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Colour Change and Tautomerism of Some Azo-Indicators on Complex Formation with Cyclodextrins

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Abstract. Complex formation of methyl orange and some other azobenzene derivatives with cyclodextrins has been studied in acidic and alkaline solutions by a spectrophotometric method. A correlation has been established between the stability constants of the complexes, the spectral changes accompanying complex formation and a tautomeric equilibrium between protonation on the azo and dimethylamino group. Our own results have been compared with some literature data, and equilibrium constants for the tautomeric rearrangement have been calculated. It has been demonstrated that the significant colour changes observed with the protonated forms only (and not with the corresponding bases) is really caused by a shift of the tautomeric equilibrium.

Key words. Methyl orange, inclusion complexes, protonation tautomerization.

1. Introduction

Complex formation with cyclodextrins is usually not accompanied by major changes in the UV–visible absorption spectra of the guest compounds, the differences are similar to those caused by changes of solvent only. Some compounds, however – mainly those used otherwise as indicators – exhibit significant spectral changes, suitable for determining the stability constants of the complexes [1–3].

A quite special case of these compounds is provided by phenolphthalein when the doubly ionized (purple) form becomes colourless (though unprotonated) when included in β-cyclodextrin [4].

In most cases compounds of an acid–base character are involved, when really the significant spectral change is caused by a shift in the protonation equilibria, if the complex formation constants for the conjugate acid and base forms are different [1, 3, 5–7].

Azo-type indicators are known to exist in alkaline solutions as unprotonated (yellow) forms and in acidic media as protonated ones. The red colour is attributed to the formation of a quinoidal structure due to protonation on the azo group. Besides the protonation tautomerism, a further complication is the possibility of *cis–trans* isomerization. These changes have been widely studied [8, 9]. Protonation can take place either on the amino group (when the —N=N— azo structure remains intact), or on the azo group. As Machida *et al.* have shown by Raman spectroscopy [9], in acidic aqueous solutions methyl orange exists almost totally as the azonium–quinoid form, protonated on the N-atom in the β-position relative to the dimethylaminophenyl moiety. (This means that protonation on the azo α-N exists only in principle, because in that case the quinoid structure could not be formed.)

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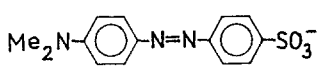
In regard to the *cis–trans* isomerization: in the state in which the compound is protonated on the azo group, no *cis* or *trans* configuration can be distinguished. In the case of the unprotonated yellow form, when the concentration of the energetically unfavourable *cis* configuration can be increased by light irradiation, back-transition to the *trans* state is considerably promoted by acids or even by weaker proton donating agents [10]. Moreover, in the complex with α-cyclodextrin no *cis* configuration can be detected in the unprotonated state either [11].

In the case of methyl orange, the greatest change in absorbance caused by complexation with cyclodextrins can be observed in solutions of pH = 2.6 at the absorption band of the acidic form, when the decrease in absorbance is caused mainly by complex formation with the unprotonated form [1]. With methyl orange and some other azo dyes, however, it has been observed that in strongly acidic solutions addition of cyclodextrins results in a significant decrease in the intensity of the red colour, without any considerable shift in the wavelength of the maximum [5, 12–14]. According to Szejtli *et al.* [1] and Matsui and Mochida [14], the explanation for this decrease in absorbance is that, on inclusion, the red coloured azonium-quinoid form of the indicator is transformed via a tautomeric equilibrium into a form protonated on the Me_2N-group, and this form is colourless.

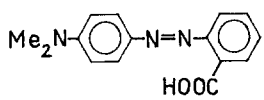
Because of the different sizes of the α-, β- and γ-cyclodextrins, the fit of the guest in the CD ring will be different, so the effect of complex formation with the different cyclodextrins on the tautomeric equilibria are also different.

Our aim was to investigate the connection between the tautomeric equilibrium, the apparent molar absorptivities and the stability constants of the complexes of some azo dyes with different cyclodextrins.

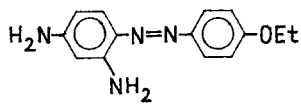
Complex formation of methyl orange (**I**) has been studied with β- and γ-cyclodextrin in acidic solutions and with α-cyclodextrin in an alkaline medium. The results have been compared to some supplementary literature values and equilibrium constants for the tautomeric rearrangement have been calculated. The investigations have been complemented with those for methyl red (**II**) and *p*-ethoxychrysoidine (4-ethoxy-2′,4′-diaminoazobenzene, **III**, see Figure 1) with β-cyclodextrin.



methyl orange, **I**



methyl red, **II**



p-ethoxy-chrysoidine, **III**

Fig. 1. Structural formulae of the indicators discussed.

2. Experimental

α-, β- and γ-cyclodextrins were obtained from the Chinoin Chemical-Pharmaceutical Works (Budapest, Hungary), and were recrystallized twice from water.

The indicators were Reanal products of analytical grade and no impurities could be detected.

All pH buffer reagents were of analytical grade.

UV–visible absorption spectra of indicators were recorded on a Zeiss Specord double-beam spectrophotometer in acidic (0.1 mol dm^{-3} HCl) and in alkaline (10^{-3} mol dm^{-3} NaOH) solutions and at a pH in the range corresponding to the acid–base transition: pH = 5.06 (phosphate buffer) for **II** and **III** and pH = 2.6 (formic acid) for **I** in pure solutions of the indicators and in the presence of 6.2×10^{-3} mol dm^{-3} β-CD as well. The concentration of the indicators was 3.0×10^{-5} mol dm^{-3}. (Dimerization of the indicators and formation of 1 : 2 complexes at this low concentration can be neglected [15, 16]; it is promoted only by γ-CD, if the concentration is at least 5×10^{-5} mol dm^{-3} [17].)

Measurements were carried out under the same conditions using a Spectromom 361 spectrophotometer at wavelengths selected according to the absorption maxima of the acid and base forms of the indicators: 505 and 455 nm for **I**, 520 and 432 nm for **II** and 473, 450 and 410 nm for **III**. The concentrations of the indicators were $2–4 \times 10^{-5}$ mol dm^{-3} and those of the α-, β- and γ-cyclodextrins were varied between $10^{-4}–2.5 \times 10^{-3}$ mol dm^{-3}, $2.4 \times 10^{-4}–8 \times 10^{-3}$ mol dm^{-3} and $10^{-3}–2.5 \times 10^{-2}$ mol dm^{-3}, respectively.

3. Results and Discussion

The changes caused by β-CD in the spectra of methyl red are shown in Figure 2. It is remarkable that the effect is greater in the case of the protonated form, although the formation constant of the unprotonated complex is larger (see later), and this difference is even more pronounced in the case of methyl orange [1].

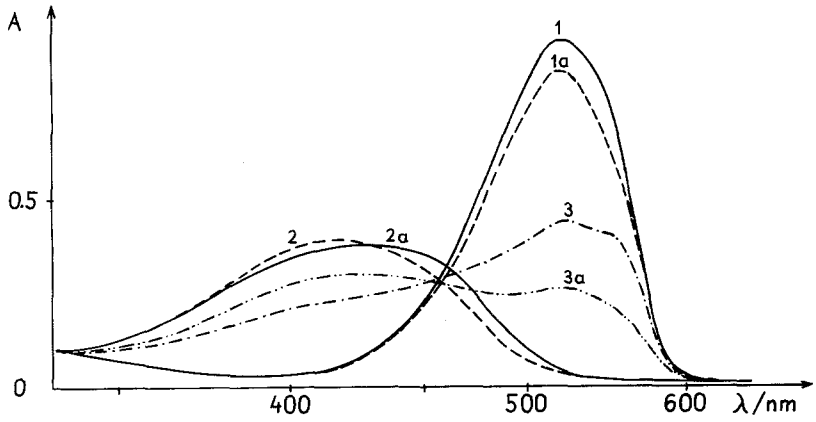


Fig. 2. UV-visible spectra of 3.0×10^{-5} mol dm^{-3} methyl red alone, and (a) in the presence of 6.2×10^{-3} mol dm^{-3} β-cyclodextrin: 1, 1a: pH = 1; 2, 2a: pH = 11; 3, 3a: pH = 5.06.

The experimental data were evaluated in two different ways. For those data obtained in strongly acidic or alkaline solutions, only a single form of the free indicator (protonated or unprotonated) had to be taken into account. So, as the cyclodextrin was present in a relatively large excess, a simple graphical solution could be employed to obtain the stability constants (K) and molar absorptivities (ε_{CDI}) of the corresponding complexes, based on the equation:

$$A = \frac{\varepsilon_I c_I - A}{K c_{CD}} + \varepsilon_{CDI} c_I \tag{1}$$

where A is the measured absorbance; c_I and c_{CD} are the total concentrations of the indicator (protonated or unprotonated) and cyclodextrin, respectively, and ε_I is the molar absorptivity of the free indicator (measured separately). (Since c_I was kept constant, the $(\varepsilon_I c_I - A)/c_{CD}$ quotient was also known.)

Data measured at pHs corresponding to the acid–base transition were evaluated by an iterative procedure with a personal computer, using the equations describing the total concentrations:

$$\begin{aligned} c_I &= [I] + [HI] + [CD \cdot I] + [CD.HI] \\ &= [I] + K_H[H^+][I] + K_I[CD][I] + K_{HI}K_H[H^+][CD][I], \end{aligned} \tag{2}$$

$$\begin{aligned} c_{CD} &= [CD] + [CD \cdot I] + [CD \cdot HI] \\ &= [CD] + K_I[CD][I] + K_{HI}K_H[H^+][CD][I] \end{aligned} \tag{3}$$

and the absorbances measured at the ith wavelengths:

$$A^i = \varepsilon^i_I[I] + \varepsilon^i_{HI}K_H[H^+][I] + \varepsilon^i_{CDI}K_I[CD][I] + \varepsilon^i_{CDHI}K_{HI}K_H[H^+][CD][I], \tag{4}$$

where K_H is the protonation constant of the indicator base (taken from the literature [18] or estimated according to the experimental pH of the colour change; in the final refinement these were also handled as adjustable parameters); K_I and K_{HI} are the complex formation constants of the unprotonated and protonated forms of the indicator, respectively; ε^is are the molar absorptivities of each species; and [ ] denote equilibrium concentrations.

ε^i_{HI} and ε^i_I were known from separate measurements in pure acidic and alkaline solutions. The procedure was similar to that followed in our previous work [7]: estimated values for ε^i_{CDHI}, ε^i_{CDI}, K_{HI} and K_I were substituted into equations (2)–(4) first, and they were varied until the best fit between experimental and calculated values of A^i was reached.

The evaluations of the different types of measurements gave the same results within the limits of experimental errors (standard deviations of the equilibrium constants are less than ± 10 per cent).

When the tautomeric rearrangement is taken into account as well, the significant equilibria and colour changes for methyl orange can be symbolized according to Figure 3. (*Cis–trans* isomerization is not indicated because participation of the *cis* form in these processes is negligible [8–11].)

Protonation on the azo group must be hindered by insertion of the guest into the cyclodextrin cavity, so, if the significant spectral change accompanying complex formation of the protonated form is really caused by a shift in the tautomeric rearrangement, it is expected to be the more pronounced the more tightly the guest

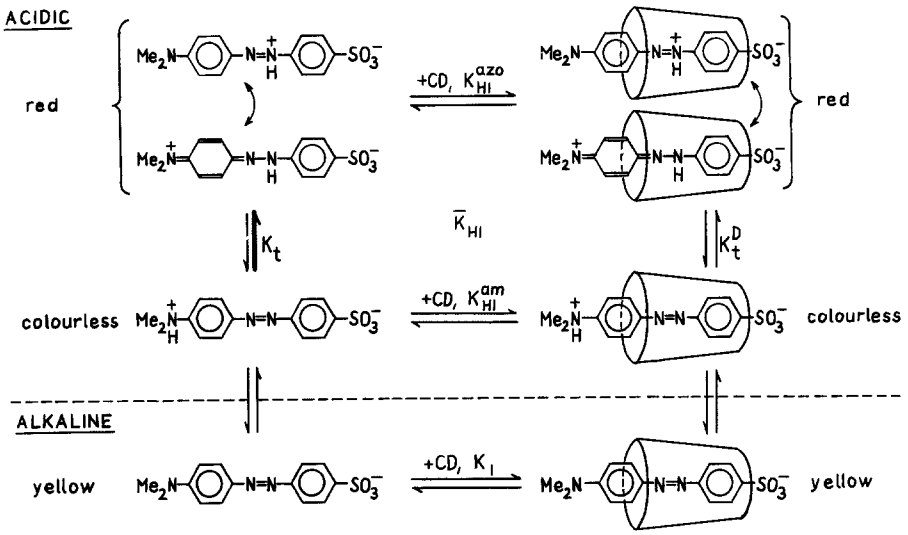


Fig. 3. Protonation and complex formation equilibria of methyl orange.

fits into the cavity, i.e. the higher the stability constant of the complex (at least, in so far as the type of complex formation is the same).

The equilibrium constant for the tautomeric rearrangement can be defined as

$$K_t = \frac{[HI_{azo}]}{[HI_{am}]},$$

where subscripts 'azo' and 'am' refer to the indicator protonated on the azo and dimethylamino group, respectively. In strongly acidic solutions without any cyclodextrin, the total concentration of the dye can be described as

$$[HI]_T = [HI_{azo}] + [HI_{am}] = [HI_{azo}](1 + 1/K_t). \tag{6}$$

Since the absorbance measured in the visible range under these conditions is provided only by the species protonated on the azo group (that is $\varepsilon_{am} \cong 0$), the measured absorbance is:

$$A = \varepsilon_{azo}[HI_{azo}] \tag{7}$$

and if it is correlated with the total concentration, the apparent character of the calculated molar absorptivity can be seen:

$$\varepsilon_{HI} = \frac{A}{c_I} = \frac{A}{[HI]_T} = \frac{\varepsilon_{azo}}{1 + 1/K_t}. \tag{8}$$

Similarly, the equilibrium constant for the tautomerism and the (apparent) molar absorptivity of the complex can be defined:

$$K_t^D = \frac{[CD \cdot HI_{azo}]}{[CD \cdot HI_{am}]} \tag{9}$$

and

$$\varepsilon_{CDHI} = \frac{\varepsilon^{D}_{azo}}{1 + 1/K^{D}_{t}}. \tag{10}$$

Combining equations (8) and (10)

$$\frac{\varepsilon_{CDHI}}{\varepsilon_{HI}} = \frac{(1 + 1/K_t)\varepsilon^{D}_{azo}}{(1 + 1/K^{D}_{t})\varepsilon_{azo}}. \tag{11}$$

Supposing that $\varepsilon^{D}_{azo} \approx \varepsilon_{azo}$ (considering that the spectral change is otherwise usually not too significant) and that the azonium-quinoid form in the free state of the indicator is practically dominating ($K_t \geqslant 100$) [9], Equation (11) can be simplified:

$$\frac{\varepsilon_{CDHI}}{\varepsilon_{HI}} \approx \frac{1}{1 + 1/K^{D}_{t}} = \frac{K^{D}_{t}}{K^{D}_{t} + 1} \tag{12}$$

and from the experimentally-determined (apparent) molar absorptivities of the free indicator and the complex, approximate values of the equilibrium constant for the protonation tautomerism of the included guest can be calculated:

$$K^{D}_{t} = \frac{\varepsilon_{CDHI}}{\varepsilon_{HI} - \varepsilon_{CDHI}}. \tag{13}$$

The results are summarized in Table I, together with some literature values, for the sake of comparison. The connection of different equilibrium constants can be seen in Figure 3. (The K^{azo}_{HI} and K^{am}_{HI} microconstants could not be calculated separately, since the constant K_t was only assumed – although $K_t \geqslant 100$ seems to be a good approximation.)

As the ammonium-type forms have absorption maxima at about 320 nm [8], we checked our results at this wavelength, too. A large increase in absorbance has been observed (for methyl orange at 325–330 nm) on addition of cyclodextrins, proving that the concentration of species protonated on the amino group has increased significantly. The relative values and the order of the calculated equilibrium constants were very similar to those obtained earlier.

Table I. Stability and protonation tautomeric equilibrium constants of cyclodextrin complexes of the indicators investigated

Indicator	ε_{HI} (λ)	Cyclodextrin	ε_{CDHI}	K^{D}_{t}	K_{HI}	K_I
methyl orange (**I**)	4.2×10^4 (505 nm)	α-CD	0.3×10^4[a]	0.08	925[a]	6300
		β-CD	2.46×10^4	1.4	300 280[b]	2820[b]
		γ-CD	2.75×10^4	1.9	100	
methyl red (**II**)	3.03×10^4 (520 nm)	β-CD	2.45×10^4	4.3	240	550
p-ethoxychrysoidine (**III**)	2.70×10^4 (473 nm)	β-CD	2.47×10^4	11	420	1050

[a] From Ref. 10; [b] From Ref. 1.

From the first part of the table concerning methyl orange and different cyclodextrins it can be seen that the theory of tautomeric equilibrium is justified. The size of the α-CD cavity is the most appropriate for tight space filling with the molecule of methyl orange, therefore the stability constant of the complex is the largest and, accordingly, the smallest value for K_t^D has been obtained. This means that the protonation tautomeric equilibrium is shifted almost completely to the side of the ammonium form (the complex is practically colourless). γ-CD, on the other hand, binds methyl orange the most loosely, protonation of the azo group is less hindered, and this results in the largest value of K_t^D.

It can be easily understood by this concept why much more significant changes are caused by complex formation with cyclodextrins in the spectra of the protonated forms of the indicators than in those of the unprotonated forms.

Methyl red and *p*-ethoxychrysoidine were investigated with β-CD to study the effect of *ortho*-substituents, which prevent penetration into the smaller α-CD cavity [2]. With β-CD, complex formation have been proved with both the protonated and unprotonated species, though the changes in spectra as well as in the complex formation constants of the acid and base forms are less pronounced. These differences, as well as the larger values obtained for K_t^D, can be explained by the effect of substituents: (i) deep penetration of the azo group is hindered by the —COOH and $-NH_2$ substituents in *ortho* positions relative to it, (ii) protonation on the azo group is also favoured by the possibility of intramolecular hydrogen bonding in this state and, in the case of *p*-ethoxychrysoidine, by the weaker basicity of the amino group relative to that of the dimethylamino group of methyl orange.

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