Complex Formation of Phenol, Aniline, and their Nitro Derivatives with β-Cyclodextrin

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The interactions of phenol, aniline, and their nitro derivatives with β-cyclodextrin (cyclohepta-amylose) have been studied by a spectrophotometric method. The complex formation constants have been determined for both the conjugate acid and base forms. The *para* isomer of nitrophenol is the only case when a more stable complex is formed with the ionic species than with the undissociated one, supporting the concept that resonance charge delocalization and London dispersion interactions are important factors influencing the stability of the complexes.

Cyclodextrins are cyclic oligosaccharides with the ability to form inclusion complexes with a great variety of different guest molecules.[1] Based on this fact cyclodextrins have increasing importance both in pharmaceutical and food industries for formulating various active ingredients, and in the research of biological processes as model enzymes for a series of catalytic reactions, *e.g.* hydrolysis of phenyl esters.[1]

In 1976 Connors and Lipari[2] observed that the acid dissociation constants of several organic compounds of acid–base character were shifted in the presence of cyclodextrins. This shift was often regarded as a measure of complex formation, though it is observable only when the stability constants related to the conjugate acid and base forms are different. Usually the complex of the ionic species is much less stable than that of the un-ionized one, the hydrophobic cyclodextrin cavity favouring uncharged molecules.[2–6]

In the case of *p*-nitrophenol, however, the shift in pK_a is of opposite sign;[2] the formation constant of the complex of *p*-nitrophenolate anion is higher, especially with α-cyclodextrin, than that of *p*-nitrophenol.[3–7] Dipolar interactions,[3] the polarity of the binding site,[5,6] charge delocalization, and dispersion forces[5–7] have been proposed to account for this phenomenon.

Although much work has been published on complex formation of substituted benzene derivatives including phenols and anilines,[8–15] in most cases either only the *para*-isomer,[6–12] or the un-ionized molecules without the conjugate base (or acid)[13–15] are dealt with. No investigation involving all three positional isomers along with their protonated or dissociated forms has been found so far.

In the present work we have investigated the complex formation of *o*-, *m*-, and *p*-nitrophenol and their anions and aniline and *p*-nitroaniline and their protonated forms with β-cyclodextrin (cyclohepta-amylose) (β-CD) by a spectrophotometric method. The results will be discussed in terms of the binding forces.

Experimental

β-Cyclodextrin was from CHINOIN (Budapest) and was recrystallized from water twice.

Nitrophenols and nitroanilines were of analytical grade (VEB Jenapharm, Laborchemie Apolda) and no impurities could be detected. Phenol was purified by freezing out from the melt, and aniline was distilled under reduced pressure.

Buffers of different composition, chosen to be as simple as possible, were used. All buffer materials were of analytical grade.

The spectrophotometric method was based on the fact that protonation equilibria are shifted in the presence of cyclodextrin, and the spectra of the ionized and neutral forms are significantly different, while complex formation causes only minor changes (see Figure).

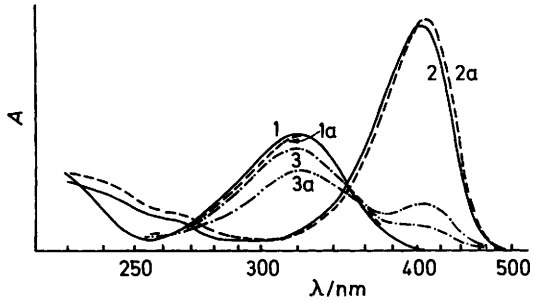


Figure. The u.v.–visible spectra of *p*-nitrophenol: 1, pH 2; 1a, pH 2, + CD; 2, pH 12; 2a, pH 12, + CD; 3, pH 6; 3a, pH 6, + CD. Concentrations: *p*-nitrophenol; 4.10×10^{-5} mol dm^{-3}, CD in case a: 10^{-2} mol dm^{-3}

Spectra were recorded on a Zeiss Specord double-beam spectrophotometer. Measurements were carried out with Spektromom 202 and 361 instruments, at three or four different wavelengths selected according to the absorption maxima of the acidic and basic forms and/or the isosbestic points of the spectra of the free and complexed guests.

The concentration of β-cyclodextrin was varied from zero to 1×10^{-2} mol dm^{-3} and absorbances were measured in solutions of different pH around the pK_a value of the given guest compound. The exact conditions are summarized in Table 1. The temperature was 23 ± 2 °C.

Results and Discussion

The Figure shows the changes caused in the spectra of *p*-nitrophenol in alkaline, nearly neutral, and acidic medium by the addition of β-CD. It is remarkable that *p*-nitrophenol is the only case when the acid–base equilibrium is shifted toward the ionic form; in all the other cases, the opposite change could be observed.

The connection between protonation and complex formation equilibria (along with definitions of the equilibrium constants) is represented in equation (1) where HA denotes phenol or

$$\begin{array}{ccc} HA & \overset{K_a}{\rightleftharpoons} & A + H^+ \\ {\scriptstyle +CD}\ \Big\| & & \Big\|\ {\scriptstyle +CD} \\ {\scriptstyle K_{HA}} & & {\scriptstyle K_A} \\ CD.HA & \rightleftharpoons & CD.A + H^+ \end{array} \qquad (1)$$

Table 1. Experimental conditions

Guest compound (pK_a)[a]	Concentration range (mol dm^{-3})	pH	Wavelength (nm)
Phenol (9.89)	9×10^{-5}—9×10^{-4}	8.80, 9.00[b] 9.25, 9.40[b]	237, 265, 290
o-Nitrophenol (7.21)	8×10^{-5}—3×10^{-4}	6.80, 7.20, 7.40[c]	225, 337 441
m-Nitrophenol (8.38)	2×10^{-4}—5×10^{-4}	7.60[c] 8.00, 8.40, 8.60[b]	248, 274, 335, 400
p-Nitrophenol (7.14)	4×10^{-5}—8×10^{-5}	5.78[d] 6.40, 6.80, 7.00[c]	314, 329 396
Aniline (4.58)	9×10^{-5}—8×10^{-4}	4.81, 4.41[d] 4.06[e]	233, 253 259, 279
p-Nitroaniline (1.0)	5×10^{-5}—1×10^{-4}	0.31, 0.61[f] 1.00, 1.30[f]	252, 273

[a] From ref. 16. The pH values sustained by the following buffers. [b] NH_3–NH_4Cl. [c] Phosphate buffer after Clark and Lubs. [d] Acetic acid–sodium acetate. [e] Formic acid–sodium formate. [f] Hydrochloric acid.

anilinium ion, and A denotes phenolate ion or aniline (the charges are omitted). (It follows that this method can be applied only when complexes of different stability are formed with the conjugate acid and base forms. This is why *m*-nitroaniline is missing. In the case of *o*-nitroaniline, other problems are caused by the necessity of high acid concentration.)

The basic equations used for the evaluation are (2) and (3)

$$C_A = [HA] + [A] + [CD.HA] + [CD.A] = [HA] + \frac{K_a}{[H^+]}[HA] + K_{HA}[HA][CD] + \frac{K_a}{[H^+]}K_A[HA][CD] \quad (2)$$

$$C_{CD} = [CD] + [CD.HA] + [CD.A] = [CD] + K_{HA}[HA][CD] + \frac{K_a}{[H^+]}K_A[HA][CD] \quad (3)$$

with C_A and C_{CD} the total concentrations of the guest molecules and CD, respectively. K_a, K_A, and K_{HA} are the equilibrium constants in equation (1) and [ ] denotes the equilibrum concentration of each species.

The absorbances measured at each wavelength are given by equation (4) where ε^i is the molar absorptivity of each species at

$$A_i = \varepsilon^i_{HA}[HA] + \varepsilon^i_A[A] + \varepsilon^i_{CDHA}[CD.HA] + \varepsilon^i_{CDA}[CD.A] = \varepsilon^i_{HA}[HA] + \varepsilon^i_A\frac{K_a}{[H^+]}[HA] + \varepsilon^i_{CDHA}K_{HA}[HA][CD] + \varepsilon^i_{CDA}\frac{K_a}{[H^+]}[HA][CD] \quad (4)$$

the *i*th wavelength. (In addition, in some cases further interactions such as dimerization of the guest molecules, association with the components of the buffer, or the formation of ternary complexes has to be taken into account as well.)

ε^i_{HA} and ε^i_A were determined separately by measurements in pure acidic and alkaline solutions of the guests, and the starting values for K_a were taken from the literature.[16] Estimated values for ε^i_{CDHA}, ε^i_{CDA}, K_{HA}, and K_A (and for the additional equilibria) were first substituted into equations (2)—(4) and they were varied by an iterative procedure using a computer, until the best fit between experimental and calculated values of A_i was reached. In the final refinement, K_a values were also handled as adjustable parameters, but deviations from the literature values did not exceed 30%. The stability constants obtained this way are summarized in Table 2.

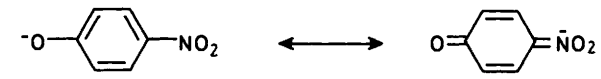
Table 2. Stability constants of the β-cyclodextrin complexes

Guest compound	Protonated from K_{HA}/dm^3 mol^{-1}	Unprotonated (dissociated) from K_A/dm^3 mol^{-1}
Phenol	129 ± 5	15 ± 1
o-Nitrophenol	145 ± 10	100 ± 5
m-Nitrophenol	130 ± 10	75 ± 10
p-Nitrophenol	130 ± 15	410 ± 40
Aniline	2.3 ± 0.2	56 ± 3
p-Nitroaniline	100 ± 10	300 ± 20

Our results show (in accord with the literature[7,8,11]) that the *p*-nitrophenolate ion forms a much more stable complex than the undissociated *p*-nitrophenol. With the other guests, however, the stability constants obtained for the ionized species are smaller.

The surprisingly high stability of the *p*-nitrophenolate complex has been interpreted[3] in terms of dipolar interactions. In this case, however, similar behaviour ought to be expected with the other isomers, too.

The data in Table 2 support an alternative explanation,[5–7] namely that London dispersion forces are the dominating factors. Resonance charge delocalization in the *p*-nitrophenolate anion (**1**) increases the electron density and polarizability, so



(1)

increasing the stability of the complex. It follows that a similar effect is not to be expected for the *meta* isomers or for the unsubstituted phenol, because delocalization of this type is possible in *para* and *ortho* substituted compounds only. The *ortho* position may be less favoured because of steric hindrance. Thus in cases other than *p*-nitrophenol, the competing effect of the stronger hydration of the charged species dominates.

The formation constant of the *p*-nitroaniline complex may seem to be surprisingly large, but it becomes reasonable as the electron distribution and polarizability of *p*-nitroaniline can be considered an intermediate between those of *p*-nitrophenol and its anion.

These results can be correlated with the observations of Eftink and Harrison[7] and of Harata,[17] and suggest a contribution of hydrogen bonding towards stabilizing these complexes. Eftink and Harrison established that the stability-increasing effect of the dissociation of the phenolic hydroxy group was much more pronounced for α- than for β-cyclodextrin, and Harata showed that in the case of hydroxybenzoic acids and nitrophenols the *meta* isomer had larger negative enthalpy for complex formation than the *para* isomer while the opposite tendency was observed with aminobenzoic acids and nitroanilines. In the cavity of α-CD, the guest molecules fit tightly, the van der Waals interactions are relatively strong, but there is no possibility of hydrogen-bonding between the hydroxy groups of CD and that of nitrophenol, especially for the *para* isomer. Dissociation of the phenolic hydroxy group merely increases the electron density and the polarizability, causing a dramatic increase in the stability of the complex. In the larger β-CD cavity the fit is not so tight; consequently, van der Waals forces are weaker, but some deviation from strictly axial insertion is possible, so hydrogen-bonding may contribute to the interaction, even with the *para* isomer. Dissociation

destroys the hydrogen bond, partly compensating for the effect of the increased dispersion forces. The *meta* isomer offers better prospects for hydrogen-bonding.

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