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COMPLEX FORMATION OF CYCLOMALTO-OCTAOSE WITH TETRABROMOPHENOLPHTHALEIN AND SOME RELATED COMPOUNDS

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ABSTRACT

The interactions of cyclomalto-octaose (γ-cyclodextrin) with some phthalein-type acid–base indicators have been investigated photometrically. Bromine substituents enhance the formation of complexes and tetrabromophenolphthalein [3,3-bis(3,5-dibromo-4-hydroxyphenyl)-3H-isobenzofuran-1-one] forms the most stable complex (K 6.0 × 10^3). This stability and other phenomena suggest that the host and guest are involved in a three-site interaction.

INTRODUCTION

The equilibrium constants of cyclomalto-oligosaccharide (cyclodextrin, CD) inclusion complexes can be determined by following a competing reaction spectrophotometrically[1]. The coloured species is generally an acid–base indicator which gives a stable complex with the CD, and the equilibrium concentration can be monitored in solution by its characteristic absorbance. Since the total concentrations of the dye, the CD, and the competing colourless guest are known as well as the equilibrium constant of the dye–CD complex, the equilibrium constant(s) for the guest can be calculated.

Spectrophotometric methods for the quantitative determination of CDs based on dye–CD interactions require a high equilibrium constant and it is advantageous if only one species absorbs at a given wavelength. Thus, the so-called "one-coloured" indicators are favoured, where the dye–CD complex is colourless. With "two coloured" indicators or dyes that give coloured adducts with CDs, the calculations are more complex and the results are generally less precise.

The interaction of the azo-type acid–base indicator Methyl Orange with cyclomaltohexaose (αCD) is well-known[1,2]. Because of the tautomeric equilibrium[3], the Methyl Orange–αCD complex is almost colourless. The formation of a complex between Methyl Orange and cyclomaltoheptaose (βCD) is less favourable and, with cyclomalto-octaose (γCD), even $(dye)_2$–γCD and $(dye)_2$–$(\gamma CD)_2$ complexes can be formed[4].

A near ideal complex is that of phenolphthalein with βCD. The complex has

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no absorbance in the visible spectrum in spite of the fact that it contains phenolphthalein in its dianion form[5], which, in the free state, is responsible for the red colour at pH >10. Moreover, the stability constant of the phenolphthalein–βCD complex is the highest among the known values. Both facts can be explained on the basis of a three-site interaction of phenolphthalein with the host[5], where one phenolic (or phenolate) ring is included in the cavity of βCD and this interaction is promoted by hydrogen bonding with the hydroxyl groups of the host. The role of the carboxylate group of phenolphthalein is also important since Phenol Red (which has a sulphonate instead of a carboxylate group) forms complexes the stabilities of which are much lower[5]. It seems that the carboxylate group interacts with the central ("methane" or carbenium-type) carbon atom (but this interaction does not mean the re-formation of the lactone ring).

The three-site interaction leads to an $sp^2 \rightarrow sp^3$ change at the central carbon atom, so that the dianion form in the inclusion complex does not absorb in the visible range, and this is the basis for its use in the competitive determination of equilibrium constants[6] and the spectrophotometric determination of βCD[7].

The analytical chemistry of CDs is based usually on chromatographic methods[1], but the reaction with phenolphthalein can be used in h.p.l.c. for sensitive post-column detection[8]. Spectrophotometric methods also have some importance. Thus, a combination of methods using Methyl Orange, phenolphthalein, and Bromocresol Green has been employed[9] to analyse mixtures of CDs.

The interactions of γCD with some indicators are now described.

EXPERIMENTAL

The commercial indicators (except tetrabromophenolphthalein) were of analytical grade, and stock, concentrated ethanolic solutions were diluted with water to (2–3) × 10^{-5}M. CDs were supplied by the Chinoin Chemical–Pharmaceutical Works (Budapest).

Most of the reactions were investigated in 0.02M sodium carbonate in order to keep the ionic strength and pH (10.5) constant. The solutions of Bromocresol Green were buffered in formic acid–sodium formate mixtures.

Tetrabromophenolphthalein was prepared[10] by reaction of hot ethanolic phenolphthalein with a solution of bromine in glacial acetic acid. The product had m.p. 282° (dec.) (from ethanol). Tetrabromophenolphthalein is poorly soluble at room temperature in ethanol and is nearly insoluble in water. Its solutions were prepared by dissolving ~0.16 g in M Na_2CO_3 (2.5 mL) and diluting with doubly distilled water to 25 mL, and were used within 1 h. The solutions investigated spectrophotometrically contained 0.02M sodium carbonate and 0.002M dye.

The absorption spectra were recorded at 22 ±2° on a Zeiss Specord double-beam spectrophotometer and the absorbances were measured on a Spectromom 195D instrument.

TABLE I

STABILITY CONSTANTS (K) OF INDICATOR–CD COMPLEXES AT 22 ±2°

Indicator (I)	*γCD complex*	*βCD complex*
Phenolphthalein (**1**)	1.87×10^3	2.30×10^4
o-Cresolphthalein (**2**)	1.5×10^2	4.0×10^2
Fluorescein (**3**)	2×10^2	1.20×10^3
Thymolphthalein (**3**)	$\sim 10^2$	$< 10^2$
α-Naphtholphthalein (**7**)	1.30×10^3	6×10^2
Dibromocresolphthalein (**4**)	4.0×10^2	$\sim 10^2$
Bromocresol Green (**8**)	3.10×10^3	
Tetrabromophenolphthalein (**5**)	6.00×10^3	4.0×10^2

RESULTS AND DISCUSSION

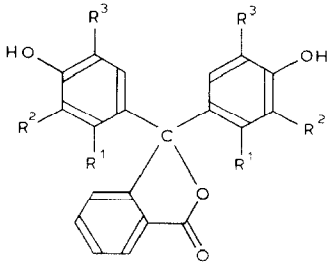
Although phenolphthalein (**1**) forms a colourless 1:1 complex with γCD in solution at pH 10.5, the equilibrium constant (1.87×10^3) is an order of magnitude less than that (2.30×10^4) with βCD (see Table I).

The size of the cavity in γCD allows the formation of $(dye)_2$–γCD or $(dye)_2$–$(\gamma CD)_2$ complexes[11], and the stoichiometries are dependent on both the absolute and relative concentrations. Some properties of γCD inclusion complexes with azo-type dyes depend on the position of the sulphonate group[12]. The azo-type calmagite metal indicator has been recommended[13] as a specific reagent for γCD, but the relatively narrow linear range of the calibration curve indicates a rather low stability. Tropaeolin 000 forms[14] three different complexes with γCD.

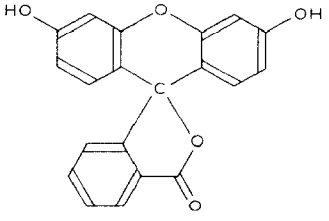
Since our aim was to find a reaction for γCD similar to that of phenolphthalein with βCD, and no problems of stoichiometry have been found in the association of γCD with phenolphthalein, phthalein-type indicators were investigated. Congo Red was also investigated qualitatively, since its complex with γCD seemed to have advantageous properties[11,12], but it was found not to be more favourable than *o*-cresolphthalein (**2**) or α-naphtholphthalein (**7**).

Replacement of the phenolic groups in phenolphthalein (**1**) by the *o*-cresol groups in *o*-cresolphthalein (**2**) dramatically decreased the equilibrium constant K (from 2.30×10^4 to $\sim 4.0 \times 10^2$). The corresponding K values for the γCD complexes were 1.87×10^3 and 1.5×10^2, respectively. The effects were similar with fluorescein (**6**), where the K values for the complexes with βCD and γCD were 1.20×10^3 and $\sim 2 \times 10^2$, respectively. The K values for γCD complexes compared with those of βCD increased when the substituent was more bulky, but the absolute values were lower.

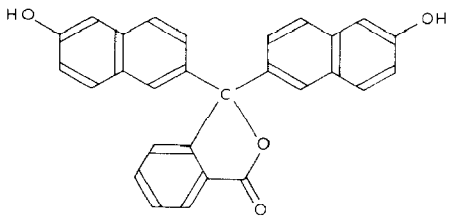
Thymolphthalein (**3**) gave no complex with βCD and the interaction with γCD was weak, *i.e.*, the Pr^i substituent is too bulky for complex formation. Two other examples were found where the complexes with γCD were more stable than those with βCD, namely, those of α-naphtholphthalein (**7**; K 1.30×10^3) and di-



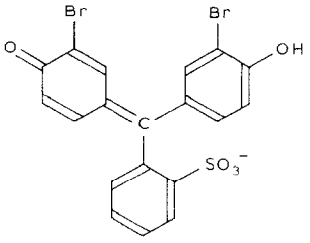
1 $R^1 = R^2 = R^3 = H$ (phenolphthalein)
2 $R^1 = R^3 = H, R^2 = Me$ (*o*-cresolphthalein)
3 $R^1 = Me, R^2 = H, R^3 = Pr^i$ (thymolphthalein)
4 $R^1 = H, R^2 = Me, R^3 = Br$ (dibromocresolphthalein)
5 $R^1 = H, R^2 = R^3 = Br$ (tetrabromophenolphthalein)



6 (fluorescein)



7 (α-naphtholphthalein)



8 (Bromocresol Green)

bromo-*o*-cresolphthalein (**4**; K 4.0×10^2). Since this last K value is about three times greater than that for the *o*-cresolphthalein (**2**), interest in bromo derivatives was stimulated.

Bromocresol Green, which is a sensitive and specific reagent for γCD[13,15], also belongs to this group, but the stability constants appear not to have been reported. Formation of the complex results in a shift in the protonation equilibrium since the complexes of the acidic and basic forms have different stabilities. The reaction can be followed by measuring the absorbance at 440 and 620 nm in solutions of pH 4.2 which corresponds to the colour change (acid–base transition) of the indicator. The K value ($3.10 \pm 0.4 \times 10^3$) of the γCD complex with the de-

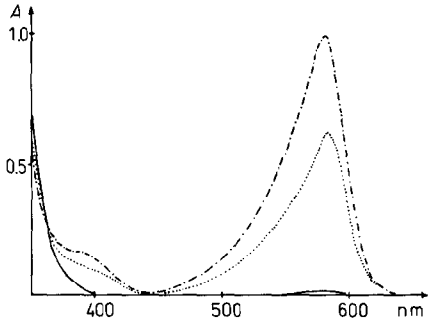


Fig. 1. The visible spectra of tetrabromophenolphthalein (**5**) in solution of pH 10.5, ·····; in the presence of 10^{-2}M γCD, ——; and in the presence of 10^{-2}M βCD, ·····.

protonated form is higher than that (8.2×10^2) of the protonated form. Unfortunately, the Bromocresol Green–γCD complexes are coloured.

Attention was then turned to tetrabromophenolphthalein (**5**). The visible spectrum of 0.002M tetrabromophenolphthalein at pH 10.5 is shown in Fig. 1. The molar absorbance of the dye is small (4.2×10^2) in contrast to those of phenolphthalein and most of its derivatives. The absorption at 583 nm was eliminated by 0.01M γCD, reduced to two-thirds by 0.01M βCD, and unaffected by 0.01M αCD.

Several stoichiometries have been found among the inclusion complexes of γCD, but a 1:1 complex was found for tetrabromophenolphthalein (**5**). The absorbances at 583 nm were linearly proportional to the concentration of free indicator in dilute aqueous solution at pH 10.5, which means that no self-association occurred. Using the method of continuous variation[16], the sum of the concentrations was 2×10^{-3}M, but the concentration of **5** was varied from 2×10^{-3}M to zero and that of γCD from zero to 2×10^{-3}M. Fig. 2a shows that γCD forms a 1:1 complex with **5**. The interaction of **5** with βCD was more ambiguous (Fig. 2b), although the data could be evaluated supposing only 1:1 association.

Although the decomposition of tetrabromophenolphthalein (**5**) at pH 10.5 is rapid, its use for spectrophotometric determination of γCD was tested. The dependence of the ΔA values (the difference between the absorbances without and with γCD) at 583 nm as a function of [γCD] shows (Fig. 3) that fairly good results can be achieved with solutions containing 0–1.2 mg/mL of γCD and 2×10^{-3}M **5** ($+2 \times 10^{-2}$M Na_2CO_3).

Measurements were made with different concentrations of dyes and γCD (or βCD) in order to determine the stability constant $K = [I - CD]/[I][CD]$, where I stands for the dye. The equilibrium concentration of **5** was determined spectrophotometrically, and those of the CD complexes were then calculated[6] (see Table I).

The γCD–**5** complex is rather stable (K $6.0 \pm 0.6 \times 10^3$), and more stable than the βCD–**5** complex (K $4.0 \pm 0.9 \times 10^2$).

Thus, the interaction of **5** with γCD seems to be similar to that[5] of phenolphthalein with βCD for which a three-site interaction has been proposed. The

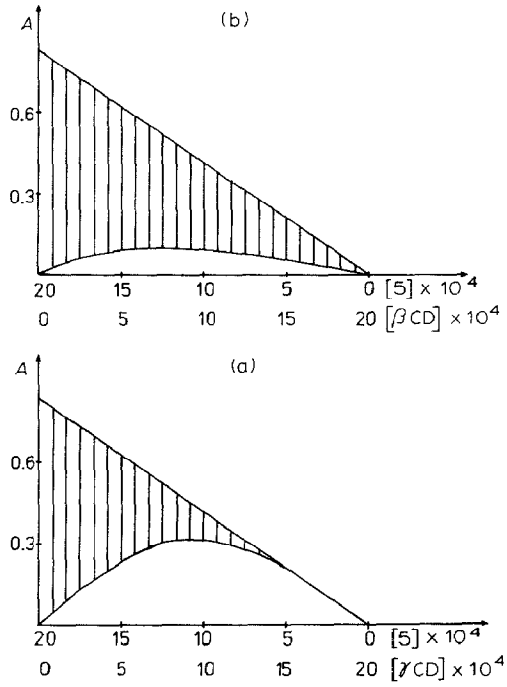


Fig. 2. The curves of Job's method[16]: tetrabromophenolphthalein (**5**) with (a) γCD, (b) with βCD.

fitting of the phenolic ring into the cavity of a CD influences the hydrogen bonds, including that with the carboxylate substituent as well as the $sp^2 \rightarrow sp^3$ change at the central carbon atom. Since the fitting is less favourable, the complex with βCD is one order of magnitude less stable. The different fittings result in the reversed ratio of stability constants for the βCD and γCD complexes of phenolphthalein (**1**) and tetrabromophenolphthalein (**5**).

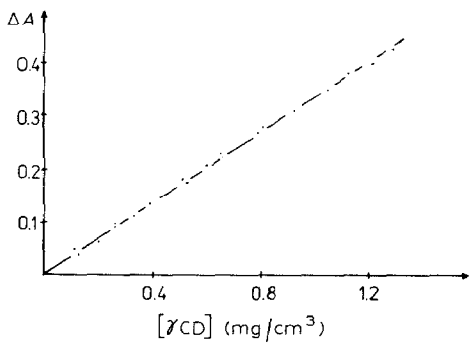


Fig. 3. Differential absorbance at 583 nm of tetrabromophenolphthalein (**5**) in solution at pH 10.5 as a function of the concentration of γCD.

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