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Determination of the stability and stoichiometry of *p*-methyl red inclusion complexes with γ-cyclodextrin

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Abstract

The effect of cyclomaltooctaose (γ-cyclodextrin, γCD) on the UV–visible spectrum of *p*-methyl red {4-[4-(dimethylamino) phenylazo] benzoic acid} in alkaline and acidic aqueous solutions was analyzed according to a 2:1 (dye:γCD) inclusion process. The inclusion process in acidic solutions was studied at different ionic strengths. The values of the Gibbs free energy, ΔG^0, obtained in alkaline and acidic solutions indicate that the 2:1 inclusion complex of the cation form of the dye is more stable than that of the anion form. The values of the thermodynamic parameters ΔH^0 and ΔS^0 of the inclusion process are given for both media. The formation of the 2:1 inclusion complexes is exothermic and entropy stabilized.

Keywords: Inclusion complexes; Cyclodextrins; Stability constants; *p*-Methyl red; γ-Cyclodextrin

1. Introduction

Cyclomalto-oligosaccharides (cyclodextrins) form inclusion complexes in aqueous solutions with a wide variety of substrates such as dyes [1–3], drugs [4], small anions [5], carboxylic acids [6], and alcohols [7]. Such substrates usually form inclusion compounds with 1:1 stoichiometry; however, other stoichiometries have been reported [8–10].

Several spectrophotometric investigations have been reported from our laboratory concerning the inclusion complexes of cyclomaltohexaose (α-cyclodextrin, αCD) and cyclomaltoheptaose (β-cyclodextrin, βCD) with the acid forms of methyl orange [11–13], methyl yellow [11,12], and *p*-methyl red [14]. The inclusion processes of αCD and βCD with the anions of methyl orange, *o*-methyl red, and *p*-methyl red, were also investigated conductometrically [15,16]. In all cases the spectrophotometric and conductance data fitted a model involving 1:1 stoichiometry.

Owing to its larger cavity, cyclomaltooctaose (γ-cyclodextrin, γCD) tends to form ternary inclusion complexes, unlike α- and β-cyclodextrins. For example, crystal violet and methylene blue have been reported to form 1:1 inclusion complexes with βCD and 2:1 (guest:host) inclusion complexes with γCD [10]. Azo dyes such as roccellin [17] that usually exhibit no inclusion complexes with αCD form 1:1 inclusion complexes with βCD and 2:1 (dye:γCD) inclusion complexes with γCD.

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The purpose of the present study was to investigate the inclusion of *p*-methyl red in alkaline solutions (anion form) and in acidic solutions (cation form) with γCD in aqueous solutions and to report the thermodynamic parameters ΔG^0, ΔH^0 and ΔS^0 of these inclusion processes.

2. Experimental

The synthesis of the acid form of *p*-methyl red, its ^{1}H NMR spectrum and its elemental analysis have been reported previously [18]. γCD was purchased from Sigma (St. Louis, MO, USA) and was used without further purification. The required amount of γCD was weighed in the hydrated form and its concentration in solution was calculated on a dry basis [13].

A typical stock solution of *p*-methyl red in water had a concentration of 3.97×10^{-4} mol dm^{-3} and a pH value of 10.5. Under these conditions, *p*-methyl red is present as the sodium salt. Stock solutions of ca. 6.0×10^{-3} mol dm^{-3} of γCD were used. Solutions for the study of the inclusion process of the anion form (structure A, Fig. 1) were prepared by using a fixed amount of *p*-methyl red stock solution while varying the amount of γCD stock solution. In a typical experiment, 1.00 cm^3 of the *p*-methyl red stock solution was transferred to 25 cm^3 volumetric flask, followed by the required amount of γCD stock solution and 0.40 cm^3 of 8×10^{-3} mol dm^{-3} NaOH solution whereby a pH value of 9.5 and an ionic strength, *I*, of 1.50×10^{-4} mol dm^{-3} were attained. The same procedure was followed for studying the inclusion process of the cation at pH 1.0. In this case aqueous solutions of HCl (with a final concentration of 0.10 mol dm^{-3}) and NaCl were used. The latter was used to vary the ionic strength in the range 0.10–1.0 mol dm^{-3}. The final concentration of *p*-methyl red (at pH 9.5 and 1.0) was fixed at 1.586×10^{-5} mol dm^{-3}, while the concentration of γCD was varied in the range 1.0×10^{-4}–4.0×10^{-3} mol dm^{-3}. The UV–visible spectra of the test solutions were recorded at 16.0, 25.0, 33.0 and 40.5°C, using a double-beam spectrophotometer (DMS 100, Varian) and a quartz cell with optical path length of 1.00 cm. Other experimental details were the same as those used previously [11].

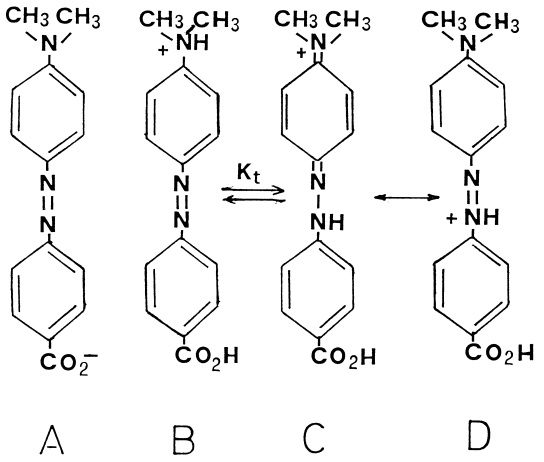

Fig. 1. Structural formulas of the anion (A) and the cation of *p*-methyl red. B is the ammonium tautomer, while C and D are the resonance structures of the azonium tautomer.

3. Results and discussion

The UV–visible spectrum of the *p*-methyl red anion shows two absorption maxima at 464 and 273 nm, which were assigned as π–π* and *n*–π* transitions of the azo linkage and the carboxylate group, respectively [18]. The addition of γCD to the aqueous solution of the *p*-methyl red anion resulted in a decrease in the intensity and a blue shift of ca. 25 nm at the longer wavelength accompanied by the formation of an isosbestic point at 447 nm. The absorption at 273 nm also decreased on the addition of γCD, but the absorption maximum remained unchanged. Fig. 2 illustrates such observations.

In 0.10 mol dm^{-3} HCl, *p*-methyl red is considered to be in the cationic protonated form [18], which is composed of the ammonium and azonium tautomers B, C and D shown in Fig. 1. The UV–visible spectrum of the *p*-methyl red cation shows two absorption maxima at 510 and 320 nm. The absorption at 510 nm is attributed to the azonium tautomer (the resonance hybrid of structures C and D, Fig. 1) and that at 320 nm to the ammonium tautomer (structure B, Fig. 1). This assignment was based on the literature of azobenzene dye spectra in acidic solutions [19]. The addition

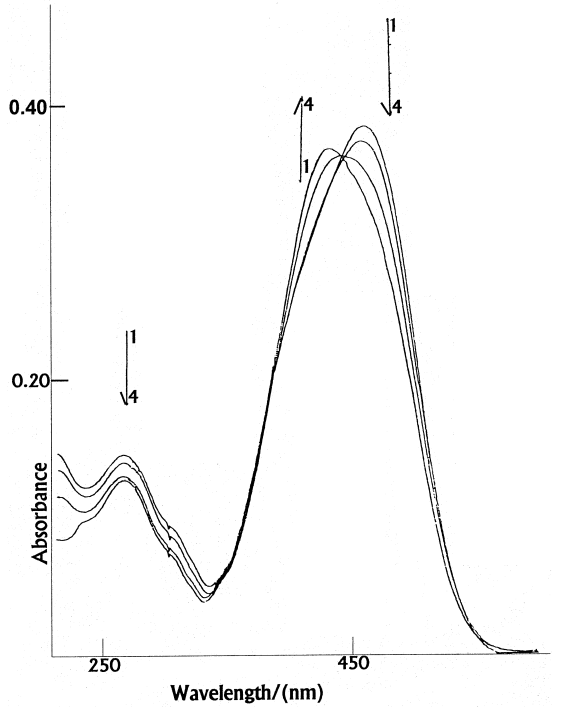

Fig. 2. The effect of γCD on the UV–visible spectrum of *p*-methyl red at pH 9.5 and 25°C. Values of 10^3[γCD] are 0.00, 0.30, 1.50 and 4.00 mol dm^{-3} for curves 1–4, respectively.

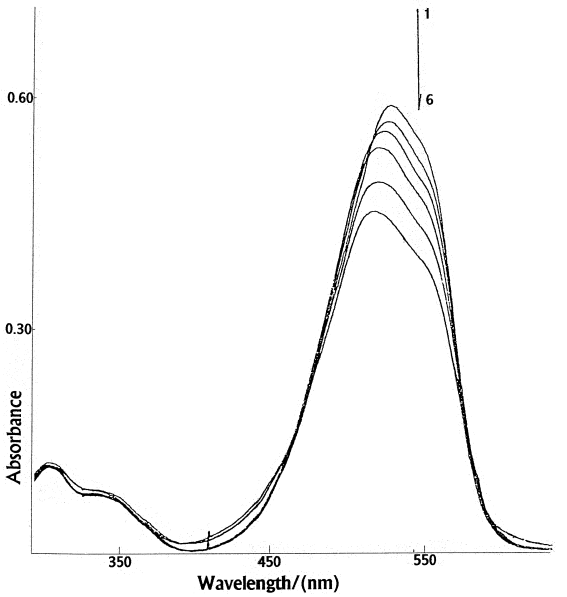

Fig. 3. The effect of γCD on the UV–visible spectrum of *p*-methyl red in 0.10 mol dm^{-3} HCl at 25°C. Values of 10^3[γCD] are 0.00, 0.50, 1.00, 1.50, 2.00, and 2.80 mol dm^{-3} for curves 1–6, respectively.

of γCD to the aqueous solution of the *p*-methyl red cation resulted in a significant reduction in the intensity of the absorption at 510 nm accompanied by a blue shift and the formation of an isosbestic point at 450 nm. The absorption at 320 nm was not affected by the addition of varying amounts of γCD. Appreciable absorption was noted for wavelengths higher than 600 nm. Fig. 3 shows the effect of γCD on the spectrum of *p*-methyl red in acidic solutions.

The spectral observations noted after the addition of γCD to both anion and cation solutions of *p*-methyl red indicate the occurrence of an inclusion process, where the anion or the cation bind to the γCD cavity. In principle, more than one stoichiometry can be proposed for the binding of *p*-methyl red to the γCD cavity. In addition, the cis-trans isomerism of the azo linkage and the tautomerism of the cation could lead to several inclusion complexes for a given stoichiometry. The investigation of all these possibilities was not feasible in the present study. The inclusion of *p*-methyl red by αCD and βCD was previously analyzed according to a 1:1 stoichiometry [14]. The Benesi–Hildebrand equation for 1:1 stoichiometry is as follows:

$$l.C_o.S_o/\Delta A = 1/(K_f.\Delta\varepsilon) + C_o/\Delta\varepsilon \quad (1)$$

where l is the optical path length of the cell used, C_o and S_o represent the initial molar concentrations of cyclodextrin and *p*-methyl red, respectively. ΔA is the change in the absorbance of *p*-methyl red following the addition of a cyclodextrin, K_f is the stability constant of the inclusion complex and $\Delta\varepsilon$ is the difference in the molar absorptivities between free and complexed *p*-methyl red. Eq. (1) is a straight line equation with slope equal to $1/\Delta\varepsilon$ and intercept equal to $1/K_f.\Delta\varepsilon$. For both the anion and the cation complexes of *p*-methyl red with αCD and βCD, the value of the ratio slope/intercept of Eq. (1) was found to be independent of the initial concentration of *p*-methyl red [14]. This evidence supports the assumption of 1:1 stoichiometry. However, a modification of Eq. (1) has been suggested for evaluating the stability constant of

2:1 (dye:cyclodextrin) inclusion complexes from spectrophotometric data [10]. The modified equation is:

$$l.C_o.S_o^2/\Delta A = 1/(K_f.\Delta\varepsilon) + 2S_oC_o/\Delta\varepsilon \quad (2)$$

Three possibilities can lead to Eq. (2). The first is where the dye is included as a dimer species from the aqueous solution. The second is a step-wise inclusion process, where the 1:1 complex forms first, then second dye monomer is added to the cavity to give the ternary 2:1 complex. The third possibility is the one-step formation of the 2:1 complex. All these possibilities can lead to Eq. (2) with certain approximations [10]. Since the dimerisation of *p*-methyl red is negligible under the present experimental conditions [18] and the third possibility involves a low probability third-order molecular reaction, the second possibility seems the most realistic for analyzing the spectrophotometric data of both the anion and cation complexes of *p*-methyl red with γCD. It should be pointed out that plotting ($l.C_o.S_o/\Delta A$) as ordinate vs. C_o, as abscissa cannot distinguish between 1:1 [Eq. (1)] and 2:1 [Eq. (2)] stoichiometries, since they both predict linear relationships. However, dividing both sides of Eq. (2) by S_o shows that the slope/intercept ratio of Eq. (2) depends on the initial concentration of the dye, S_o. Such dependence cannot be obtained for Eq. (1). We have verified these expectations for the binding of the anion and cation of *p*-methyl red with γCD. For example, doubling the concentration of *p*-methyl red anion at pH 9.5 and 25°C resulted in a corresponding doubling of the slope/intercept ratio of Eq. (2) for data at 470, 480, and 490 nm. The average values of K_f calculated from these ratios at different wavelengths and different values of S_o were within ±2.8% of the average value at 25°C. The dependence of the slope/intercept ratio of Eq. (2) on S_o was also verified for the binding of the *p*-methyl red cation with γCD at 520,525 and 530 nm. Based on these findings, the equilibrium spectrophotometric data were fitted using equation 2 on the assumption that the formation of the 2:1 inclusion complex occurs in a step-wise manner as found by Clarke et al. [9] for the methyl orange/γCD system. As illustrated in Fig. 4 the

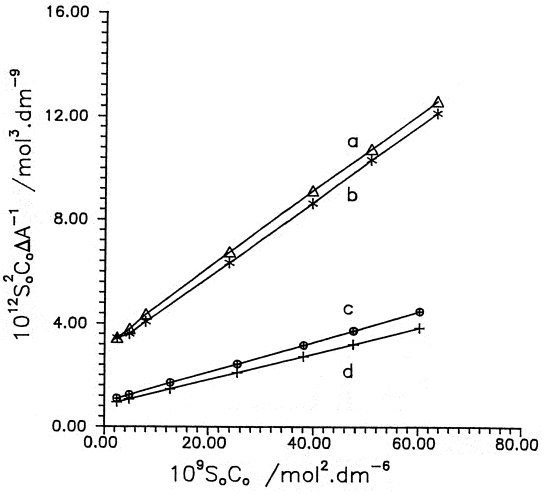

Fig. 4. Plot of equation 2 at 25°C with initial concentration of *p*-methyl red = 1.586×10^{-5} mol dm^{-3}. Lines a and b represent anion data at 490 and 470 nm, while lines c and d represent cation data at 520 and 530 nm, with an ionic strength of 0.10 mol dm^{-3} for the cation solutions.

data fit with Eq. (2) in all cases. The corresponding values of K_f (an overall equilibrium constant) were calculated from the linear least-squares analysis of the spectrophotometric data. The average values of K_f for the anion complex obtained at 16.0, 25.0, 33.0, and 40.5°C are $(3.16\pm0.09)\times10^7$, $(2.41\pm0.01)\times10^7$, $(2.07\pm0.09)\times10^7$, and $(1.68\pm0.07)\times10^7$ mol^{-2} dm^6, respectively. Since the ionic strength of the anion/γCD solutions was low (ca. 2×10^{-4} mol dm^{-3}), the K_f values were considered as thermodynamic ones. Consequently, the values of ΔH^0 and ΔS^0 of the 2:1 inclusion process of the anion form were obtained according to the following thermodynamic equation from the values of K_f obtained at several temperatures:

$$(2.303)R\ \log K_f = -\Delta H^0/T + \Delta S^0 \quad (3)$$

A linear least-squares analysis of the log K_f vs. $1/T$ data gave the following results: $\Delta H^0 = -19.0\pm1.1$ kJ mol^{-1} and $\Delta S^0 = 77.8\pm3.6$ J mol^{-1} K^{-1}. The value of ΔG^0, the Gibbs free energy, is -42.18 ± 0.03 kJ mol^{-1}.

Typical data for the calculation of K_f of the 2:1 inclusion complex of the *p*-methyl red cation with γCD at 25°C and at different ionic strengths showed that the ionic strength affects the value of K_f. This

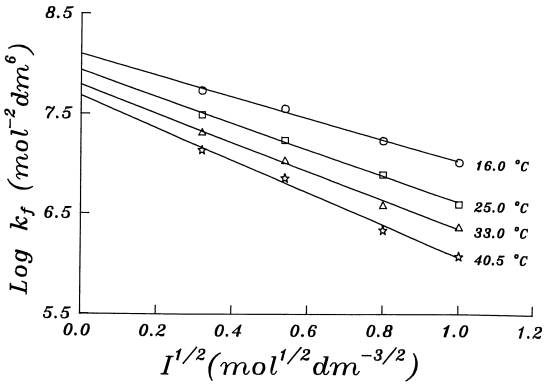

Fig. 5. The effect of ionic strength on the values of K_f for the inclusion of the *p*-methyl red cation by γCD at different temperatures.

finding is in accord with our work on αCD and βCD [14] and with the work of Smetana and Popov [20] concerning the effect of ionic strength on the equilibrium constant (defined in terms of concentrations) of an ion-molecule reaction. The influence of the ionic strength on K_f was examined at four different ionic strengths in the range of 0.10–1.0 mol dm^{-3} at each temperature. The data obtained are plotted in Fig. 5. At each temperature the value of the actual thermodynamic stability constant, K, can be obtained by extrapolation to zero ionic strength (actual values were obtained from the intercept of the linear least-squares analysis of the data of log K_f vs. $I^{1/2}$). Fig. 5 indicates that K_f decreases as the ionic strength increases. This result is in accord with other studies [14,20].

The values of log K, as obtained from the data of Fig. 5, are 8.09±0.04, 7.93±0.03, 7.79±0.06, and 7.68±0.07 at 16.0, 25.0, 33.0, and 40.5°C, respectively. These values of log K were subjected to a linear least-squares analysis according to Eq. (3). The values of ΔH^0 and ΔS^0 associated with the K value of the inclusion complex of the *p*-methyl red cation with γCD are $\Delta H^0 = -29.5 \pm 0.6$ kJ mol^{-1} and $\Delta S^0 = 52.8 \pm 2.0$ J mol^{-1} K^{-1}. The corresponding value of ΔG^0 is -45.1 ± 0.4 kJ mol^{-1}, indicating that the cation complex is more stable than the anion complex. It has been reported [12] that the addition of γCD to aqueous solutions of the acid forms of methyl orange and methyl yellow (at pH 1.1) causes no measurable changes in the absorbances of these azo dyes in the UV–visible region. However, in the present study, the structurally-related azo dye, *p*-methyl red was found to behave differently under the same experimental conditions.

Acknowledgements

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