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A Spectrophotometric Determination of the Formation Constants of the Inclusion Complexes of α- and β-Cyclodextrins with the Azonium and Ammonium Tautomers of Methyl Orange and Methyl Yellow

KHALID M. TAWARAH* and HAKAM M. ABU-SHAMLEH
Department of Chemistry, Yarmouk University, Irbid, Jordan

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Abstract. The color fading caused by the addition of α-cyclodextrin or β-cyclodextrin to an aqueous solution of a tautomeric mixture of methyl orange or methyl yellow is studied spectrophotometrically at pH 1.1 and 25.0°C. A model involving 1 : 1 stoichiometry has been used to analyze the spectrophotometric data. The addition of a cyclodextrin shifts the tautomeric mixture towards the side of the ammonium tautomer. An expression allowing the calculation of the tautomeric equilibrium constant of the inclusion complexes is derived. The formation constants of the inclusion complexes of the individual tautomers are determined. Both α- and β-cyclodextrins bind the ammonium tautomer stronger than the azonium tautomer. The inclusion complexes of α-cyclodextrin are more stable than the corresponding ones of β-cyclodextrin.

Key words. Methyl orange, methyl yellow, α-cyclodextrin, β-cyclodextrin, formation constants, inclusion complexes.

1. Introduction

The first conjugate acids of many *N*-substituted 4-aminoazobenzene dyes give a mixture of two tautomers in acid solutions [1–3]. An ammonium tautomer results when a proton is attached to the amino nitrogen and is responsible for the appearance of a UV band with λ_{max} at about 320 nm. An azonium tautomer (a resonating form) results when a proton is attached to the β-nitrogen of the azo linkage and is responsible for the appearance of a band in the visible region with λ_{max} at about 516 nm. The possibility of a third tautomer, where a proton may be attached to the α-nitrogen of the azo linkage, has been ruled out on the basis of the argument given by Yeh and Jaffe [4, 5], who showed that no α–β-tautomerism occurs in the conjugate acids of simple azobenzene derivatives not containing an amino group. It has also been reported that the position of the tautomeric equilibrium depends on the acid concentration in a manner favouring the azonium form as the acid concentration is increased [1].

Methyl yellow has two absorption maxima in 50% alcoholic 1N HCl [2]. The maximum at 516 nm was associated with the azonium tautomer while the maximum at 320 nm was associated with the ammonium tautomer. In aqueous H_2SO_4 solutions (6–24 wt.%) methyl yellow exhibited two absorption maxima, one at λ_{max}

* Author for correspondence.

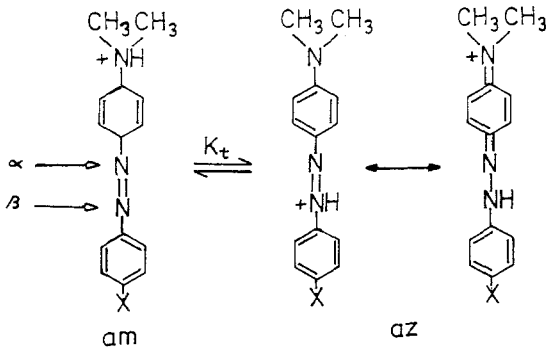

Fig. 1. Structural formulas of the ammonium tautomer (am) and the azonium tautomer (az) of the monoprotonated forms of methyl orange ($X = SO_3^-$) and methyl yellow (X = H).

of 516 nm and one at λ_{max} of 316 nm [4]. Likewise methyl orange exhibited two absorption maxima in aqueous solutions of 0.1 N H_2SO_4 [6]. The maximum appearing in the visible region at λ_{max} of 508 nm was associated with the azonium tautomer of the first conjugate acid of methyl orange, while the maximum appearing in the UV region at λ_{max} of 316 nm was associated with the ammonium tautomer. The tautomeric equilibrium constant, K_t, describing the ammonium (am) – azonium (az) tautomerism of the first conjugate acid of methyl yellow or methyl orange (see Figure 1) had been estimated to be 1–3 for methyl yellow [4] and 3–5 for methyl orange [6].

In spite of the availability of such literature information, the subject of ammonium-azonium tautomerism has not been thoroughly treated in connection with the formation of inclusion complexes between cyclodextrins and the monoprotonated forms of aminoazobenzene dyes. Recently, Buvari and Barcza [7] reported on the inclusion complexes of α-, β-, and γ-cyclodextrins with some azo dyes including methyl orange in aqueous 0.1 M HCl solutions. One of their assumptions was that $K_t \geqslant 100$ for the azo dyes they studied. Such a value is far from being realistic for the type of azo dyes in their work and is in contradiction with the value reported by Reeves [6] for methyl orange. In addition we could not find a numerical value for K_t in Ref. [9] of their paper, which seemed to be the source of their assumption. The formation constants of the inclusion complexes of the individual tautomers were not reported. Other investigators [8, 9] had also dealt with the inclusion complexes of the first conjugate acid of methyl orange with cyclodextrins and had only reported apparent formation constants of the inclusion complexes.

The purpose of the present work is to investigate the effect of α- and β-cyclodextrins on the UV–visible spectra of methyl orange and methyl yellow in aqueous HCl solutions, and to present a quantitative treatment of the various equilibria involved in a manner allowing the determination of the individual formation constants of the inclusion complexes of each tautomer.

2. Experimental

Methyl orange (sodium salt, $C_{14}H_{14}N_3SO_3Na$) and methyl yellow (base form, $C_{14}H_{15}N_3$) were purchased from Sigma and used without further purification.

Before its use, an azo dye sample was dried at 90°C in an oven for 16 hours. Methyl yellow is sparingly soluble in pure water. The solubility was enhanced in the presence of aqueous HCl. A typical stock solution of methyl yellow prepared in this manner had a concentration of 1.87×10^{-4} mol dm^{-3}, an ionic strength of 0.01 mol dm^{-3}, and a pH value of 2. A typical stock solution of methyl orange had a concentration of 1.86×10^{-4} mol dm^{-3}, an ionic strength of 1.86×10^{-4} mol dm^{-3}, and a pH value of 10. The samples of α- and β-cyclodextrins (α-CD, β-CD) were obtained from Sigma. The samples were hygroscopic, therefore the cyclodextrins were weighed as stable hydrates. These hydrates were found to have 6 H_2O molecules for α-CD and 9 H_2O molecules for β-CD. The concentrations reported in this study were adjusted to the dry basis. Stock solutions of about 6×10^{-3} mol dm^{-3} were prepared by weighing the required amount of a hydrated cyclodextrin followed by dissolving in distilled, deionized water. Solutions needed for studying the formation of the inclusion complexes were prepared by transferring a fixed amount (about 5 mL) of the azo dye stock solution to a 50-mL volumetric flask followed by adding the required amount of a cyclodextrin stock solution, then diluting to the mark with aqueous HCl. The pH of such solutions was adjusted at 1.1. The concentration of a cyclodextrin was varied in the range $1 \times 10^{-4} - 4.6 \times 10^{-3}$ mol dm^{-3}. The volume measurements were carried out by using a 10-mL microburette. The UV-visible spectra of the azo dyes were recorded in the 300–600 nm range by using a double-beam spectrophotometer (DMS 100, Varian). Stoppered quartz cells with an optical path length of 1.00 cm were used. Spectra were recorded at 25.0°C. In most cases the absorbance, displayed to the nearest 0.001, of a test solution was recorded at several wavelengths where the change in absorbances, caused by the addition of a cyclodextrin, is largest as revealed by the chart recorder.

3. Results and Discussion

We found that the UV–visible spectra of both methyl yellow and methyl orange were invariant with respect to changes in the acidity of the solution in the concentration range 0.032–0.46 M (M = mol dm^{-3}). This observation indicates that both azo dyes have been converted into the monoprotonated form (the ammonium-azonium tautomeric mixture). However HCl concentrations higher than 0.46 M resulted in an increase in the intensity of the maximum absorption in the visible region ($\lambda_{max} = 518$ nm for methyl yellow and 508 nm for methyl orange) and a decrease in the intensity of the maximum absorption in the UV region ($\lambda_{max} = 316$ nm for both methyl yellow and methyl orange). The maximum at 518 or 508 nm was attributed to the azonium tautomer, while the maximum at 316 nm was attributed to the ammonium tautomer [4, 6]. The following equation describes the tautomeric equilibrium of methyl yellow or methyl orange

$$\text{am} \overset{K_t}{\rightleftarrows} \text{az} \tag{1}$$

A value for K_t, expressed in terms of molar concentrations, can be calculated from the absorbance measurements by using the following equation

$$K_t = [\text{az}]/[\text{am}] = (\varepsilon - \varepsilon_{\text{am}})/(\varepsilon_{\text{az}} - \varepsilon) \tag{2}$$

where the square brackets denote molar concentrations, ε is the apparent molar absorptivity of the tautomeric mixture, ε_{am} and ε_{az} are the molar absorptivities of the ammonium and azonium tautomers respectively. The molar absorptivities of the pure tautomers cannot be measured directly and are estimated from the spectra of model compounds that exist only in a single form [6]. Yeh and Jaffe [4] have assumed that the molar absorptivity of the 4-phenylazo-*N*,*N*,*N*-trimethyl anilinium ion, $C_6H_5—N=N—C_6H_4—N^+(CH_3)_3$, is the same as ε_{am} for the ammonium tautomer of methyl yellow at 316 nm since the two cations differ only by replacement of an H-atom by a methyl group on the quaternary nitrogen atom. The molar absorbtivity of the model tautomer, ε_{am}, is 2.00×10^4 mol^{-1} dm^3 cm^{-1} at 316 nm. Likewise, Reeves [6] considered the 4-phenylazo-*N*,*N*,*N*-trimethyl 4′-sulfonato anilinium ion, $SO_3^-.C_6H_4—N=N—C_6H_4N^+(CH_3)_3$, as a model compound for the ammonium tautomer of methyl orange with $\varepsilon_{am} = 2.50 \times 10^4$ mol^{-1} dm^3 cm^{-1} in H_2O at 316 nm. At [HCl] = 0.46 M we have found that the apparent molar absorptivities of methyl yellow and methyl orange at $\lambda_{max} = 316$ nm are 9.24×10^3 and 6.84×10^3 mol^{-1} dm^3 cm^{-1} respectively. By using these values and the corresponding values of the model compounds, Equation 2 (with the assumption that $\varepsilon_{az} = 0$ at 316 nm) gives $K_t = 1.16$ for methyl yellow and $K_t = 2.65$ for methyl orange which are in good agreement with the literature values [4, 6]. However, further refinement of these values will be given later in this discussion.

The effect of adding α-CD on the UV–visible spectra of an aqueous monoprotonated methyl orange (HMO) and methyl yellow (HMY) is shown in Figures 2 and 3. Both figures indicate a large decrease in the intensity of the azonium band (visible region), a large increase in the intensity of the ammonium band (UV region), a small blue shift in the azonium band, an appreciable red shift (about 8 nm) in the ammonium band, and the formation of an isosbestic point at about 380 nm. The effect of β-CD on the UV–visible spectra of the monoprotonated forms of methyl orange and methyl yellow is indicated in Figures 4 and 5. A common observation regarding the effect of both α-CD and β-CD on the electronic spectra of the protonated forms of methyl orange and methyl yellow is that the intensity of the color of the solution fades with successive addition of a cyclodextrin. This color fading (red azonium form converting to colorless ammonium form) is most noticeable in the case of adding α-CD. However the color fading in the methyl yellow solutions is larger than that of the methyl orange solutions.

The monoprotonated forms of methyl yellow and methyl orange were found to obey Beer's law at several wavelengths in the concentration range $1.8 \times 10^{-6} - 6.50 \times 10^{-5}$ M. Consequently we assumed that both azo dyes exist in the monomer form at pH 1.1. It has been shown that the monoprotonated form of methyl orange forms only 1 : 1 inclusion complexes with cyclodextrins [7–9]. We have assumed this stoichiometry in this study. The formation of a 1 : 1 inclusion complex is represented by the following general equation

$$D + CD \overset{K_f}{\rightleftarrows} D.CD \tag{3}$$

where D, CD, D. CD, and K_f stand for an azo dye, a cyclodextrin, an inclusion complex, and the apparent formation constant respectively. The value of K_f can be obtained from the spectrophotometric data of Figures 2–5 by a graphical method

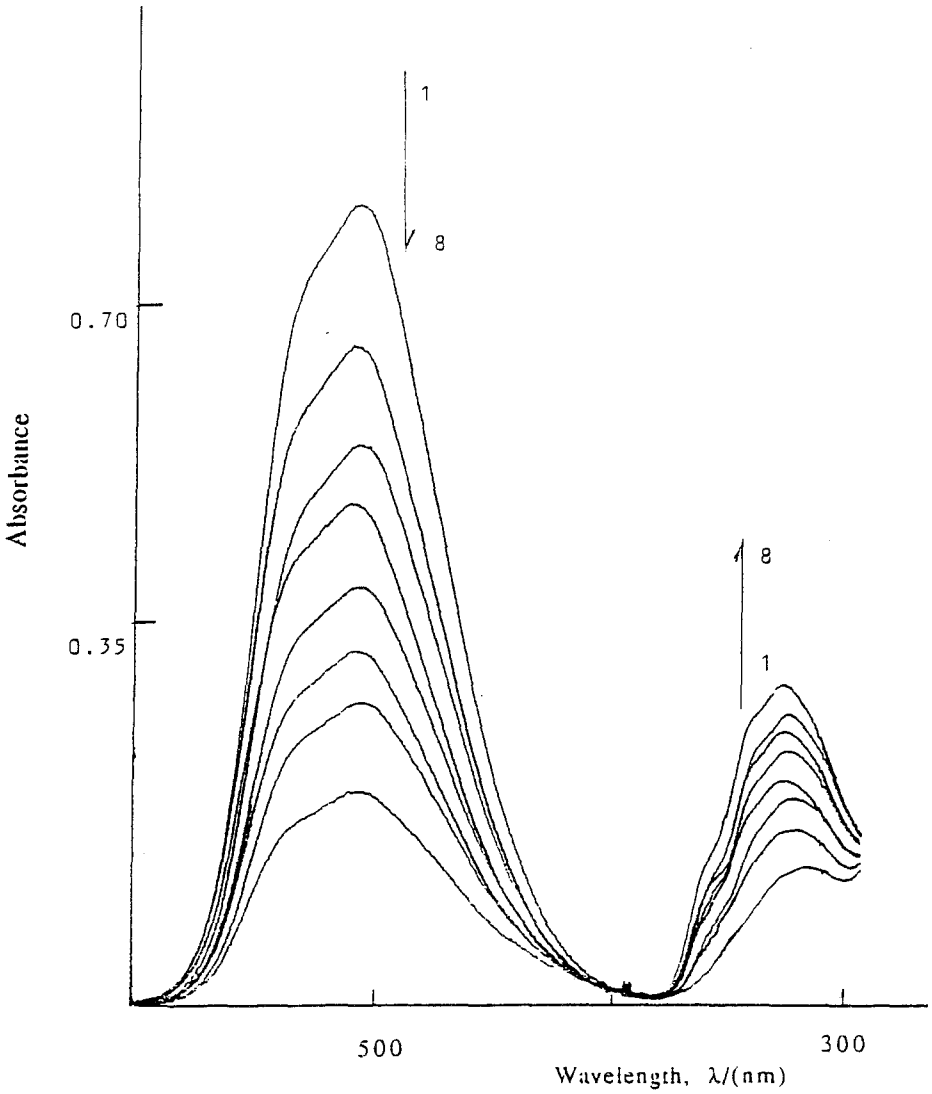

Fig. 2. The effect of α-CD on the UV-visible spectrum of 1.857×10^{-5} mol dm^{-3} methyl orange. [α-CD] $\times 10^3$: 0, 0.30, 0.60, 0.92, 1.45, 1.98, 3.96, and 4.62 mol dm^{-3} for spectra 1–8 respectively at pH = 1.1 and 25.0°C.

(or by linear regression analysis) based on the following equation

$$l \cdot C_0 \cdot S_0/\Delta A = (1/K_f \cdot \Delta\varepsilon) + C_0/\Delta\varepsilon \quad (4)$$

where l is the optical path length (in cm) of the cell used, C_0 and S_0 represent the initial concentrations (in mol dm^{-3}) of a cyclodextrin and an azo dye, ΔA is the change in the absorbance of an azo dye due to the addition of a cyclodextrin, and $\Delta\varepsilon$ is the difference in the molar absorptivities between the free and complexed azo

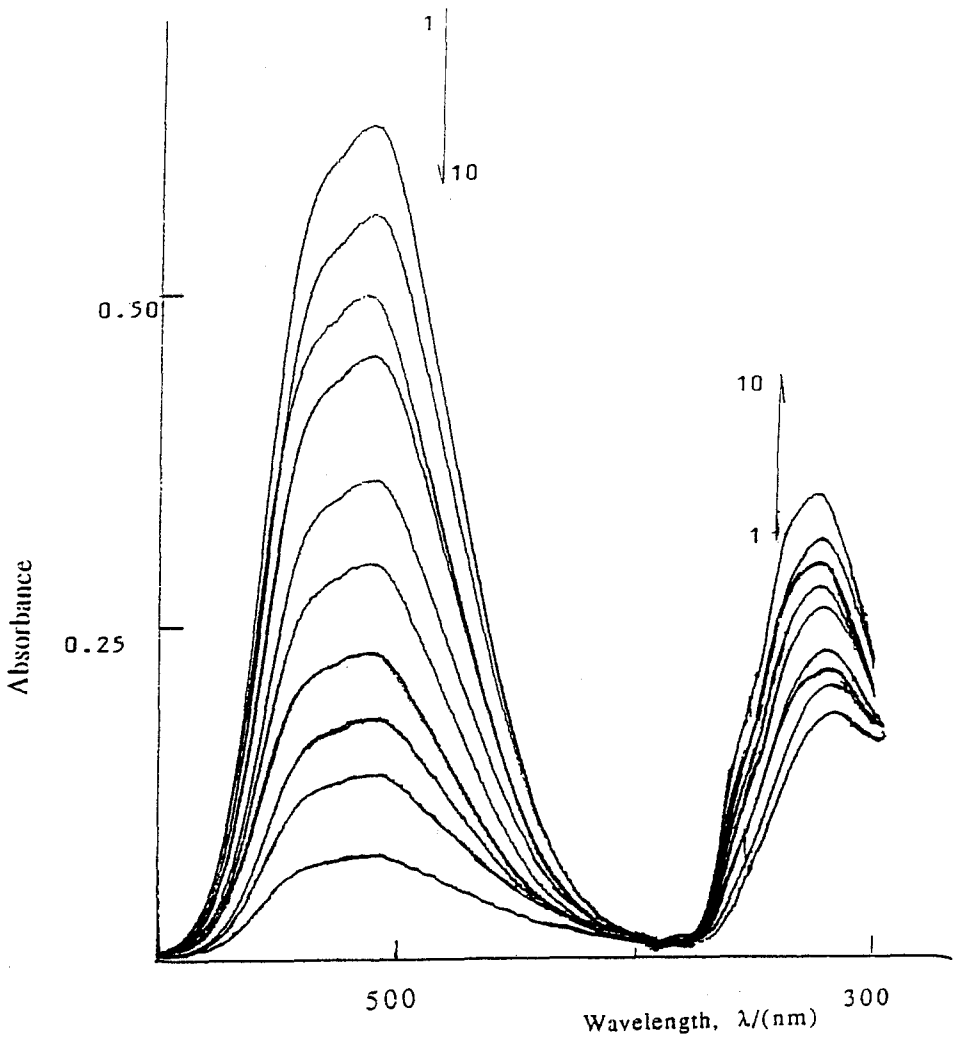

Fig. 3. The effect of α-CD on the UV-visible spectrum of 1.873×10^{-5} mol dm^{-3} methyl yellow. [α-CD] $\times 10^3$: 0, 0.22, 0.44, 0.66, 1.00, 1.30, 1.70, 2.10, 2.70, and 4.00 mol dm^{-3} for spectra 1–10 respectively at pH = 1.1 and 25.0°C.

dye. Equation 4 is usually referred to as the Benesi–Hildebrand equation [10] and is applicable if $C_0 \gg S_0$. For a graphical solution, the left-hand side of Equation 4 is the ordinate while C_0 is the abscissa. The spectrophotometric data (values of ΔA at several wavelengths) collected from the effect of α-CD and β-CD on the electronic spectra of the monoprotonated forms of methyl orange and methyl yellow at pH 1.1 (Figures 2–5) were found to be consistent with the general complexation reaction defined by Equation 3. Typical data considered for the calculations of the apparent formation constants of the inclusion complexes of the monoprotonated forms of methyl orange and methyl yellow with α-CD and β-CD

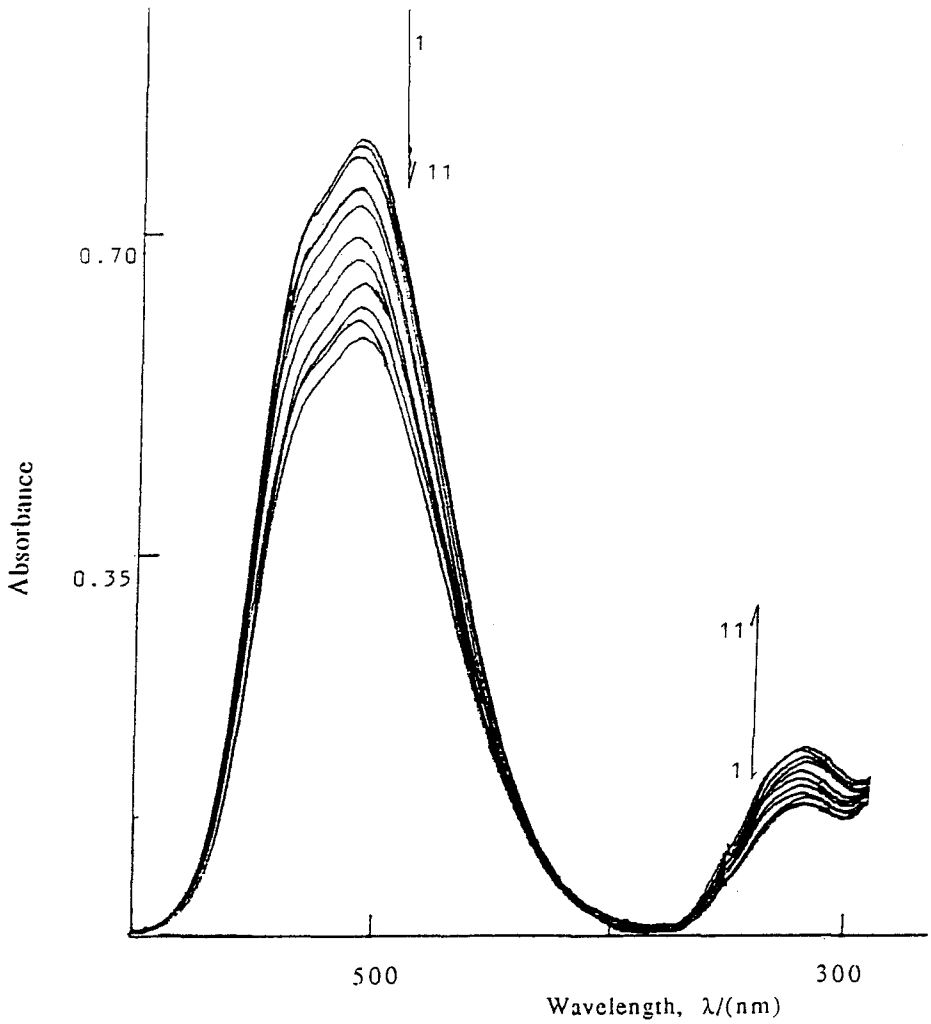

Fig. 4. The effect of β-CD on the UV-visible spectrum of 1.857×10^{-5} mol dm^{-3} methyl orange. [β-CD] $\times 10^3$: 0, 0.10, 0.18, 0.36, 0.56, 1.30, 1.80, 2.40, 3.00, 3.60, and 4.20 mol dm^{-3} for spectra 1–11 respectively at pH = 1.1 and 25.0°C.

are shown in Tables I and II, respectively. The data of these tables indicate that K_f is wavelength independent. This is true for the UV region and the average K_f values are given in Table III.

We propose the following two equilbria to account for the formation of 1:1 inclusion complexes of the individual tautomers

$$\text{am} + \text{CD} \overset{K_{\text{am}}}{\rightleftharpoons} \text{am} \cdot \text{CD} \tag{5}$$

$$\text{az} + \text{CD} \overset{K_{\text{az}}}{\rightleftharpoons} \text{az} \cdot \text{CD} \tag{6}$$

where K_{am} and K_{az} are the formation constants of the ammonium complex and the

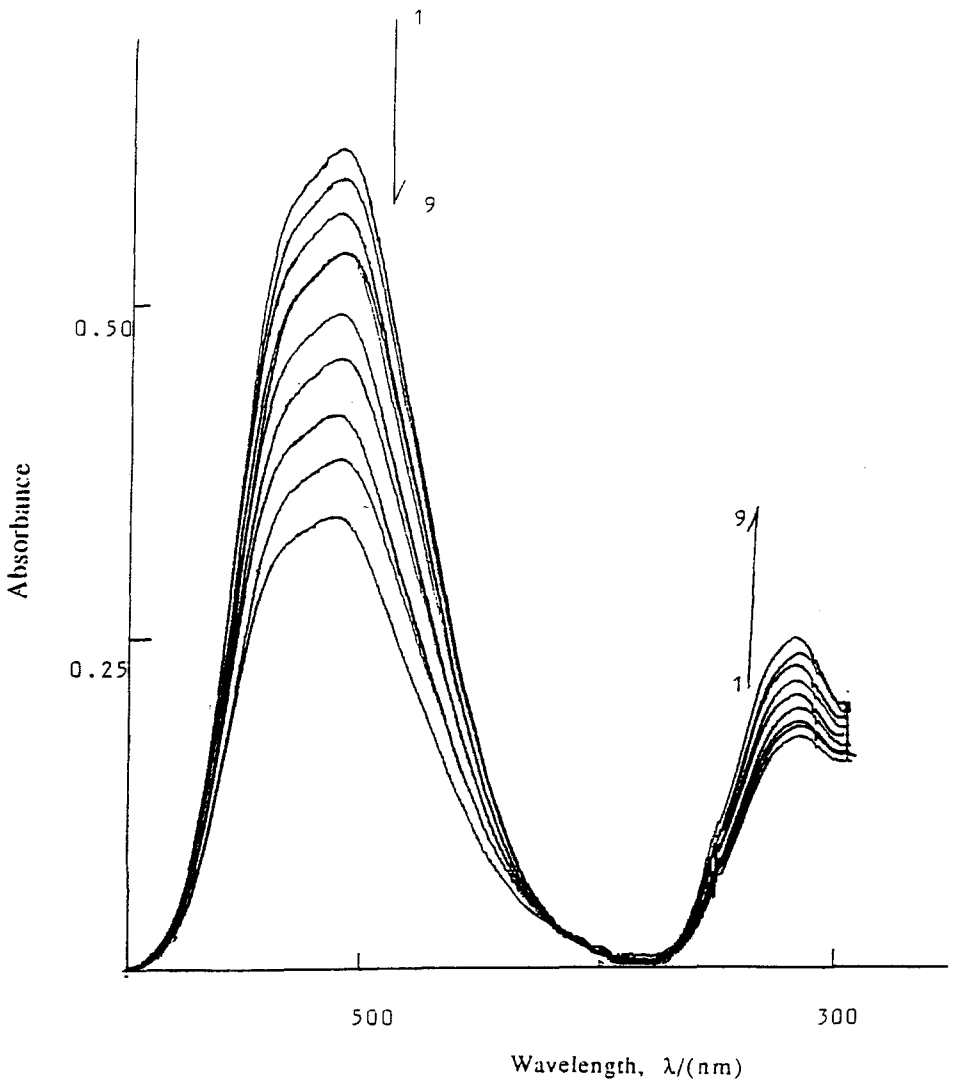

Fig. 5. The effect of β-CD on the UV-visible spectrum of 1.873×10^{-5} mol dm^{-3} methyl yellow. [β-CD] $\times 10^3$: 0, 0.12, 0.24, 0.48, 0.84, 1.20, 1.80, 2.40, and 3.6 mol dm^{-3} for spectra 1–9 respectively at pH = 1.1 and 25.0°C.

azonium complex, respectively. The equilibria of Equations 3, 5, and 6 define K_f as follows

$$(K_f)^{-1} = [\mathrm{CD}]\{[\mathrm{az}] + [\mathrm{am}]\}\{[\mathrm{am} \cdot \mathrm{CD}] + [\mathrm{az} \cdot \mathrm{CD}]\}^{-1} \quad (7)$$

The reasoning behind our proposal of the two equilibria given by Equations 5 and 6 is based on the following argument. Since the addition of a cyclodextrin resulted in a decrease in the intensity of the azonium band and an increase in the intensity of the ammonium band (as in Figures 2–5), the equilibrium of Equation 6 cannot

Table I. Spectrophotometric data at different wavelengths for calculating K_f for HMO/α-CD complex at 25.0°C

10^3C_0	A_{505}	$\frac{10^3C_0}{\Delta A_{505}}$	A_{525}	$\frac{10^3C_0}{\Delta A_{525}}$	A_{530}	$\frac{10^3C_0}{\Delta A_{530}}$	A_{535}	$\frac{10^3C_0}{\Delta A_{535}}$
0.000	0.808	–	0.754	–	0.733	–	0.697	–
0.330	0.668	2.357	0.621	2.481	0.603	2.538	0.574	2.683
0.660	0.569	2.762	0.528	2.920	0.511	2.973	0.486	3.128
0.924	0.508	3.080	0.470	3.254	0.455	3.324	0.433	3.500
1.452	0.425	3.791	0.391	4.000	0.379	4.102	0.360	4.309
1.979	0.365	4.467	0.334	4.712	0.323	4.827	0.307	5.074
2.639	0.308	5.278	0.280	5.568	0.270	5.700	0.257	5.998
3.299	0.269	6.121	0.243	6.456	0.235	6.624	0.223	6.960
3.959	0.240	6.970	0.215	7.345	0.208	7.541	0.198	7.934
4.619	0.218	7.829	0.194	8.248	0.188	8.475	0.179	8.917

$C_0 = [\alpha\text{-CD}]$ in mol dm^{-3}, $[\text{HMO}] = 1.857 \times 10^{-5}$ mol dm^{-3}, and A_n is the absorbance at wavelength n in nm.

Table II. Spectrophotometric data at different wavelengths for calculating K_f for HMY/β-CD complex at 25.0°C.

10^3C_0	A_{518}	$\frac{10^3C_0}{\Delta A_{518}}$	A_{525}	$\frac{10^3C_0}{\Delta A_{525}}$	A_{540}	$\frac{10^3C_0}{\Delta A_{540}}$	A_{545}	$\frac{10^3C_0}{\Delta A_{545}}$
0.000	0.624	–	0.609	–	0.554	–	0.503	–
0.24	0.579	5.33	0.565	5.45	0.515	6.15	0.468	6.86
0.84	0.497	6.61	0.487	6.88	0.444	7.64	0.404	8.48
1.20	0.466	7.60	0.456	7.84	0.415	8.63	0.379	9.68
1.80	0.422	8.91	0.413	9.18	0.377	10.17	0.344	11.32
2.40	0.392	10.34	0.385	10.71	0.351	11.82	0.321	13.19
3.60	0.351	13.19	0.344	13.58	0.314	15.0	0.287	16.67

$C_0 = [\beta\text{-CD}]$ in mol dm^{-3}, $[\text{HMY}] = 1.873 \times 10^{-5}$ mol dm^{-3}, and A_n is the absorbance at wavelength n in nm.

be considered as the only inclusion process because it will lead to an increase in the intensity of the azonium band. The same applies for the case of having the two equilibria of Equations 5 and 6 with $K_{az} > K_{am}$. The last possibility which might be considered is to have the equilibrium of Equation 5 as the only inclusion process. Although this possibility does not contradict the observed changes in the intensities of the azonium and ammonium bands, it does not account for the observed shifts in the maximum of each band. Therefore the only scheme which can be in accord with the observed changes in the intensities, the shifts in the two maxima, and the presence of the azonium band even at relatively high concentrations of a cyclodextrin is the one given by Equations 5 and 6 with $K_{am} > K_{az}$.

K_{am} and K_{az} can be obtained by considering the following equilibria

$$\begin{array}{ccc} \text{az} + \text{CD} & \overset{K_{az}}{\rightleftharpoons} & \text{az} \cdot \text{CD} \\ \downarrow\uparrow K_t & & \downarrow\uparrow K^* \\ \text{am} + \text{CD} & \underset{K_{am}}{\rightleftharpoons} & \text{am} \cdot \text{CD} \end{array} \qquad (8)$$

Table III. Values of the apparent formation constants of the 1:1 inclusion complexes of the monoprotonated forms of methyl orange (HMO) and methyl yellow (HMY) at 25.0°C.

Azo dye	Cyclodextrin	$K_f^a \pm S^b$	Reference
HMO	α-CD	670 ± 10	This work
		925[c]	[7]
		800	[9]
		470	[11]
	β-CD	270 ± 10	This work
		300[c]	[7]
		300	[8]
		370	[9]
		230 ± 40	[12]
HMY	α-CD	1200 ± 10	This work
	β-CD	490 ± 10	This work

[a] K in $mol^{-1}\ dm^3$.
[b] Standard deviation.
[c] Temperature was not specified.

where K^* is the tautomeric equilibrium constant of the tautomerism of the az · CD and am · CD complexes and is defined by Equation 9

$$K^* = [\text{az} \cdot \text{CD}]/[\text{am} \cdot \text{CD}] \tag{9}$$

It can be shown that

$$K_{az}/K_{am} = K^*/K_t \tag{10}$$

The combination of Equations 7, 9 and 10 yields the following equations

$$K_{am} = K_f(1 + K_t)(1 + K^*)^{-1} \tag{11}$$

$$K_{az} = K_f(1 + K_t)(1 + K^*)^{-1}(K^*/K_t) \tag{12}$$

Since K_f and K_t are known, Equations 11 and 12 allow the determination of K_{am} and K_{az} provided that a value for K^* is available. An equation defining K^* in terms of K_t and molar absorptivities can be derived as follows. The apparent molar absorptivity, ε, of the tautomeric mixture is related to the individual molar absorptivities of the uncomplexed tautomers, ε_{az} and ε_{am}, by the relation

$$\varepsilon(1 + K_t) = \varepsilon_{az} \cdot K_t + \varepsilon_{am} \tag{13}$$

In the presence of a cyclodextrin, the analogue of Equation 13 is

$$\varepsilon^*(1 + K^*) = K^* \cdot \varepsilon^*(\text{az} \cdot \text{CD}) + \varepsilon^*(\text{am} \cdot \text{CD}) \tag{14}$$

where ε^* is an apparent molar absorptivity of a tautomeric mixture of complexes, ε^* (az · CD) and ε^* (am · CD) are the individual molar absorptivity of the azonium and ammonium complexes respectively. The value of ε^* can be obtained from the slope of Equation 4 ($\Delta\varepsilon = \varepsilon - \varepsilon^*$). Subtracting Equation 13 from Equation 14 yields

$$K^* = \{\varepsilon^* - \varepsilon^*(\text{az} \cdot \text{CD})\}^{-1}\{\varepsilon - \varepsilon^* + \varepsilon^*(\text{am} \cdot \text{CD}) - \varepsilon_{am} + K_t(\varepsilon - \varepsilon_{az})\} \tag{15}$$

The unavailability of model compounds suitable for estimating the molar absorptivities of the individual tautomers, whether free or complexed, in the visible region, requires that certain simplifying assumptions are to be introduced in order to deal with Equation 15. If we let $\varepsilon_{am} = \varepsilon^*$ (am · CD) = 0 and $\varepsilon_{az} = \varepsilon^*$(az · CD) = ε in the visible region, then Equation 15 gives a negative value for K^* since $\varepsilon > \varepsilon^*$ in the visible region. However, if we consider data in the UV region and rule out the assumption that $\varepsilon^* = \varepsilon^*$ (am · CD), since it leads to $\varepsilon^* = \varepsilon^*$ (az · CD) according to Equation 14 which is unrealistic, then the approximations $\varepsilon_{az} = \varepsilon^*$ (az · CD) and $\varepsilon_{am} = \varepsilon^*$ (am · CD) transform Equation 15 into the form

$$K^* = \{\varepsilon^* - \varepsilon_{az}\}^{-1}\{\varepsilon(1 + K_t) - K_t \cdot \varepsilon_{az} - \varepsilon^*\} \tag{16}$$

The approximations leading to Equation 16 require that the formation of an inclusion complex has no dramatic effect on the molar absorptivity of a given tautomer. This seems reasonable since weak bonding is usually involved in the formation of such inclusion complexes. A further simplification can be introduced into Equation 16 by assuming that $\varepsilon_{az} = 0$ at 316 nm. However, some authors [4, 6] have used the approximation $\varepsilon_{az} = 2 \times 10^3$ cm^{-1} mol^{-1} dm^3, a value obtained at 320 nm by Wepster [13] for the first conjugate acid of 4-amino-3, 5-di-*t*-butylazobenzene which exists solely as the azonium species. We have used Wepster's value for calculating K_t and K^* as given by Equations 2 and 16, respectively. By using the values of K_f, K_t, and K^* Equations 11 and 12 were solved for K_{am} and K_{az}. The results of our calculations are shown in Table IV together with the values of ϵ and ϵ^* at 316 nm.

The data of Table IV indicate that the values of K_{am} for the α-CD complexes are higher than their counterparts for the β-CD complexes and K_{az} is less than K_{am} for a given azo dye/cyclodextrin system. These findings are in agreement with the strong color fading noted after the addition of α-CD as compared to that of β-CD and in agreement with the spectral changes evident in Figures 2–5. The values of K^* for α-CD complexes are less than the corresponding ones of β-CD complexes and all of them are less than K_t for methyl orange or methyl yellow. This means that the addition of a cyclodextrin shifts the tautomeric equilibrium of an azo dye in favour of the ammonium tautomer, i.e. stronger complexation is achieved by the ammonium tautomer. Our values for K^* are different from those reported by Buvari and Barcza [7] who, we believe, oversimplified the calculation of K^*.

Table IV. Values of the apparent molar absorptivities, tautomeric equilibrium constants, and the formation constants of the ammonium and azonium inclusion complexes at 25.0°C.

Azo dye	$10^{-4}\,\varepsilon^a$ (316 nm)	K_t	CD	$10^{-4}\varepsilon^{*a}$ (316 nm)	K^*	K_{az}^b ($\times 10^{-2}$)	K_{am}^b ($\times 10^{-2}$)
HMO	0.684	3.75	α	1.86	0.38	2.3	23
			β	1.43	0.87	1.6	6.8
HMY	0.924	1.49	α	1.79	0.13	2.3	26
			β	1.64	0.25	1.6	9.8

[a]in mol^{-1} dm^3 cm^{-1}.
[b]in mol^{-1} dm^3.

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