may exist as a neutral molecule, No, as a monocation, NoH^+, or as a dication, NoH_2^{+2}.

In a strong acid medium (pH < 2), the majority of species is the diprotonated molecule, NoH_2^{+2}, and conversely, in an alkaline medium (pH > 10), the neutral form (No) predominates; between these two extremes (2 < pH < 10), the tertiary amine group is protonated (NoH^+). With regard to the host molecule, β-CD ionizes at pH > 11, and the pK_a of a secondary OH group is 12.3;[22] consequently, in an alkaline medium, the β-CD molecules bear a negative charge (CD^-).

With the previous considerations in mind, the present investigation was planned by defining the following possible different situations.

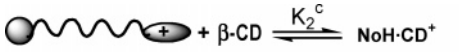
1. Strong acid medium (pH < 2). Aqueous solutions of hydrochloric acid were used. The guest is a dication, NoH_2^{+2}, and the host is a neutral molecule (eq 1). Changes in the spectra



(1)

of UV−vis absorption of fluorescence emission were used to study the complexation process. Chloride ions form inclusion complexes with β-CD; nevertheless, the stability constant is very small (K_c = 2.56 mol^{-1} dm^3)[23] and allows us to avoid its consideration at low chloride ion concentrations.[24]

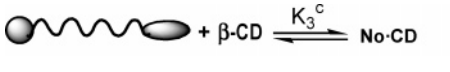
2. Mild acid medium (pH > 4). In aqueous buffered solutions of acetic acid−acetate, the guest is a cation, NoH^+, and the host is neutral (eq 2). To study the complex formation, we



(2)

carried out the kinetic study of the nitrosation reaction of the primary amine group to give diazonium ions, the electrical conductivity of the guest, and changes on the fluorescence emission spectrum of the guest. The nitrosation reaction of novocaine has been studied in water in the absence of cyclodextrin.[20] Acetic acid molecules and acetate ions are also competitors for the β-CD cavity; however, as for chloride ions, the small stability constant (K_c < 7 mol^{-1} dm^3)[24] makes it possible for us to avoid its consideration under low buffer concentrations.

3. Mild basic medium (pH ∼ 10). Both the guest and the host are neutral molecules (eq 3). The basic medium was



(3)

obtained from aqueous solutions of carbonate−bicarbonte buffer of pH 10.4. The complexation process was investigated from

(11) Nadai, T.; Ueda, H. In *Comprehensive Supramolecular Chemistry*; Szejtli, J., Osa, T., Eds.; Pergamon: New York, 1996; Vol. 3, Chapter 14.
(12) Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035.
(13) Konno, A.; Misaki, M.; Toda, J.; Wada, T.; Yasumatsu, K. *Agric. Biol. Chem.* **1986**, *46*, 2203.
(14) Nakanishi, K.; Masukawa, T.; Nadai, T.; Yoshii, K.; Okada, S.; Miyajima, K. *Biol. Pharm. Bull.* **1997**, *20*, 68.
(15) Brown, N. O.; Butler, D. L.; Chiang, P. K. *J. Pharm. Pharmacol.* **1993**, *45*, 666.
(16) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(17) Liu, Y.; Zhang, Y.-M.; Qi, A.-D.; Chen, R.-T.; Yamamoto, K.; Wada, T.; Ionue, Y. *J. Org. Chem.* **1997**, *62*, 1830.
(18) Iglesias, E. *J. Am. Chem. Soc.* **1998**, *120*, 13057. Iglesias, E.; Casado, J. *Int. Rev. Phys. Chem.* **2002**, *21*, 37.
(19) Csernak, O.; Buvari-Barcza, A.; Samu, J.; Barcza, L. *Inclusion Phenom. Macr. Chem.* **2005**, *51*, 59.
(20) Iglesias, E.; Brandariz, I.; Penedo, F. *Chem. Res. Toxicol.* **2006**, *19*, 594.
(21) Kamaya, H.; Hayes, J. J.; Ueda, I. *Anesth. Analg. (Baltimore)* **1983**, *62*, 1025. See also: CAS Database, registry number 59-46-1.
(22) Geld, R. I.; Schwarts, L. M.; Laufer, D. A. *Bioorg. Chem.* **1982**, *11*, 274.
(23) Rohrbach, R. P.; Rodríguez, L. J.; Eyring, E. M.; Wojclk, J. F. *J. Phys. Chem.* **1977**, *81*, 944.
(24) Iglesias, E. *J. Org. Chem.* **2000**, *65*, 6583.

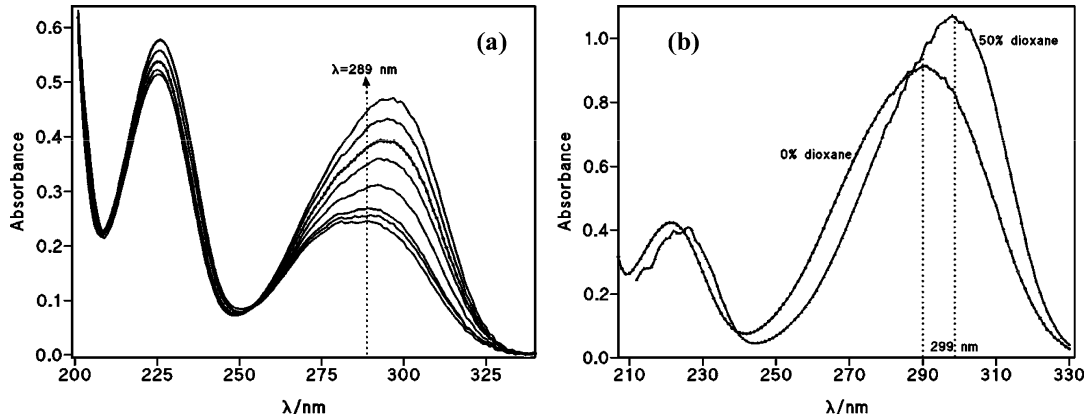


FIGURE 1. (a) UV−vis absorption spectra of novocaine (5×10^{-5} M) at $[H^+] = 0.025$ M (HCl) as a function of the β-CD concentration increase. (b) The UV−vis spectra of novocaine (5×10^{-5} M) in water and in water/dioxane (1:1 v/v) solution showing the effect of the polarity of the medium on the position of the maximum wavelength absorption.

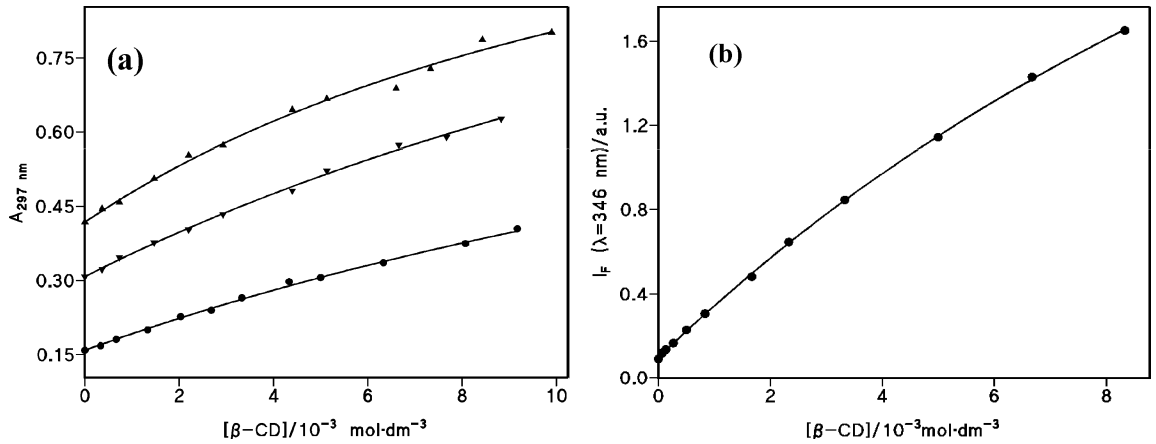
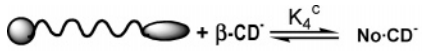


FIGURE 2. (a) Variation of the absorbance of aqueous hydrochloric acid solutions of novocaine (6.5×10^{-5} M) as a function of [β-CD] at [H^+] equal to (●) 0.050, (▼) 0.025, and (▲) 0.0167 M. (b) Variation of the fluorescence emission intensity, I_F, at 346 nm of aqueous hydrochloric acid ([H^+] = 0.060 M) solutions of novocaine (9×10^{-6} M) as a function of [β-CD].

the analysis of the changes in fluorescence emission spectra of the guest by the presence of β-CD.

4. Alkaline medium ([OH^-] > 0.1 M). Aqueous solutions of NaOH were used to get the conditions in which the guest is a neutral molecule and the host is an anion (eq 4). To follow the



(4)

complex formation, the hydrolysis reaction of the ester function has been studied.

Results

1. Strong Acid Medium. The UV−vis absorption spectrum of novocaine shows two absorption bands centered at ~220 nm ($\pi \rightarrow \pi^*$ transition) and at ~290 nm that markedly decrease after acidifying the solution and shift to shorter wavelengths (blue shifts) in high dielectric media; these all are common features of an $n \rightarrow \pi^*$ transition (from the H_2N group to the π-system of the phenyl ring), which disappears on protonation of the auxochrome H_2N group, as observed experimentally. By adding β-CD to an aqueous acid solution of novocaine, the optical intensity of this band enhances as the β-CD concentration is being increased (Figure 1a) and, at the same time, the λ_{max} shifts to higher wavelengths due to the inclusion of novocaine into the β-CD cavity, which provides a low-polarity environment for the chromophore. This effect can be clearly seen in Figure 1b which compares the position of λ_{max} in water with that in an aqueous/dioxane mixture of 50% v/v.

A. Absorbance Measurements. On the basis of the preceding observations, we studied the formation of inclusion complexes between novocaine and β-CD in a strong acid medium of HCl from the measurements of the absorbance at ~290 nm of aqueous solutions of 6.5×10^{-5} M of the drug as a function of the concentration of β-CD. The results, displayed in Figure 2a, show that the absorbance increases with the concentration of β-CD and also that the overall effect is higher at the lower [H^+]. The increase in absorbance is due to the increase in NoH^+ concentration as it is being included in the β-CD cavity. The results agree quite well considering the formation of 1:1 inclusion complexes between the host and the monocation of novocaine, which is evidenced by the saturation limits at high cyclodextrin concentration. Then, Scheme 2 could be proposed to account for the experimental facts.

Taking into account that, under the experimental conditions, $[nov]_o = [NoH_2^{+2}] + [NoH^+]$, that $A_{287} = \epsilon l[NoH^+] +$

SCHEME 2

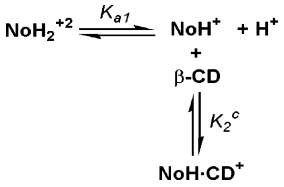


TABLE 1. Values of (ϵ/ϵ') and K_2^c Obtained in the UV−Vis Spectra Titration of Novocaine by β-Cyclodextrin Aqueous Solutions and the Input Values of A_o and α, in Accordance with Eq 7[a]

$[H^+]$/M	A_o^{exptl}	$\alpha(=K_{a1}/(K_{a1}+[H^+]))^{exptl}$	(ϵ/ϵ')	K_2^c/mol^{-1} dm^3
0.0167	0.418	0.23	0.80 ± 0.10	265 ± 55
0.025	0.308	0.16	0.87 ± 0.09	252 ± 50
0.050	0.159	0.09	0.86 ± 0.07	265 ± 9

[a] The pK_{a1} = 2.31 has been previously determined.[20]

$\epsilon' l[NoH{\cdot}CD^+]$, and the expressions of K_{a1} and K_2^c of eqs 5 and 6, one gets eq 7 which gives the variation of absorbance, A, as a function of [β-CD] at fixed $[H^+]$.

$$K_{a1} = \frac{[NoH^+][H^+]}{[NoH_2{}^{+2}]} \tag{5}$$

$$K_2{}^c = \frac{[NoH{\cdot}CD^+]}{[NoH^+][\beta\text{-CD}]} \tag{6}$$

The nonlinear correlation analysis of eq 7 to the experimental points resulted in the lines drawn in Figure 2 when the optimized values of the unknown parameters, ϵ'/ϵ and K_2^c, were those reported in Table 1, along with the input values of the controlled variables $[H^+]$, A_o, and α {$=K_{a1}/(K_{a1}+[H^+])$ was determined for pK_{a1} and $[H^+]$ was given in Table 1}.

$$A_{287} = \frac{A_o\{1 + (\epsilon'/\epsilon)K_2^c[\beta\text{-CD}]\}}{1 + \dfrac{K_{a1}}{K_{a1}+[H^+]}K_2^c[\beta\text{-CD}]} \tag{7}$$

B. Fluorescence Measurements. The stability equilibrium constant of complex formation was also determined from fluorescence measurements. Figure 2b shows the variation of the fluorescence emission intensities, I_F, read at 346 nm as a function of [β-CD] and keeping constant the novocaine (9×10^{-6} M) and the H^+ (=0.060 M, HCl) concentrations.

The principal attraction of the luminescence method is the extremely high sensitivity of this technique. On the fluorescence time scales, cyclodextrins and most of the molecular self-assembled aggregates can be considered as rigid host systems carrying the solubilized probe molecules or frequently the fluorophore. Novocaine behaves as a freely moving non-covalently bound probe for the β-CD cavity, thus providing information on the hydrophobic cavity as well as on the structural details on guest conformation. The existence of a single broad fluorescence band in nonpolar solvents centered at 350 nm is due to a twisted internal charge-transfer state (TICT). The polar nature of this TICT state causes the wavelength of the emission maximum to depend on the polarity of the medium and to shift to a shorter wavelength with respect to that in the bulk water environment as the polarity of the medium decreases.[25,26] These features were experimentally observed for novocaine as shown in Figure 3, which displays the effect of increasing the dioxane percentage in the aqueous solutions. Such characteristics observed in the fluorescence emission of aromatic amines make these compounds good candidates for estimating the polarity of microenvironments such as the cyclodextrin cavity.[27,28]

Therefore, taking into account that the emission is due to a TICT state, the quantitative effect of β-CD on the fluorescence spectrum can be explained by considering eq 8, where $I_F{}^w$ represents the fluorescence intensity of the drug molecules in the bulk water medium and $I_F{}^c$ represents that of the complexed drug.

$$I_F = I_F{}^w[NoH^+] + I_F{}^c[NoH{\cdot}CD^+] \tag{8}$$

On the other hand, the stoichiometric novocaine concentration is

$$[nov]_o = [NoH_2{}^{+2}] + [NoH^+] + [NoH{\cdot}CD^+] \tag{9}$$

By combining eqs 8 and 9, along with the definition of the equilibrium constants K_{a1} and K_2^c of Scheme 2, we achieve eq 10 which relates the variation of I_F with the host concentration. The nonlinear regression analysis of this equation to the experimental points shown in Figure 2b yields the following results: $I_F{}^w = 0.115 \pm 0.010$ for the fluorescence intensity in the absence of cyclodextrin; $I_F{}^c = 0.83 \pm 0.01$; $K_{a1}/(K_{a1}+[H^+]) = 0.089$, and $K_2^c = (278 \pm 30)$ dm^3 mol^{-1} (cc = 0.999$_4$) (see Table 2, first row). For comparison purposes, it can be noted that the binding constant in the strong acid medium, K_1^c, can be obtained as the product $K_{a1} \times K_2^c$ (=1.4 mol^{-1} dm^3).

$$I_F = \frac{I_F{}^w + I_F{}^c K_2^c[\beta\text{-CD}]}{1 + K_2{}^c \dfrac{K_{a1}}{K_{a1}+[H^+]}[\beta\text{-CD}]} \tag{10}$$

2. Mild Acid Medium. Aqueous buffered solutions of acetic acid−acetate were used to obtain the mild acid medium (pH ∼ 4.5), except for in those experiments in which the electrical conductance technique was used to measure the complexation process. In performing electrical conductance measurements, we used no added acid, and the novocaine hydrochloride salt was the only additive to the aqueous cyclodextrin solutions. The inclusion complex formation was recorded by fluorescence, reaction kinetics of the nitrosation of the H_2N group, and electrical conductance measurements.

A. Fluorescence Measurements. The emission of fluorescence was performed in aqueous solutions of acetic acid−acetate buffer (0.017 M) of pH = 4.35. The excitation of samples ([nov] = 9 μM) was done at 300 nm, and the fluorescence emission intensity was collected at 346 nm for varying [β-CD]. The experimental results, depicted in Figure 4a, show a strong increase of the fluorescence emission intensities with the cyclodextrin concentration leading to saturation levels, which indicates the formation of 1:1 inclusion complexes. The charge-transfer state undergoes radiationless decay via molecular relaxation, which can be slowed by increasing the molecular

(25) Retting, W. *Angew. Chem., Int. Ed. Engl.* **1983**, *15*, 971.
(26) Kalyanasundaram, K. In *Photochemistry in Organized & Constrained Media*; Ramamurthy, V., Ed.; VCH Publishers: U.K., 1991; Chapter 2.
(27) Nag, A.; Bhattacharyya, K. *Chem. Phys. Lett.* **1988**, *151*, 474.
(28) Harada, A.; Furue, M.; Nozakura, S. *Macromolecules* **1977**, *10*, 676.

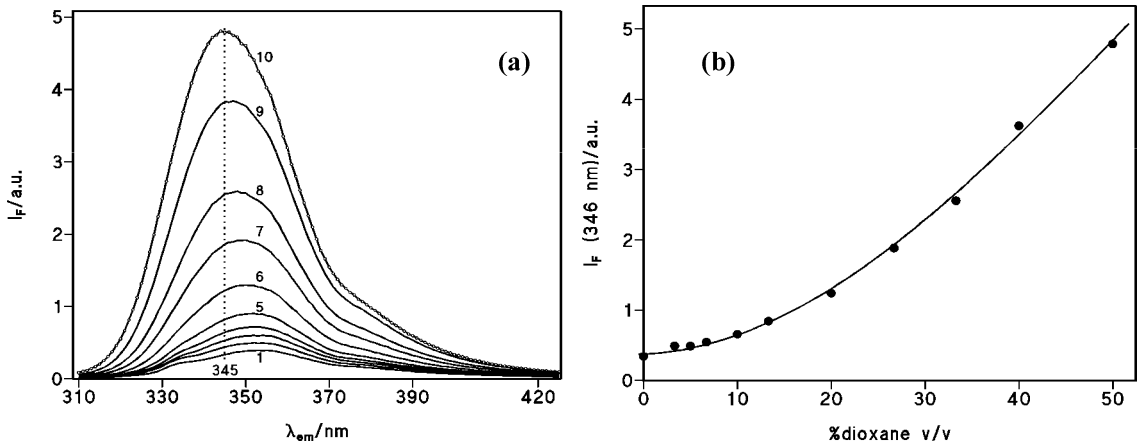


FIGURE 3. (a) Fluorescence emission spectra of novocaine as a function of the percentage of dioxane. (b) Plot of the fluorescence emission intensities read at 346 nm as a function of % v/v dioxane.

TABLE 2. Stability Equilibrium Constants of the Inclusion Complexes Formed between Novocaine and β-CD (K_n^c, $n = 2$ (eq 2) and 3 (eq 3)) under Different Experimental Conditions Obtained from Fluorescence Emission Measurements

aqueous medium	I_F^w/au^{exptl}	I_F^c/au^{optimized}	K_n^c/mol^{-1} dm^3	$\lambda_{ex}/\lambda_{em}$ (nm)
HCl, 0.060 M	0.115	0.83 ± 0.01	278 ± 30 ($n = 2$)	300/346
acetic acid/acetate (pH 4.33)	0.243	3.26 ± 0.06	282 ± 12 ($n = 2$)	300/346
carbonate/bicarbonate (pH 10.41)	0.437	4.75 ± 0.05	1536 ± 67 ($n = 3$)	300/346
50% v/v dioxane/water		4.789		300/346

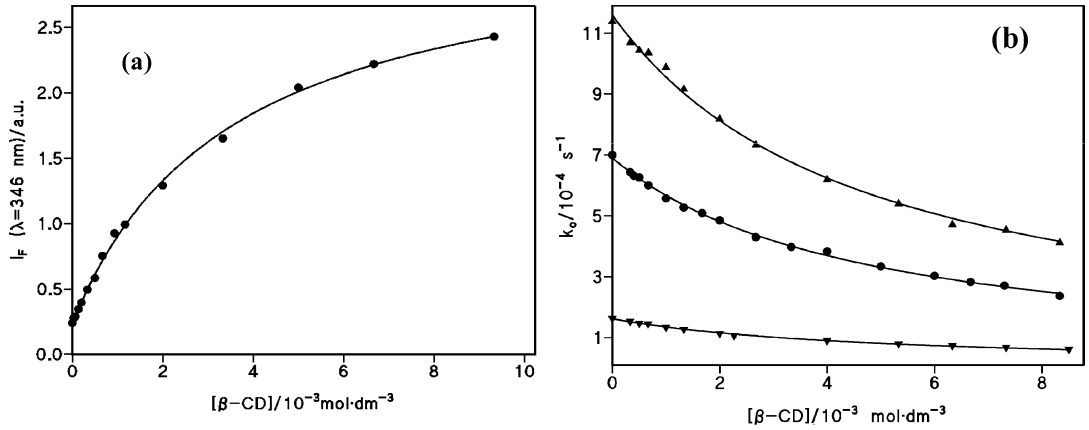


FIGURE 4. (a) Variation of the fluorescence emission intensities, I_F, read at 346 nm, as a function of β-CD concentration at pH = 4.33 in acetic acid−acetate buffer (0.017 M). (b) Influence of β-CD concentration on the pseudo-first-order rate constant obtained in the nitrosation of novocaine (6.5×10^{-5} M) at [nitrite] = 3.3×10^{-3} M and a pH of (▲) 4.30, [buffer] = 0.040 M; (●) 4.50, [buffer] = 0.040 M, and (▼) 4.90 [buffer] = 0.052 M.

rigidity of the fluorophore when, e.g., it is included in the CD cavity; this fact, along with the lower polarity of the CD cavity sense by the fluorophore, increases strongly the fluorescence emission.

Under these conditions, only the tertiary amine group is protonated ($pK_{a2} = 9.30$);[6,21] therefore, the total novocaine concentration is the sum of the free (NoH^+) and complexed ($NoH{\cdot}CD^+$) monoprotonated forms of novocaine; that is, the $[H^+]$ term in eq 10 is negligible against K_{a1}. Fitting the resulting expression of eq 11 to the experimental points shown in Figure 4a yields the following values: $I_F^w = 0.238$; $I_F^c = (3.26 \pm 0.06)$; $K_2^c = (282 \pm 12)$ dm^3 mol^{-1} (cc = 0.999$_7$) (collected in Table 2, second row, for comparative purposes). Note the good concordance of K_2^c values with those obtained in the previous section from the experiments performed in a strong acid medium.

$$I_F = \frac{I_F^w + I_F^c K_2^c[\beta\text{-CD}]}{1 + K_2^c[\beta\text{-CD}]} \qquad (11)$$

B. Reaction Kinetics. The influence of β-CD on the nitrosation reaction of novocaine has been studied in aqueous solutions of acetic acid−acetate buffer of pH 4.33, 4.50, and 4.90 and a fixed [nitrite] of 3.3 mM and a [nov] of 0.065 mM. Figure 4b shows representative results. In every case, the rate of the reaction is diminished by the presence of cyclodextrin leading to saturation levels due to the formation of 1:1

SCHEME 3

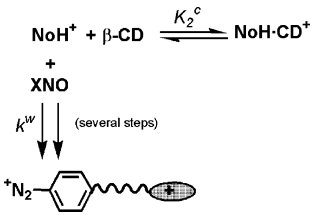


TABLE 3. Experimental Conditions and Stability Constants of the Inclusion Complexes Formed between the Novocaine Cation and β-CD Obtained in the Kinetic Study of the Nitrosation ([nitrite] = 3.3 mM) of Novocaine in Aqueous Acid Medium at 25 °C

[buffer]/M	pH	$k_o^w/10^{-4}$ s^{-1}	K_2^c/mol^{-1} dm^3
0.042	4.33	11.6 ± 0.1	214 ± 7
0.040	4.50	7.02 ± 0.05	227 ± 5.5
0.052	4.90	1.62 ± 0.01	220 ± 6
average			220

nonreactive inclusion complexes, in which the primary amine group attached to the phenyl ring must be protected from the attack of the nitrosating agents, NO^+ and N_2O_3. In every case, the reciprocal plot of k_o against [β-CD] draws good straight lines (graphs not shown), which is indicative of a rapid establishment of the equilibrium between free and complexed novocaine cations with cyclodextrin and the low nitrosation path through only the substrate existing in the bulk water phase (Scheme 3). Then, starting from this scheme, one arrives to eq 12 to express the variation of the pseudo-first-order rate constant with the [β-CD].

$$k_o = \frac{k_o^w}{1 + K_2^c[\beta\text{-CD}]} \quad (12)$$

The solid lines in Figure 4b were obtained in the fit of eq 12 to the experimental points with the results of k_o^w and K_2^c reported in Table 3. As can be see, K_2^c values are slightly lower than those measured from fluorescence experiments. The comparison of either Figures 2a and 2b or Figures 4a and 4b clearly indicates the higher sensitivity of fluorescence over kinetic results, which could explain the small deviation in K_2^c values. However, smaller deviations could be found if the complexed drug was reactive toward N_2O_3; then, the addition in the numerator of eq 12 of the corresponding new term that accounts for the reaction through the complex increases slightly the values of K_2^c, but the rate constant via the complexed drug is not significant (deviations are comparable to the optimized value).

Even though the effect of cyclodextrin in the electrical conductance is small, vide infra, the results of K_2^c agree perfectly with those of fluorescence, and this is a expected result if one considers the absence of interferences in using the former technique.

C. Conductivity Measurements. The electrical conductance of aqueous solutions of novocaine hydrochloride (NoH^+Cl^-) salt (0.98 mM) was measured to monitor the formation of inclusion complexes. The aqueous solutions of the drug were prepared by dissolving the desired amount of novocaine hydrochloride salt in the appropriate volume of water. Figure 5a displays the values of the specific conductivity, κ, for the binary drug/water solutions for different drug concentrations at various temperatures ranging from 13 to 25 °C. The conductivity of the solution can be expressed as a function of the ionic molar conductivities, λ_i, and the concentration of the charged species, according to eq 13, with κ_o being the conductivity of water (at pH ∼ 7 the contribution of the electrical conductivity of OH^- and H^+ was neglected in comparison with that of NoH^+ and Cl^- at $\sim 10^{-3}$ M). A linear square fit of the experimental points yields the values of κ_o and $\Sigma\lambda_i$ ($=\lambda_{NoH} + \lambda_{Cl}$) reported in Table 4.

$$\kappa = \kappa_o + \lambda_{NoH^+}[NoH^+] + \lambda_{Cl^-}[Cl^-] = \kappa_o + (\lambda_{NoH^+} + \lambda_{Cl^-})[nov]_o \quad (13)$$

Figure 5b shows the plot of specific conductivity or aqueous solutions of NoHCl at a constant concentration of about 1 mM as a function of β-cyclodextrin concentration. The decrease in κ when β-CD is added evidences the inclusion of NoH^+ in the neutral host cavity because the mobility of the associated cation is expected to be less than that of the free cation. Then, eq 14 applies to report all contributions to the conductivity by the different charged species.

$$\kappa = \kappa_o + \lambda_{NoH^+}[NoH^+] + \lambda_{Cl^-}[Cl^-] + \lambda_{NoH\cdot CD^+}[NoH\cdot CD^+] \quad (14)$$

Taking into account the expression of K_2^c (Scheme 3) and the total novocaine concentration, $[nov]_o$, eq 14 may be rearranged into eq 15, where $\kappa^w = \kappa_o + \Sigma\lambda_i[nov]_o$ and $\kappa^c = (\lambda_{Cl} + \lambda_{NoH\cdot CD})[nov]_o$. Nonlinear regression analysis of this equation to the experimental data gives the values of the adjusted parameters reported in Table 4.

$$\kappa = \frac{\kappa^w + \kappa^c K_2^c[\beta\text{-CD}]}{1 + K_2^c[\beta\text{-CD}]} \quad (15)$$

Noteworthy is, first, the good concordance between K_2^c values obtained here and by fluorescence measurements (see Table 2, second row), second, that the optimized κ^w values agree quite well with that measured at the same drug concentration (∼1 mM) as that used in the presence of cyclodextrin, and, finally, that κ^c takes lower values than κ^w because the mobility of the complexed novocaine cation, $NoH\cdot CD^+$, is lower than that of the free NoH^+ cation.

3. Mild Basic Medium. The inclusion complex formation was also investigated at pH 10.41 in aqueous solutions of carbonate−bicarbonate buffer (3.3 mM). Under these conditions, either the guest (novocaine, $pK_{a2} = 9.31$) or the host (β-CD, $pK_a = 12.3$) exists in the majority as neutral molecules. We measured the steady-state emission of the fluorescence of novocaine ([nov] = 9μM) at 346 nm (excitation at 300 nm) as a function of β-CD concentration. Figure 6a shows the corresponding titration spectra, where the strong intensity increase and the blue shift in λ_{max} as the guest occupies the host cavity, a less-polar microenvironment than the bulk water phase, can be seen. Figure 6b shows the plot of emission intensities against the [β-CD].

Starting with eq 3 and taking into account that the fluorescence emission is due to both free and complexed neutral novocaine ($I_F = I_F^w[No] + I_F^c[No\cdot CD]$), it is easy to arrive at eq 16, where K_3^c is the stability equilibrium constant between neutral novocaine and neutral cyclodextrin. Fitting of this equation to the experimental points results in the solid line in Figure 6b with the results $I_F^w = 0.43 \pm 0.02$ (the experimental

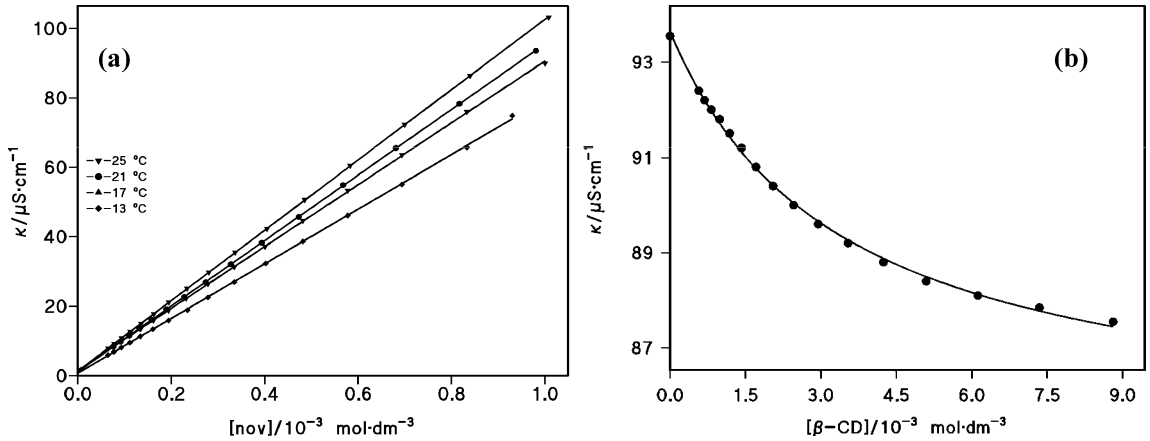


FIGURE 5. (a) Plot of the specific electrical conductance of aqueous solutions of novocaine hydrochloride as a function of novocaine concentration and temperature. (b) Decrease of the electrical conductance of aqueous solutions of novocaine hydrochloride (0.98 mM) at 21 °C on increasing the β-CD concentration.

TABLE 4. Parameters Obtained in the Electrical Conductance Experiments of Aqueous Solutions of 0.98 mM Novocaine Performed in the Absence and Presence of β-Cyclodextrin

	$\kappa = \kappa_o + \sum \lambda_i[\text{nov}]_o$		$\kappa = \{\kappa^w + \kappa^c K_2^c[\beta\text{-CD}]\}/\{1 + K_2^c[\beta\text{-CD}]\}$		
temp/°C	κ_o/μS cm^{-1}	$\sum \lambda_i/\Omega^{-1}$ cm^2 mol^{-1}	κ^w/μS cm^{-1}	κ^c/μS cm^{-1}	K_2^c/mol^{-1} dm^3
	[β-CD] = 0; [nov] = variable		[β-CD] = variable; [nov] = 1×10^{-3} M		
13	0.76 ± 0.05	78.7 ± 0.8	76.70 ± 0.06	70.27 ± 0.02	295 ± 9
17	1.50 ± 0.10	89.1 ± 0.2	86.60 ± 0.05	77.68 ± 0.12	294 ± 11
21	1.12 ± 0.03	94.38 ± 0.08	93.64 ± 0.06	85.32 ± 0.34	293 ± 14
25	1.27 ± 0.04	101.35 ± 0.08	102.15 ± 0.06	93.60 ± 0.08	282 ± 8

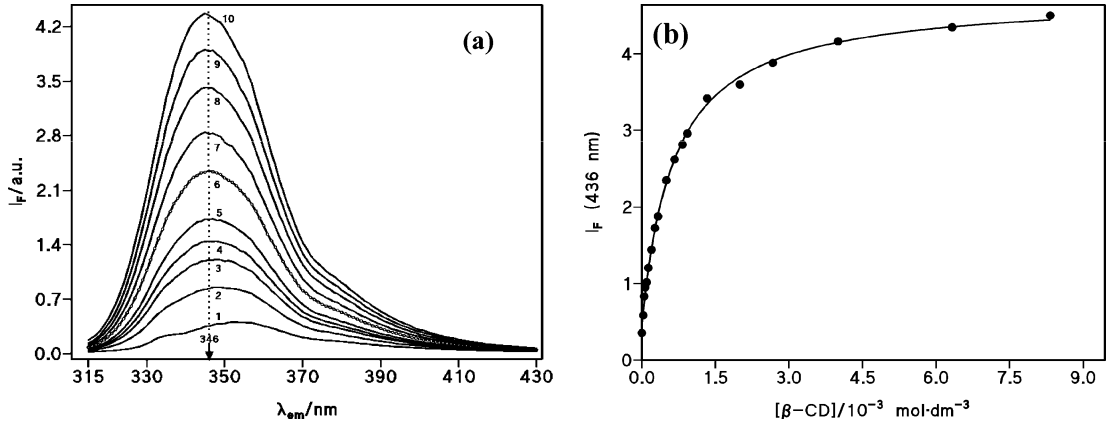


FIGURE 6. (a) Fluorescence emission spectra of novocaine (9×10^{-6} M) in aqueous buffered solutions of carbonate−bicarbonate buffer (3.3 mM) of pH = 10.41 as a function of [β-CD] equal to: (1) 0.0; (2) 0.05; (3) 0.133; (4) 0.20; (5) 0.27; (6) 0.5; (7) 0.83; (8) 1.33; (9) 2.67; and (10) 6.33 mM. (b) Plot of fluorescence intensities read at 346 nm as a function of the concentration of β-CD; the curved line is the fit of eq 16 to the experimental points. For parameters, see Table 2, third row.

$I_F^w = 0.426$), $I_F^c = 4.75 \pm 0.05$, and $K_3^c = 1450 \pm 70$ mol^{-1} dm^3 (cc = 0.999_8) (see row 3 of Table 2).

$$I_F = \frac{I_F^w + I_F^c K_3^c[\beta\text{-CD}]}{1 + K_3^c[\beta\text{-CD}]} \qquad (16)$$

It is important to remark, first, the high value of K_3^c, the stability inclusion complex formation between the neutral guest and the neutral host, which is approximately 5-fold K_2^c that measures the stability of the inclusion complex between the novocaine cation, NoH^+, and neutral β-CD and, second, the extremely high sensitivity of the fluorescence emission of novocaine to the microenvironment polarity.

4. Alkaline Medium. In aqueous solutions of NaOH, the ester function of novocaine hydrolyzes at moderate reaction rates to give *p*-amino-benzoate and diethylaminoethanol. The rate of the reaction in the absence of OH^- or even in aqueous buffered solutions, e.g., of carbonate−bicarbonate of pH 10.4, is negligible but increases proportionally with [OH^-] (Table 5).[20] Therefore, we investigated the formation of inclusion complexes in an alkaline medium of [OH^-] > 0.1 M by recording by UV−vis spectroscopy the rates of the ester hydrolysis of novocaine as a function of β-CD concentration.

Under pseudo-first-order conditions ([No] = 6.5×10^{-5} M and [OH^-] > 0.1) we measured the decrease in absorbance at 285 nm. The absorbance−time data fit perfectly the integrated

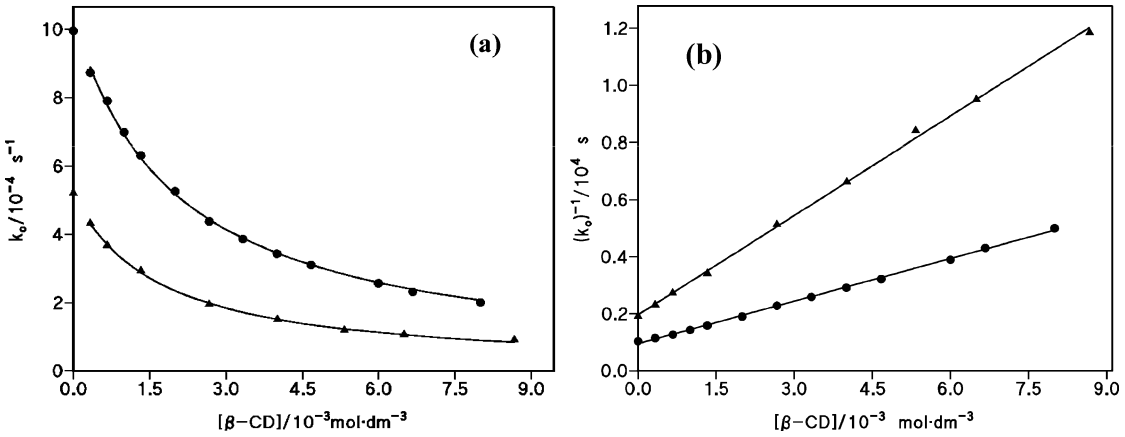


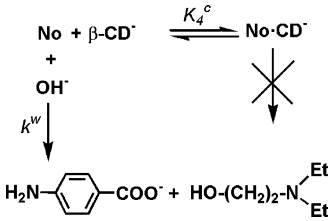
FIGURE 7. (a) Influence of β-CD concentration on the pseudo-first-order rate constants corresponding to the alkaline hydrolysis of novocaine measured at [OH$^-$] equal to (●) 0.27 and (▲) 0.13 M. (b) Reciprocal plot of k_o against [β-CD] according to eq 17; for parameters, see Table 5.

TABLE 5. Rate Constants and Stability Constants of the Inclusion Complexes Formed between Novocaine and β-CD in an Aqueous Alkaline Medium Obtained from the Kinetic Study of Ester Hydrolysis of Novocaine (6.5×10^{-5} M) at Variable Concentrations of Cyclodextrin[a]

[OH$^-$]/M	$k_o^w/10^{-4}$ s^{-1}	k^w/mol^{-1} dm^3 s^{-1}	K_4^c/mol^{-1} dm^3
0.13	5.08	3.91×10^{-3}	772 ± 24
0.20	7.87	3.93×10^{-3}	708 ± 14
0.25	9.66	3.86×10^{-3}	675 ± 29
0.30	11.54	3.85×10^{-3}	664 ± 9

[a] See Scheme 4 and eq 17.

SCHEME 4



first-order equation, from which the pseudo-first-order rate constant, k_o, was obtained. At fixed novocaine and OH$^-$ concentrations, we measured the effect of [β-CD] on reaction rates. Figure 7a shows typical results of the variation of k_o with the host concentration: k_o decreases as a consequence of the formation of the inclusion complexes because the encapsulated substrate is protected from the attack of OH$^-$ until a saturation limit that corresponds to an all-substrate inclusion. The fact that the reciprocal plot of k_o varies linearly with [β-CD], shown in Figure 7b, means the complexes of 1:1 stoichiometry are nonreactive.

Then, Scheme 4 may be proposed to explain the experimental observations, from which eq 17 is derived by taking into account that rate $= k^w$[OH$^-$][No]$_w$ and that [No]$_o$ = [No]$_w$ + [No·CD$^-$].

$$k_o = \frac{k_o^w}{1 + K_4^c[\beta\text{-CD}^-]} \qquad (17)$$

The nonlinear regression analysis of the experimental points according to eq 17 yields the values of k_o^w ($=k^w$[OH$^-$]), listed in Table 5; from which it can be determined an average value for $k^w = 3.9 \times 10^{-3}$ mol^{-1} dm^3 s^{-1}. On the other hand, K_4^c decreases smoothly on increasing the [OH$^-$], probably as a consequence of the fact that the real measured constant is a mixed value of K_4^c and K_3^c because at [OH$^-$] = 0.13 M only 87% of the total β-CD concentration is ionized and the association to neutral β-CD molecules (K_3^c) is much higher than that to ionized molecules: the extrapolated value of K_4^c to the 100% ionized β-CD is 600 mol^{-1} dm^3 (Figure 8), i.e., lower than half of the K_3^c value.

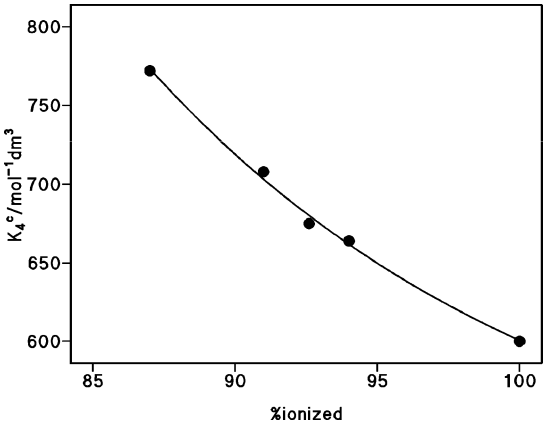


FIGURE 8. Extrapolated K_4^c at 100% ionized β-cyclodextrin molecules.

Discussion

In this study, we have made use of three experimental techniques for measuring four different parameters that include the optical absorption, fluorescence emission, electrical conductivity, and reaction rate constants to investigate molecular recognition by β-cyclodextrin. The required guest concentration was in every case lower than that of the host and varied from 9 μM in fluorescence emission to 65 μM in absorbance measurements or to 980 μM in performing electrical conductance. Despite the wide gamut of both experimental conditions and techniques, the measured binding constants for the same process are in very good agreement regardless of the property of the guest being registered, even though the emission of fluorescence shows undoubtedly the highest sensibility and versatility.

On the other hand, the results obtained from measurements in a strong acid medium (pH < 2) show that full protonated novocaine molecules (NoH_2^{+2}) do not form inclusion complexes

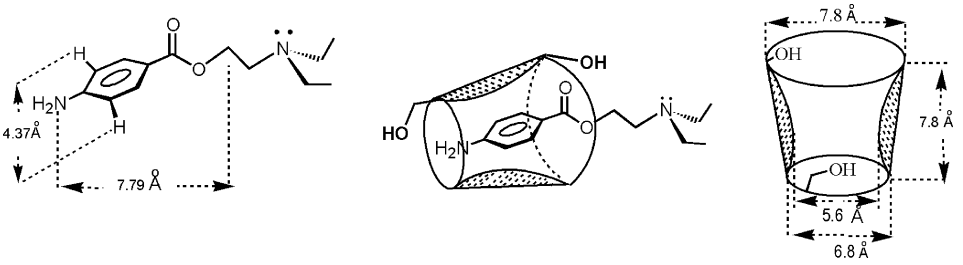


FIGURE 9. Postulated structure for the complex formed between neutral (No) or monoprotonated (NoH^+) novocaine and β-CD.

with neutral β-CD; however, the presence of cyclodextrin increases the acidity of the NoH_2^{+2} species ($K_1^c = K_{a1} \times K_2^c$) as a consequence of the complexation of novocaine molecules that are protonated only on the tertiary amine group. The existence of this complex, NoH^+·CD, is clearly recognized from electrical conductance experiments. However, neutral novocaine molecules show a higher affinity for the host cavity than any other substrate form. In addition, the influence of β-CD on the nitrosation reaction of the H_2N group, to give the diazonium ion, indicates a good protection of this group in the encapsulated drug from the attack of the nitrosating agents (NO^+, N_2O_3) residing in the bulk water phase. In the same manner, the ester group is also fully protected from the nucleophilic attack of OH^- because the included drug is unreactive in alkaline hydrolysis. These results together indicate that novocaine penetrates the β-CD cavity by the wider ring with the H_2N group (and not the H_3N^+ group) end first, allowing the aromatic ring of the drug and the ester group inside the cavity with 1:1 stoichiometry. This picture agrees also with the strong increase of fluorescence emission from the encapsulated guest, in which state the molecular rigidity of the fluorophore reduces the radiationless decay via molecular relaxation of the TICT state. Molecular mechanics calculations[29] and experimental results led to the structure of the complex novocaine−cyclodextrin sketched in Figure 9.

Given that no hydrogen bonds between the host and the guest are evident from the postulated complex structure because both the H_2N group and the COO group are far (more than 4 Å) from the CD hydroxyl groups, only van der Waals interactions are responsible for the host−guest interactions because the ion−dipole interactions that should be more important between NoH_2^{+2} and CD, higher charge on the cation, do not contribute to the inclusion complexation. Thus, NoH_2^{+2} does not form inclusion complexes, and in the complex NoH^+·CD, the charged moiety of the guest remains outside the cavity immersed in the bulk water solvent. The hydrophobicity of a substrate was considered to be the result of the enhanced structure of the water molecules in the near vicinity of a nonpolar solute. With this in mind, the driving forces in the inclusion complexation of novocaine to β-CD must be van der Waals interactions and hydrophobicity.

In a strong acid medium, the hydration of novocaine dication strong ion−dipole (water molecules) interactions overwhelms host−guest van der Waals interactions and hydrophobicity effects, resulting in the noninclusion of NoH_2^{+2} ($K_1^c \sim 0$); by contrast, in mild basic media, the hydrophobicity of neutral novocaine molecules and host−guest van der Waals interactions operates in the same direction as the formation of inclusion complexes, and we measure the higher value of the stability

(29) *HyperChem*, version 7; HyperCube software: 2002.

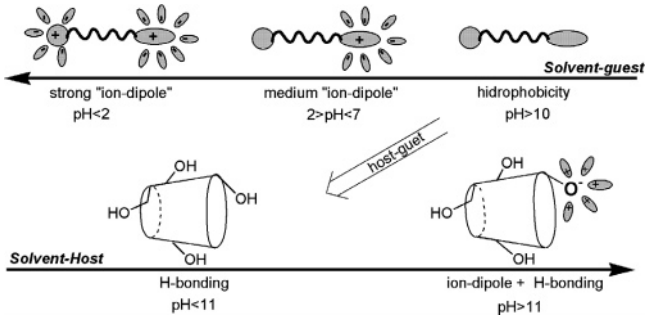


FIGURE 10. Schematic representation of molecular interactions among solvent−guest−host molecules.

inclusion constant ($K_3^c = 1500\ mol^{-1}\ dm^3$), which is more than 5-fold the stability constant measured in a mild acid medium. Like water molecules, β-CDs are also dipoles, but the number of ion−dipole interactions is decisive in the neat driving force of inclusion, and the balance drops on the side of water.

Under the conditions of ionized β-CD, the hydration of secondary alkoxide groups (O^-), ion−dipole interactions, on the main entrance of the host cavity and the release of these highly structured solvent molecules previously to the guest inclusion oppose guest hydrophobicity. Figure 10 tries to illustrate the variation of all driving forces and the region where host−guest van der Waals interactions and guest hydrophobicity operate in the same direction.

Conclusions

Formation of cyclodextrin inclusion complexes is known to involve mainly nonspecific, weak interactions, and no covalent binding, that are of paramount importance in supramolecular chemistry and in biological chemistry. The results obtained in this study evidence that van der Waals interactions and hydrophobic interactions constitute the major driving forces for cyclodextrin complexation provided that the size and the conformation of the guest are adequate to the host cavity. In the last sense, electrostatic interaction and H-bonding can significantly affect the conformation of a particular inclusion complex. On the other hand, the TICT emission of novocaine makes this compound a good choice in studies of microenvironment polarity, e.g., in the cavity interior of cyclodextrins.

Experimental Section

Materials. Novocaine hydrochloride (NoHCl) and β-cyclodextrin (β-CD) were of the highest available purity (>99%) and were used without further purification. Hydrochloric acid, acetic acid, sodium acetate, and sodium carbonate or hydrogen carbonate, sodium nitrite, and sodium hydroxide were obtained in maximum purity. The concentrations of aqueous solutions of HCl and NaOH were

determined by titration against standards, whereas the acidity of aqueous buffered solutions of acetic acid/acetate or carbonate/bicarbonate was obtained from pH measurements. All the solutions were freshly prepared with double distilled water over potassium permanganate. The conductivity of pure water was lower than 3 μS/cm (1 S = 1 Ω^{-1}).

UV−vis Measurements. The UV−vis spectra and kinetic experiments were recorded with a double beam UV−vis spectrophotometer fitted with thermostated cell holders at 25 °C following the decrease in absorbance at 285 nm. The concentration of novocaine was about 6.5×10^{-5} M. Data acquisition and analysis of both UV−vis spectra and kinetics were performed with software supported by the manufacturer, and data were converted to ASCII format for their analysis with common packet programs. The kinetic measurements of absorbance vs time always fit the first-order integrated rate equation quite satisfactorily ($r > 0.999$); in what follows, k_o denotes the pseudo-first-order rate constant.

Fluorescence Measurements. Steady-state fluorescence spectra were recorded on a spectrofluorometer at 25 °C. Data acquisition and analysis of fluorescence spectra were performed with the Fluorescence Data Manager Software supported by the manufacturer. Excitation and emission slits were fixed at 4 and 2 nm, respectively. The excitation wavelength was set at 300 nm, and the emission intensity of fluorescence was recorded at 346 nm. In all measurements, the emission spectra, collected from 315 to 400 nm, were obtained from solutions with a constant novocaine concentration of ~9 μM, chosen because the absorbance of the sample at 300 nm (the excitation wavelength) was lower than 0.2 absorbance units, and a variable β-CD concentration ranging from 0 to ~0.01 M.

Conductance Measurements. Specific conductivities were measured in a microprocessor conductivimeter provided with a four-pole measuring cell (the measured cell factor was equal to 1.10 cm^{-1}) and a temperature sensor, using solutions prepared with doubly distilled water and following the extraction−dilution method.[30] The solutions were thermostated in the conductivity cell equipped with a magnetic stirring device, and the temperature was held constant within ±0.01 °C. The conductivity of the aqueous solutions was measured as a function of the drug concentration for the binary drug/water system and as a function of cyclodextrin concentration, keeping constant the concentration of the drug (~1 mM) for the ternary cyclodextrin/drug/water system. In the latter case, the β-CD concentration was much higher than that of the drug, No, through the entire range to ensure a proper binding constant determination.

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(30) Domínguez, A.; Fernández, A.; González, N.; Iglesias, E.; Montenegro, L. *J. Chem. Educ.* **1997**, *74*, 1227.