Complex Formation of Phenolphthalein and Some Related Compounds with β-Cyclodextrin

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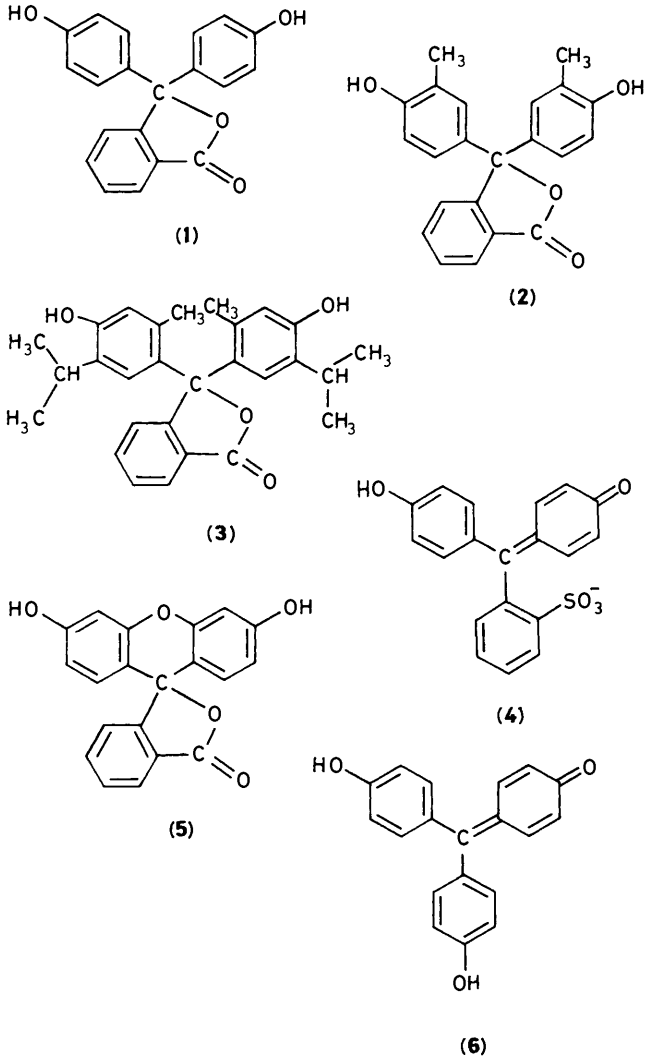
The complex formation of phenolphthalein and β-cyclodextrin (cyclohepta-amylose) has been investigated by spectrophotometric, c.d., and potentiometric methods. It has been shown that complexes are formed with the protonated and dissociated forms of phenolphthalein as well; the dianion is complexed in alkaline solution in a colourless form. The complexes formed have the largest stability constants among cyclodextrin complexes known up to now. The unusual stabilities and colour change can be explained by the combined effect of the accessibility of the phenolic–phenolate moiety and presence of the carboxy–carboxylate group simultaneously, providing optimal space filling and the possibility for the cyclodextrin molecule to interact with three functional groups of phenolphthalein at the same time.

Cyclodextrins are cyclic oligosaccharides with the ability to form inclusion complexes with a great variety of different guest molecules. Complex formation is not usually accompanied by major changes in the u.v.–visible spectra of the guests;[1] minor changes like the effect of solvent can be observed. There are, however, some compounds (mainly used as indicators) where the spectra are significantly changed on addition of cyclodextrins[2–4] and which are often used as competing reagents for determining the stability constants of other inclusion complexes.[5–8]

Some years ago a spectrophotometric method was worked out for the determination of the formation constants of β-cyclodextrin complexes using phenolphthalein (**1**) as competing reagent.[8] It was based on the well known fact that only a 1:1 complex is formed between phenolphthalein and β-cyclodextrin and (in alkaline solutions, pH *ca.* 10.5) complex formation causes a dramatic decrease in the absorptions of light.[2] The apparent stability constant of the complex has been found to be 2.3×10^4 (in 2×10^{-2}M-Na_2CO_3 solution at 25 °C). This value is at least one order of magnitude greater than those for simpler aromatic molecules,[1,9–11] in spite of the fact that only one aromatic ring of the phenolphthalein can be accommodated in the β-cyclodextrin cavity.

Our aim was to clarify the reasons for the unusual stability and spectral change. For this purpose spectrophotometric, c.d., and potentiometric measurements were carried out, and for comparison other triphenylmethane derivatives such as *o*-cresolphthalein (**2**), thymolphthalein (**3**) phenol red (**4**), fluorescein (**5**), and aurin (**6**) were also investigated. Our results were presented at a conference[12] and are a part of a thesis.[13]



Experimental

β-Cyclodextrin was from the Chinoin Chemical-Pharmaceutical Works (Budapest, Hungary) and was recrystallized from water twice. The indicators were recrystallized from ethanol–water mixtures. All the other materials were of analytical grade and were used without further purification.

U.v.–visible absorption spectra were recorded on a Zeiss Specord double-beam spectrophotometer in alkaline solutions (2×10^{-2}M-Na_2CO_3), in pure distilled water and in some cases (phenol red, fluorescein) also in acidic media (0.1—0.001M-HCl) and in the range of the colour change of the indicator (pH 7.6

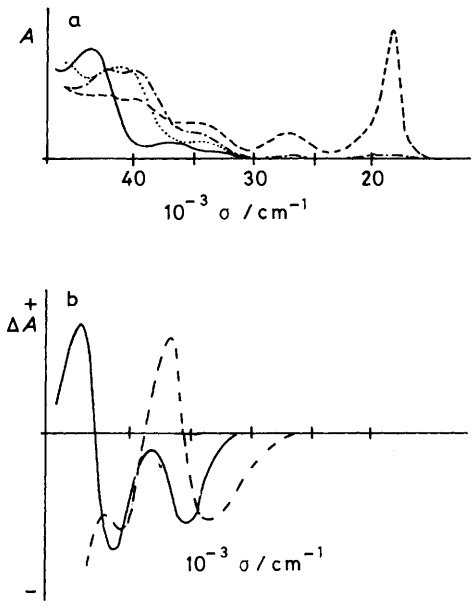


Figure 1. U.v.–visible absorption and c.d. spectra of phenolphthalein: a, absorption spectra: —— pH *ca.* 6; - - - - pH 10.5; — · — · — · — pH 10.5, in the presence of excess of β-cyclodextrin; · · · · pH *ca.* 14; b, induced c.d. in the presence of β-cyclodextrin: —— pH *ca.* 6; - - - - pH 10.5

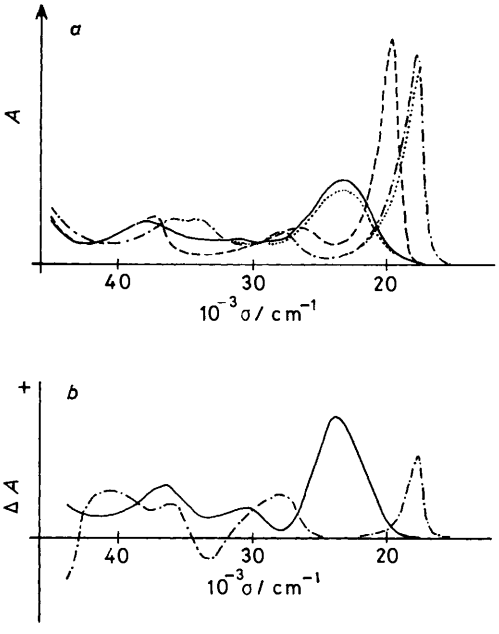


Figure 2. U.v.–visible absorption and c.d. spectra of phenol red: a, absorption spectra: —— 3 < pH < 7; — · — · — pH 10.5; - - - pH < 1; · · · · pH *ca.* 6 and 10.5, respectively, in the presence of excess of β-cyclodextrin; b, induced c.d. in the presence of β-cyclodextrin: —— pH *ca.* 6; — · — · — pH 10.5

phosphate buffer), with and without cyclodextrin. The concentration of the dyes was 2—3 × 10^{-5}M and that of the cyclodextrin was 5 × 10^{-3}—10^{-2}M.

C.d. spectra of cyclodextrin-containing solutions were recorded on a Roussel–Jouan Dichrograph no. III (Jobin–Yvon). The temperature of the optical measurements was 23 ± 2 °C.

pH-Potentiometric titrations were carried out using a Radiometer PHM 4d-type pH-meter with an Ag–AgCl reference and Radiometer G 202 B-type glass electrode, combined with a W-type salt bridge. Because of solubility problems, all the solutions were prepared in 35% (v/v) ethanol–water mixtures. The ionic strength was 0.1M-NaCl and the temperature was kept at 25.0 ± 0.1 °C. Portions (10.00 cm^3) of 1.0 × 10^{-3}M-phenolphthalein solution were titrated with 2.0 × 10^{-2}M-NaOH solution under nitrogen, without cyclodextrin and in the presence of 1.0 × 10^{-2} and 1.3 × 10^{-2}M β-cyclodextrin, respectively. To determine the E_0 value, the solutions also contained hydrochloric acid in known concentration. Separate titrations were also made in solutions containing HCl only, with and without cyclodextrin, so that the interaction between the cyclodextrin and hydroxide ions (when pH >10)[14] can be corrected.

Results

Figure 1a shows the u.v.–visible absorption spectra of phenolphthalein under various circumstances. The curves (surprisingly) indicate that the disappearance of the purple colour accompanying complex formation is not caused by re-formation of the protonated form, as seems plausible at first sight, as with other compounds capable of acid dissociation.[15] Only the absorption band in the visible range disappears. The u.v. spectrum remains different from that of the acidic form, in which no significant change is caused by the addition of cyclodextrin. It is worth mentioning that the spectrum of the complex most resembles that of the colourless carbinol base formed in strongly alkaline medium.

More detailed information about the complex formation can be obtained from the c.d. measurements.[16]

Induced c.d. spectra (Figure 1b) prove that the complex formed in alkaline solution is colourless. Moreover, complex formation occurs in neutral and acidic solution, but not the same form of guest is included.

The spectra of phenol red (**4**) are shown in Figure 2. Phenol red can exist in three different forms: as a red dianion in strongly alkaline solution, a yellow monoanion in neutral and moderately acidic media, and an orange undissociated acid.[17,18] No significant change is caused by β-cyclodextrin in the absorption spectra of either form, nor is the ratio of the intensities corresponding to the red and yellow forms changed at pH 7.6. In the pH range corresponding to the transition between the yellow and orange species, however, an increase in the concentration of the cyclodextrin at a constant pH results in a significant decrease in the absorbance at 513 nm and an increase at 440 nm. From these measurements the complex formation constants for the undissociated form and the monoanion have been found to be 30 ± 5 and 440 ± 20, respectively. This means that complexes of nearly similar stability are formed with both the red dianion and the yellow monoanion. This conclusion is supported by the induced c.d. spectra, but in contrast to the case of phenolphthalein (**1**) [and aurin (**6**)] the electronic system responsible for light absorption in the visible range is not essentially affected by interaction with the cyclodextrin, although the corresponding part of the molecule is included.

Similar phenomena to those observed with phenolphthalein (**1**) are shown only by the most similar *o*-cresolphthalein (**2**), but the stability constant found for its complex is much smaller: 400 ± 50. With thymolphthalein (**3**) only some very weak interaction could be detected in alkaline solution.

With fluorescein (**4**), a nearly colourless complex is formed in a rather narrow pH range (10^{-2}—10^{-3}M-HCl). Its formation constant has been found to be 1 100 ± 100.

The results obtained for the series phenolphthalein (**2**), *o*-cresolphthalein (**3**), and thymolphthalein (**4**) clearly show that insertion of the phenolic group is essential. If it can penetrate the cyclodextrin cavity, complexes can be formed with both the

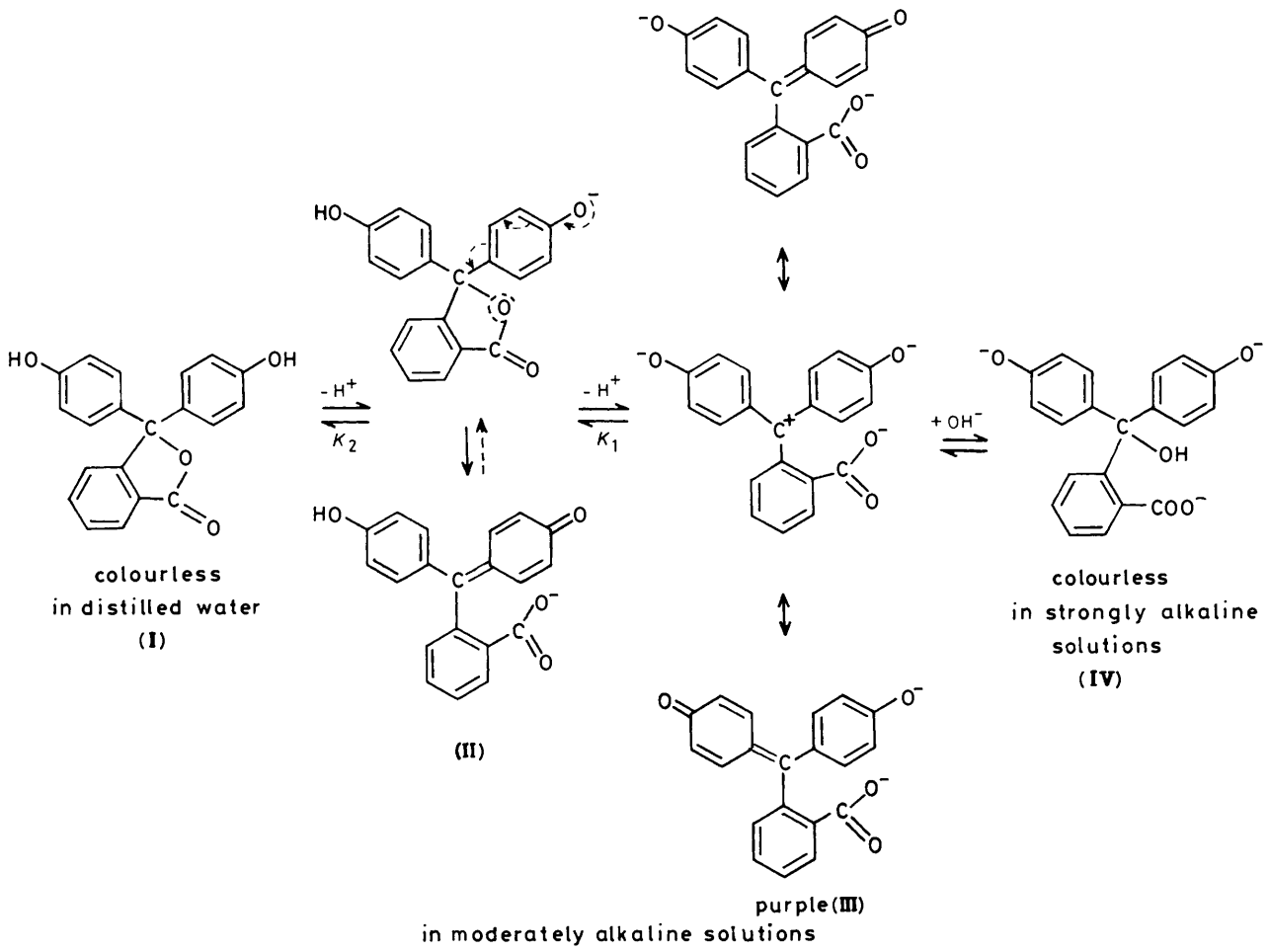


Figure 3. Deprotonation equilibria and colour changes of phenolpthalein

acidic and basic forms. If the substituents are too bulky, some weak interaction, if any, is only possible with the remaining third ring in alkaline solution and not with the lactonoid structure [formula (**I**) in Figure 3]. Compared with the other indicators, it can be concluded that the presence of the carboxy or carboxylate group at the same time is important as well, and the simultaneous effect of these two factors results in the unique behaviour of phenolphthalein.

It is also interesting that a significant decrease in absorbance on complex formation is shown by those indicators which fade in strongly alkaline solutions. Although both processes are reversible, the essential difference between them is that complex formation is rapid while with strong alkali the equilibrium is established rather slowly.

The protonation state of phenolphthalein in the complex was studied by potentiometric titration. From computerized evaluation of the titration curves the relationships (1)—(4) were

$$K_1 = \frac{[HI^-]}{[I^{2-}][H^+]} = 7.9 \pm 0.7 \times 10^9 \quad (1)$$

$$K_2 = \frac{[H_2I]}{[HI^-][H^+]} = 3.4 \pm 0.2 \times 10^9 \quad (2)$$

$$K_1^{CD} = \frac{[CD{\cdot}HI^-]}{[CD{\cdot}I^{2-}][H^+]} = 6.3 \pm 0.6 \times 10^9 \quad (3)$$

$$K_2^{CD} = \frac{[CD{\cdot}H_2I]}{[CD{\cdot}HI^-][H^+]} = 2.1 \pm 0.1 \times 10^9 \quad (4)$$

obtained for 35% ethanol–water (see Figure 3). The protonation constants for the free indicator are in good agreement with those of Gaizer *et al.*,[19] the differences being attributed to the solvent mixture.

The general definition of the complex formation constants with β-cyclodextrin (CD) is given by equation (5). In the

$$\beta_i = \frac{[CD{\cdot}H_iI^{i-2}]}{[H_iI^{i-2}][CD]} \quad (5)$$

evaluation combined with the results of the spectrophotometric measurements at pH 10.5, the stability constants of equations (6)—(8) have been obtained.

$$\beta_0 = 3.1 \pm 0.3 \times 10^4 \quad (6)$$

$$\beta_1 = 2.4 \pm 0.1 \times 10^4 \quad (7)$$

$$\beta_2 = 1.5 \pm 0.1 \times 10^4 \quad (8)$$

In solutions of pH 10.5, most phenolphthalein exists in the unprotonated form (**III**), and protonation is not favoured by the complex formation. Consequently, the complex of the dianion must be colourless.

Discussion

From the literature,[17,18] it seems most likely that phthalein indicators exist in acidic aqueous solutions as well as in apolar solvents as colourless lactonoids; no lactone ring is formed, however, in the sulphophthaleins. The most probable scheme for the colour change of phenolphthalein is in Figure 3.

Machida *et al.*[18] have shown that the yellow form of phenol red (**4**) contains one quinonoid ring and that the red colour of aurin (**6**) in alkaline solutions is the consequence of the loss of two protons. This is in accord with our potentiometric results related to the dissociation of phenolphthalein seemingly in one step and, on the other hand, it means that the red colour is bound with the presence of at least two equivalent (unprotonated) phenolic rings with continuous resonance electron delocalization between them involving the possibility of the formation of a carbenium ion as a transition state. If this conjugation is hindered by any kind of interaction, this results in the disappearance (or at least significant diminution or shift) of the colour.

β-Cyclodextrin can manifest these interactions in several ways. One of the phenol moieties, which is included into the cyclodextrin cavity, can form a hydrogen bond with its primary hydroxy group. On the wider rim of the cyclodextrin torus H-bonds are possible between the secondary hydroxys and other phenolic group or the carboxylate, respectively, at the same time. These possibilities can be demonstrated by space-filling models. These simultaneous interactions contributing to other forces effective in inclusion complex formation may lead to the unusual stability of the phenolphthalein–β-cyclodextrin complexes. They provide hindrance to resonance charge delocalization in themselves, or in addition the carboxylate oxygen may be forced close to the central carbon atom of phenolphthalein, providing an explanation for the disappearance of the colour upon complex formation in alkaline solution.

Therefore our conclusions are: (i) there is three-site contact between phenolphthalein and β-cyclodextrin; (ii) one of the phenolic rings is included in the cavity of β-cyclodextrin; (iii) inclusion complex formation is promoted by hydrogen bonds, first of all by the proton-donor ability of phenolic hydroxy groups or by the proton-acceptor ability of either phenolate or quinonoid oxygens; (iv) the carboxy or carboxylate substituent plays a central role; (v) the three-site interaction leads to an $sp^2 \longrightarrow sp^3$ change at the central ('methane') carbon atom; (vi) there must be some interaction between the central ('methane', or carbenium-type) atom and the carboxylate substituent (possibly through a water molecule having both electron pair and/or hydrogen-donor abilities), but no lactone ring is formed.

Taguchi[20] reached very similar results using ^{13}C n.m.r. spectroscopy and temperature-jump kinetics. First, assumptions (i) and (v) were proved by his measurements. If we accept Taguchi's definition,[20] 'the binding is characterized by what might be termed as atypical or nonclassical hydrophobic interaction between relatively polar substrates', as a rather odd description of hydrogen bonding, our assumption (iii) is supported by the new data. As Taguchi did not investigate any sulphophthaleins,[20] statement (iv) is neither proved nor disproved by the new results. As our results point unambiguously to the inclusion of a phenolic–phenolate–quinonoid ring (ii), they seem to provide us with additional information to the ^{13}C n.m.r. results in ref. 20, where the conclusion was, 'this point requires further study with the PP–β-CD complex'.

It seems that a difference exists between statement (vi) and the central result in ref. 20. There is no direct proof that any 'colourless lactonoid dianion' exists if we compare the assignments of Taguchi[20] and Berger.[21] The statement, that the ^{13}C chemical shifts are equal between the 'lactonoid form' and 'transient form in the complex', is in no case true, but, taking Berger's assignments into consideration, the chemical shifts of the 'transient form in the complex' can always be observed between those of the 'lactonoid form' and 'red coloured form', and using Berger's data, they are generally nearer to the red form than to the lactonoid form.

It seems, unfortunately, that the existence of a new phenolphthalein form, the 'colourless lactonoid dianion',[20] is not proved. There does not exist an 'enormous rate of lactonization'[20] and β-cyclodextrin, at least in the reaction with phenolphthalein, does not 'mimic enzymic processes'.[20]

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