

Cyclodextrin effects on physical–chemical properties of novocaine

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Abstract The UV-vis absorption and the fluorescence emission spectra of novocaine were analysed in aqueous cyclodextrin (CD) solutions. Either the absorbance read at λ_{\max} 290 nm or the fluorescence emission intensity at λ_{ems} 346 nm increase in the presence of both α - and β -CD due to the formation of 1:1 inclusion complexes. The lower polarity of the CD-cavity sensed by the included drug enhances the emitted fluorescence; in fact, the same effect was observed in aqueous mixtures of acetonitrile, dioxane, or dimethylsulfoxide. The inclusion complex formation between the monocation of novocaine and CDs diminishes the electrical conductance of aqueous solutions of novocaine hydrochloride (NoHCl). Both the nitrosation reaction in aqueous acid medium and the ester hydrolysis in alkaline medium are retarded in the presence of CDs. The strongest effect was observed with β -CD as a consequence of the higher stability inclusion complex.

Keywords Cyclodextrins · Electrical conductance · Ester hydrolysis · Fluorescence emission · Inclusion complexes · Nitrosation · Novocaine

Introduction

The possibility of inclusion complex formation with a selected cyclodextrin (CD) depends mostly on the

properties of the guest. Its polarity and geometry are crucial factors in determining the extent of the complex formation and the kind of guest molecules which can penetrate into the CD cavity.

Complexation of a guest often results in a small shift of the UV absorption maximum and also in the molar absorption coefficient. The increase in the optical density is frequently observed in the selective complexation of a given form of the guest that, e.g., may be an acid or a base. UV-vis spectroscopy is routinely used for determination of the guest content. The fluorescence emitted by the included guest in the CD-complex increases markedly in most cases, mainly due to the local micro-environment provided by the apolar CD-cavity. The electrical conductance of the binding ionic guest by CDs in water decreases significantly, and the molecular conductance values for each 1:1 inclusion complex are also lower than the corresponding of the free guest. The selectivity exercised by CDs over chemical reactions is essentially governed by the selective blocking of some active sites of an included guest molecule against attack by other reactants. Cyclodextrins can protect an included guest against reactants, accommodating part of a guest molecule sensitive. By contrast, if CD is dissolved in a moderately alkaline aqueous solution where some secondary hydroxyl groups are deprotonated ($\text{p}K_{\text{a}} \sim 12$), the ester function of active ester substrates is susceptible to hydrolytic cleavage by the strong nucleophile of the alkoxide anion residing at the secondary hydroxyl rim of CD. [1, 2]

We show in this report that working under different experimental conditions and with the same substrate, the local anaesthetic novocaine (*nov*), the presence of CDs modifies both the UV-vis absorption and the

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fluorescence emission spectra of nov, the electrical conductance, and the rate of the reaction corresponding to both the nitrosation of H_2N -group in acid medium and the ester hydrolysis in alkaline medium. The effect of the presence of β -CD is higher than that of α -CD.

Experimental

Novocaine hydrochloride (NoHCl), and CDs were of the highest available purity ($> 99\%$) and used without further purification. The concentration of aqueous solutions of HCl and NaOH were determined by titration against standards, whereas the acidity of aqueous buffered was obtained from pH measurements. All the solutions were freshly prepared with double distilled water. The conductivity of pure water was lower than $3 \mu\text{S/cm}$ ($1 \text{ S} = 1 \Omega^{-1}$). The UV-vis spectra and kinetic experiments were recorded with a double beam UV-vis spectrophotometer fitted with thermostated cell holders at 25°C and following the decrease in absorbance at 285 nm . The concentration of nov was about $6.5 \times 10^{-5} \text{ M}$. Kinetic measurements of absorbance versus time fit the first-order integrated rate-equation ($r > 0.999$) and k_o denotes the pseudo-first order rate constant. Steady-state fluorescence spectra of $\sim 10 \mu\text{M}$ nov aqueous solutions were recorded at 25°C . Excitation and emission slits were fixed at 4 and 2 nm , respectively; the excitation wavelength was set at 300 nm and the emission intensity was read at 346 nm . Specific conductivities were measured in a conductivimeter provided with a four-pole measuring cell (the cell-factor equal to 1.10 cm^{-1}) and temperature sensor, using solutions prepared with doubly distilled water and following the extraction–dilution method.[3] The solutions were thermostated within $\pm 0.01^\circ\text{C}$ in the conductivity cell equipped with a magnetic stirring device.

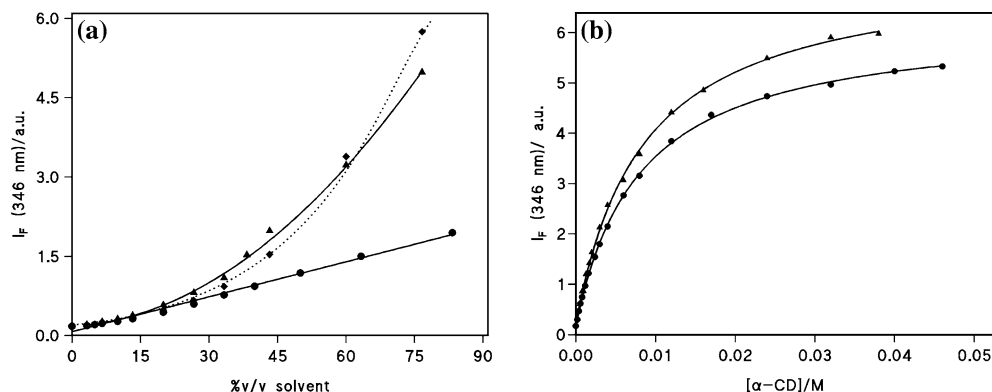
Results and discussion

The novocaine molecule possesses both a primary amine ($\text{p}K_a = 2.31$) and a tertiary amine ($\text{p}K_a = 9.30$) groups. Then, depending on the acidity of the medium, nov may exist as a neutral molecule (No , at $\text{pH} > 10$), as a dication (NoH_2^{2+} , at approximately $\text{pH} < 2$), or as a monocation (NoH^+ , in between those pH interval).

The UV-vis absorption spectrum of $6.5 \times 10^{-5} \text{ M}$ aqueous solutions of nov showed two absorption bands centered approximately at 220 and 290 nm . In strong acid medium (e.g. $[\text{H}^+] = 0.02 \text{ M}$) the intensity of the 290 -band increased by the addition of either α - or β -CDs meanwhile the maximum wavelength absorption shifted to longer wavelengths, $\sim 300 \text{ nm}$. The neat observed effect increases when the $[\text{H}^+]$ is small, which means that the NoH_2^{2+} species does not form inclusion complexes [4, 5].

By contrast, the addition of CDs to aqueous solutions of nov shifted the maximum wavelength of fluorescence emission to shorter values: $\lambda_{\text{em}} \sim 354 \text{ nm}$ in water shifts to $\lambda_{\text{em}}^{\text{max}} \sim 341 \text{ nm}$ at high CD-concentration, and enhances strongly the fluorescence intensity emission, I_F , under what ever acidity conditions; nevertheless, the enhancement is higher in conditions of neutral host and guest, i.e. at $\text{pH} \sim 10.5$. The same behaviour is observed in water mixtures of MeCN, dioxane or DMSO. Figure 1a shows the plot of I_F read at 346 nm against the percentage of the organic solvent in the aqueous solutions. For acetonitrile–water mixtures, I_F increases proportional to the solvent percentage (or $[\text{solvent}]$), whereas in either dioxane or DMSO–water solutions an exponential increase of I_F was observed. The high effect of solvent polarity is explained by the twisted intramolecular charged transfer (TICT) emission state of nov, which is only possible in conditions where the forms NoH^+ or No of nov are present; that is, NoH_2^{2+} species is not a fluorophore and no fluorescence is observed in strong

Fig. 1 Fluorescence emission intensities of nov ($\sim 10 \mu\text{M}$) in (a) aqueous mixtures of (●) MeCN, (▲) dioxane, (◆) DMSO, and (b) as a function of $[\alpha\text{-CD}]$ for $[\text{nov}]$ equal to (●) 13 and (▲) $16 \mu\text{M}$ in 3.3 mM carbonate–bicarbonate buffer of pH 10.4 at 25°C



acid medium. On the other hand, the different behaviour showed in MeCN and DMSO or dioxane aqueous solutions can be attributed to the difference in hydrogen bonding ability of solvent molecules and the electron donor group ($\text{H}_2\text{N}-$) of *nov*, which stabilizes the twist conformer to facilitate the formation of the TICT state.

Figure 1b shows the variation of I_F as a function of the α -CD concentration. The appearance of saturation levels at high $[\text{CD}]$ is typical of complex formation of 1:1 stoichiometry. At pH 10.4, both the host and the guest are neutral, then $[\text{nov}] = [\text{No}]$, and $[\text{CD}]_0 = [\text{CD}]$, i.e. since $[\text{nov}] \ll [\text{CD}]_0$ all CD molecules can be considered as free or uncomplexed. Therefore, if K_c represents the equilibrium stability constant of the inclusion complexes, $\text{No} \cdot \text{CD}$, taking into account that the fluorescence emission intensity is $I_F = I_F^w + I_F^c$, i.e., the sum of the fluorescence emission due to *nov* free molecules (I_F^w) and *nov* included molecules (I_F^c), the Eq. 1 may be derived to simulated the curves in the Fig. 1b. The corresponding results are listed in Table 1.

$$I_F = \frac{I_F^w + I_F^c K_c [\alpha - \text{CD}]}{1 + K_c [\alpha - \text{CD}]} \quad (1)$$

The effect of CDs on the electrical conductance of aqueous solutions of NoHCl is displayed in Fig. 2. The formation of 1:1 complexes between NoH^+ (protonated *nov* at the tertiary amine-N) and β -CD reduces the specific conductivity due to the lower electrical mobility of the complexed cations than the free ones; in other words, the molar conductivity of $\text{NoH}^+ \cdot \text{CD}$ ($\lambda_{\text{NoH}^+ \cdot \text{CD}}$) is lower than the molar conductivity of NoH^+ (λ_{NoH^+}). Compare the experimental values obtained for: $\lambda_{\text{Cl}^-} + \lambda_{\text{NoH}^+} = 94.38 \pm 0.08 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ and $\lambda_{\text{Cl}^-} + \lambda_{\text{NoH}^+ \cdot \text{CD}} = 85.32 \pm 0.07 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$. Starting from data in Fig. 2b and taking into account all contributions to the specific conductance ($\kappa = \kappa_0 + \lambda_{\text{Cl}^-}[\text{Cl}^-] + \lambda_{\text{NoH}^+}[\text{NoH}^+ \cdot \text{CD}]$) and the equilibrium $\text{NoH}^+ + \text{CD} \rightleftharpoons \text{NoH}^+ \cdot \text{CD}^+$, K_c'' one arrives to Eq. 2 that describes perfectly the variation of κ with $[\beta\text{-CD}]$ (solid curve) with the parameters listed in Table 1.

$$\kappa = \frac{\kappa^w + \kappa^c K_c'' [\beta - \text{CD}]}{1 + K_c'' [\beta - \text{CD}]} \quad (2)$$

The reaction rates of both the nitrosation of the H_2N -group of *nov* in aqueous acid medium and the alkaline hydrolysis of the ester group were retarded in the presence of CDs. Figure 3 shows the variation of the observed rate constant, k_o , for the hydrolysis of the ester function in aqueous alkaline solutions of α -CD. The $[\text{OH}^-]$ was corrected by considering the acid ionisation equilibrium of α -CD ($\text{p}K_a = 12.4$), in order to maintain constant the $[\text{OH}^-]$.

The inhibition effect of CDs on both reactions of *nov* is due to inclusion complex formation of 1:1 stoichiometry (saturation levels at high host concentration) between either NoH^+ or No and neutral (CD) or ionized (CD^-) cyclodextrin. The H_2N -group of *nov* enters first on the CD-cavity, whose size allows encapsulation of the reactive groups, which remain protected from the attack of hydrophilic reagents, such as OH^- or N_2O_3 in nitrosation. In every case, the observed rate constant fits Eq. 3, where k_o^w is the pseudo-first order rate constant measured in water and K_c' is the stability constant of the complex formed between cationic (or neutral) *nov* and neutral (or ionized) CD, respectively to the case of nitrosation or hydrolysis reaction. The linear relationship obtained in the plot of the reciprocal k_o versus $[\alpha\text{-CD}]$ can be seen in Fig. 3b for the results listed in Table 1.

$$k_o = \frac{k_o^w}{1 + K_c' [\alpha - \text{CD}]} \quad (3)$$

Table 1 Values of the stability constants, K , of the inclusion complexes formed between *nov* and CDs under various experimental conditions determined from different methods

Method	Medium	Equilibrium	Host	K/M ⁻¹
Fluorescence emission	Alkaline, $[\text{OH}^-] = 0.05 \text{ M}$, 25 °C	$\text{No} + \text{CD}^- \xrightleftharpoons{K_c'} \text{No} \cdot \text{CD}^-$	α -CD	55 ± 2
	Buffered solutions of $\text{CO}_3^{2-}/\text{HCO}_3^-$, 3.3 mM, pH 10.4, 25 °C	$\text{No} + \text{CD} \xrightleftharpoons{K_c} \text{No} \cdot \text{CD}$	β -CD	660 ± 10
			α -CD	124 ± 2 120 ± 3
	Buffered solutions of acetic acid (pH 4.3), 25 °C	$\text{NoH}^+ + \text{CD} \xrightleftharpoons{K_c''} \text{NoH}^+ \cdot \text{CD}^+$	β -CD	1536 ± 67 282 ± 12
Ester hydrolysis	Alkaline, $[\text{OH}^-] = 0.20 \text{ M}$, 25 °C	$\text{No} + \text{CD}^- \xrightleftharpoons{K} \text{No} \cdot \text{CD}^-$	α -CD	49 ± 1
Conductance	Aqueous at 17 °C	$\text{NoH}^+ + \text{CD} \xrightleftharpoons{K_c'} \text{NoH}^+ \cdot \text{CD}^+$	β -CD	708 ± 14
			β -CD	294 ± 11
Nitrosation	Buffered solutions of acetic acid (pH 4.3), 25 °C	$\text{NoH}^+ + \text{CD} \xrightleftharpoons{K_c''} \text{NoH}^+ \cdot \text{CD}^+$	β -CD	214 ± 7

Fig. 2 Specific electrical conductance of aqueous nov solutions measured (a) at 21 °C as a function of $[nov]$ in the absence of β -CD (\bullet) and in the presence of 9.33 mM of β -CD (\blacktriangle) and (b) at 17 °C as a function of $[\beta$ -CD] and fixed $[nov] = 0.98$ mM

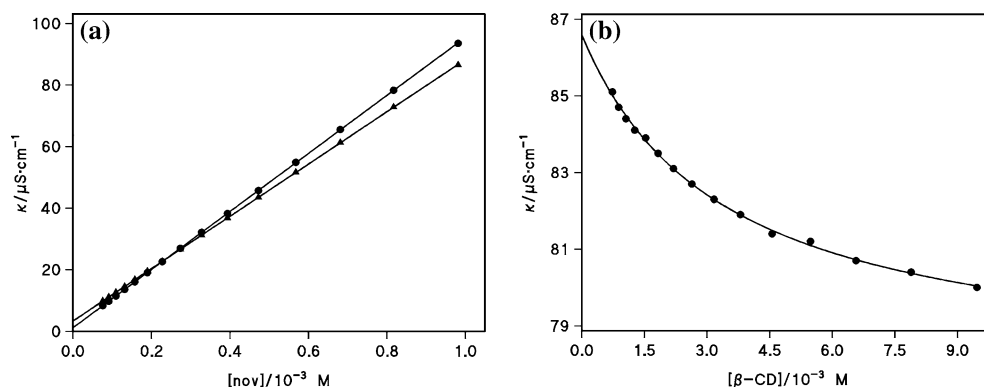
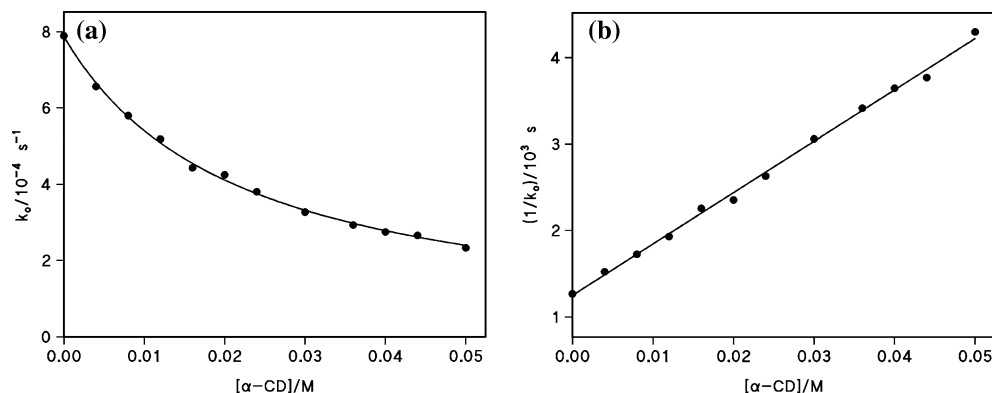


Fig. 3 (a) Variation of observed rate constant of the alkaline hydrolysis ($[\text{OH}^-] = 0.20$ M) of nov (65 μM) as a function of $[\alpha$ -CD]; (b) reciprocal plot of k_o against $[\alpha$ -CD]



The higher inhibition effect observed in the alkaline hydrolysis than in the nitrosation reaction can be explain because, first, the stability of the complex $\text{No} \cdot \text{CD}^-$ (neutral nov and ionized CD) is higher than that of $\text{NoH}^+ \cdot \text{CD}$ (monocation of nov and neutral CD), and, second, in alkaline medium the secondary CD rim is negatively charged, due to ionisation of secondary OH-groups; then, the electrostatic repulsive effects between like charge ions (OH^- and CD^-) prevent the reaction through the complexes.

Conclusions

In this work, we have shown that the fluorescence emission of novocaine was significantly increased in the presence of both α -CD and β -CD. The highest enhancement was observed at approximately pH 10, i.e. under conditions where both the host and the guest are neutral molecules. The presence of CDs reduces the electrical conductance of aqueous solutions of novocaine hydrochloride; as well as, the reaction rates of both the nitrosation of H_2N -group in aqueous acidic medium, and the hydrolysis of the ester in alkaline medium. Changes in these physical and chemical

properties are due to the formation of 1:1 inclusion complexes; however, the corresponding value of the stability constant depends of the pH and, in every case, is more than 10-times higher for β -CD than for α -CD.

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