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Spectral Studies on the Cyclodextrin Inclusion Complexes of Toluidine Blue O and Meldola's Blue in Aqueous Solution

M. ILANCHELIAN, C. RETNA RAJ and R. RAMARAJ*
School of Chemistry, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. The absorption and emission spectral properties of toluidine blue O (TBO) and Meldola's blue (MDB) in the presence of cyclodextrins (CDs) were studied. Formation of 1 : 1 and 1 : 2 (CD-Dye) inclusion complexes were observed with β-CD and γ-CD, respectively. An increase in the emission intensity was noticed in the presence of β-CD due to the formation of 1 : 1 (CD-Dye) inclusion complexes. However, a decrease in the emission intensity was observed in the presence of γ-CD due to the formation of 1 : 2 (CD-Dye) inclusion complexes. Dimerization of dye molecules in the presence of γ-CD lead to a decrease in the emission intensity. The formation constants for the 1 : 1 and 1 : 2 (CD-Dye) inclusion complexes were calculated.

Key words: Toluidine blue O, Meldola's blue, cyclodextrin, inclusion complex

1. Introduction

In recent years, there has been considerable interest in cyclodextrin (CD) inclusion complexes, because they can alter photophysical and photchemical properties of the included guest molecules [1, 2]. CDs are cyclic oligomers comprised of six, seven or eight (α, β or γ) glucopyranose residues linked by α-(1—4) bonds. The cyclic structure forms a torus shaped hydrophobic cavity with inner diameters of 4.5, 7.8 and 9.2 Å for α, β and γ-CD, respectively. The most interesting feature of these compounds is that they can accommodate guest molecules having the correct size in their hydrophobic cavity to form inclusion complexes without forming any covalent bonds [1–3]. In aqueous solution the guest and host molecules combine to form two or more inclusion complexes that differ in their stoichiometry and degree of stabilization. The stability of the inclusion complex mainly depends on the size and hydrophobicity of the guest molecule. Since the cavity diameter of γ-CD is nearly more than twice that of α-CD and larger than that of β-CD, γ-CD seems to be able to include larger molecules or to include two guest molecules in its cavity [4]. However, the stability of the inclusion complex of γ-CD remains equivocal since the cavity diameter of γ-CD is much larger than that of α- and β-CDs in

* Author for correspondence. E-mail: rrraj@pronet.xlweb.com

spite of their common depths. Spectroscopic studies of CD inclusion complexes reveal that the CDs provide a protective and more constrained environment to an electronically excited molecule [5].

It has been observed that the emission intensity of the included guest molecule is enhanced in the presence of CD [1]. The enhanced emission observed in the presence of CDs is due to (i) the shielding of the guest molecule inside the cavity from quenching by water molecules or solvent-borne quenchers, (ii) increased local viscoscity in the CD cavity with concomitant reduction of oxygen quenching, (iii) the less polar rigid microenvironment provided by the CD cavity and (iv) deaggregation of non-emittive dimer dye into emittive monomer in the case of molecules having a dimer-monomer equilibrium in aqueous solution. The inclusion of different molecules into the cavity of CDs and its influence on the photophysical and photochemical properties of the guest molecules have been studied using different methods [1–5].

The phenothiazine and oxazine dyes are important in the field of solar energy conversion and electrocatalysis [6–10]. Toluidine blue O (TBO), a phenothiazine dye and Meldola's blue (MDB), an oxazine dye (Figure 1) have been used as electrocatalysts for the electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH) [9–11]. The phenothiazine dyes are photoinactive in the dimer form and become photoactive in the monomer form [12–13]. The ability of CDs to alter dye solubilities and shift equilibria between the photoactive monomer and photoinactive dimer dye molecules make CD complexation particularly important in the field of photochemistry and photoelectrochemistry. In this paper we report the influence of CD complexation on the absorption and emission spectral properties of TBO and MDB.

2. Experimental

2.1. MATERIALS

Cyclodextrins (α, β and γ) (Aldrich) were used as received and the dye TBO (Aldrich) was purified by column chromatography on neutral alumina using ethanol : benzene (7 : 3 v/v) containing 0.4% glacial acetic acid and then recrystallized from ethanol [14]. Meldola's blue (Aldrich) was thrice recrystallised from methanol [15].

2.2. APPARATUS AND PROCEDURE

Absorption spectral studies were carried out by using a JASCO 7800 spectrophotometer. Fluorescence studies were performed on a HITACHI F4500 spectrofluorometer. The TBO and MDB molecules were excited at 595 and 567 nm, respectively and the emission spectra were recorded with 10 and 5 nm excitation and emission slits, respectively. The 3D spectra for the dyes were recorded as described earlier [16]. All the measurements were carried out at room temperature (25°C). Water

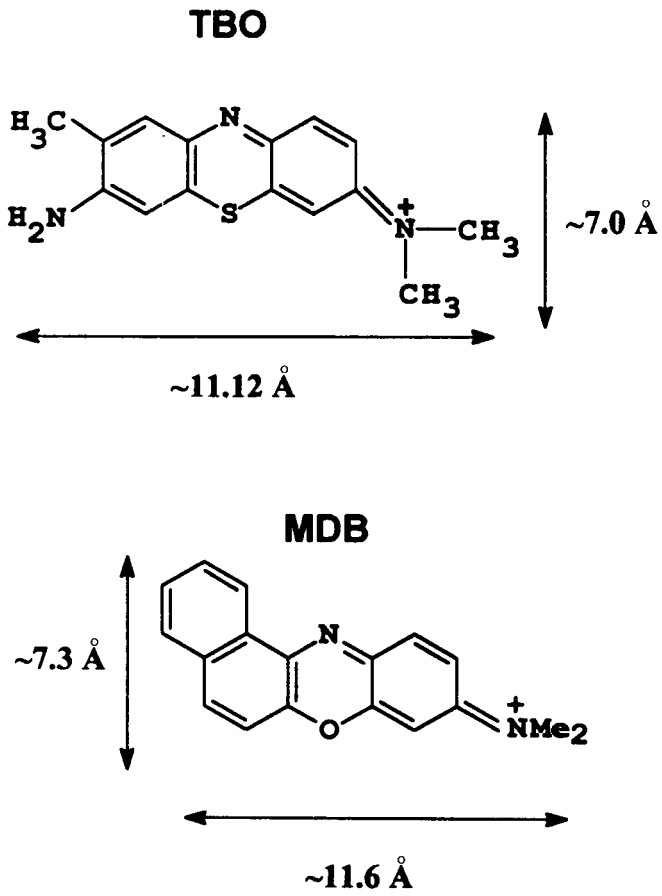

Figure 1. Structures of Toluidine blue O (TBO) and Meldola's blue (MDB).

used in this investigation was doubly distilled over alkaline potassium permanganate using an all-glass apparatus. A 10^{-5} mol dm^{-3} solution of TBO and MDB were prepared daily for experiments. For the study of the influence of β-CD and γ-CD, the CD solutions were prepared daily from the stock solution. The various concentrations of CD solutions were prepared by pipetting an aliquot of the stock solution into a 10 mL flask and then the solutions were made up to the mark with distilled water. The mixture of dye and CD solutions were stirred uniformly for 30 minutes and allowed to equilibriate for 15 minutes before recording the absorption and emission spectra. The molecular dimensions of the TBO and MDB molecules were measured by simulating the molecular model using Biosym-Insight II molecular modelling software on a silicon graphics computer system.

3. Results and Discussion

3.1. SPECTRAL STUDIES ON CD INCLUSION COMPLEXATION OF TBO

The absorption spectrum of TBO in aqueous solution showed an absorption band at 626 nm [17]. The absorption spectra of TBO at different concentrations of β-CD are shown in Figure 2A. An increase in the absorbance at 626 nm with a small red shift in the absorption maximum was observed upon the addition of β-CD due to the inclusion of TBO into the cavity of β-CD. The structure and molecular dimensions of the dyes are shown in Figure 1. From the molecular dimension (Figure 1), it is clear that only one molecule of TBO can be included into the cavity of β-CD. It has been observed that the phenothiazine dyes form dimers and higher aggregates even in dilute solution [6]. The selective inclusion of monomer TBO into the cavity of β-CD leads to the decomposition of dimer TBO into monomer. This leads to an increase in the monomer absorbance at 626 nm. The absorption band due to dimer TBO is not prominent in the absorption spectra at lower concentrations of TBO (Figure 2A). However, at higher concentrations a shoulder band around 590 nm was observed due to dimer TBO and this shoulder band decreased upon the addition of β-CD due to the decomposition of dimer dye to monomer. An isosbestic point was observed at 595 nm in the presence of different concentrations of β-CD and this clearly indicates the conversion of dimer into monomer. The emission spectra obtained for TBO at various concentrations of β-CD by exciting at the isosbestic point are shown in Figure 2B. The emission spectra recorded in the presence of different concentrations of β-CD show a twofold increase in the emission intensity which is ascribed to the formation of a 1 : 1 CD-dye inclusion complex. The selective inclusion of monomer TBO into the cavity of β-CD to form a 1 : 1 inclusion complex and the less polar hydrophobic microenvironment experienced by the TBO molecule in the β-CD cavity caused an increase in the emission intensity of the TBO molecule. When the absorption and emission spectra of TBO and MDB were recorded in less polar solvents like ethanol a significant shift in the absorption and emission bands was not noticed in comparison to water. However, an increase in the emission intensity was noticed for the dye molecules in less polar solvents like ethanol.

The absorption and emission spectral studies were also carried out in the presence of γ-CD. The absorption spectra recorded for TBO at different concentrations of γ-CD are shown in Figure 3A. Addition of γ-CD into a solution of TBO caused a decrease in monomer absorbance at 626 nm and the appearance of a new band at 590 nm. The inclusion of two dye molecules into the cavity of γ-CD caused a decrease in the absorbance at 626 nm. Inclusion of two dye molecules into the cavity leads to a decrease in the monomer concentration. The new band observed at 590 nm is very similar to the dimer absorption band noticed in the absorption spectra of higher concentrations of TBO in the absence of γ-CD. This clearly indicates that the absorption band which appeared at 590 nm in the presence of γ-CD is due to the formation of dimer TBO (1 : 2 CD-dye inclusion complex).

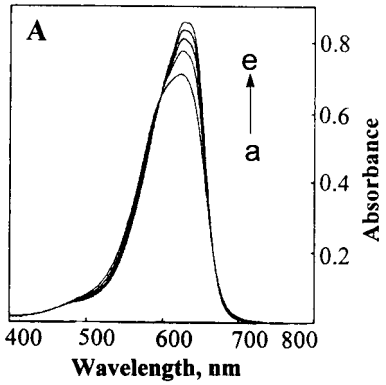

Figure 2A. Absorption spectra of TBO (1.38×10^{-5} mol dm^{-3}) at various β-CD concentrations. [β-CD]: (a) 0, (b) 7.05×10^{-4}, (c) 1.41×10^{-3}, (d) 2.12×10^{-3} and (e) 2.82×10^{-3} mol dm^{-3}.

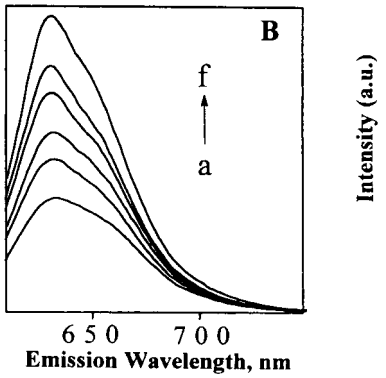

Figure 2B. Emission spectra of TBO (3.42×10^{-6} mol dm^{-3}) at various β-CD concentrations. [β-CD]: (a) 0, (b) 3.52×10^{-4}, (c) 7.05×10^{-4}, (d) 1.40×10^{-3}, (e) 2.11×10^{-3}, (f) 2.82×10^{-3} and (g) 4.23×10^{-3} mol dm^{-3}.

An isosbestic point was observed at 595 nm showing the conversion of monomer TBO into dimer TBO in the presence of γ-CD. At higher concentrations of γ-CD the absorption band observed at 626 nm due to monomer TBO almost disappeared and only a single band at 590 nm was observed. This clearly shows that the γ-CD complexation of TBO induces dimerization of TBO.

The emission spectra of TBO at various γ-CD concentrations are shown in Figure 3B. An observable decrease in the emission intensity upon the addition of γ-CD was observed. This decrease in the emission intensity was attributed to the formation of non-emittive dimer dye in the cavity of γ-CD (1 : 2) as observed in the absorption spectra. Since the γ-CD cavity diameter (9.2 Å) is large enough to accommodate two molecules in its cavity, the inclusion of two TBO molecules is possible. The γ-CD complexation aggregates the emittive monomer into non-emittive dimer. The excitation spectra recorded for TBO at different γ-

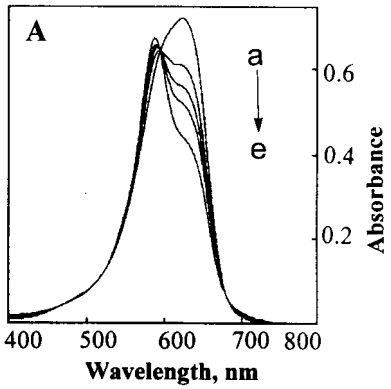

Figure 3A. Aborption spectra of TBO (1.4×10^{-5} mol dm^{-3}) at various γ-CD concentrations. [γ-CD]: (a) 0, (b) 1.50×10^{-4}, (c) 3.08×10^{-4}, (d) 4.62×10^{-4} and (e) 7.70×10^{-4} mol dm^{-3}.

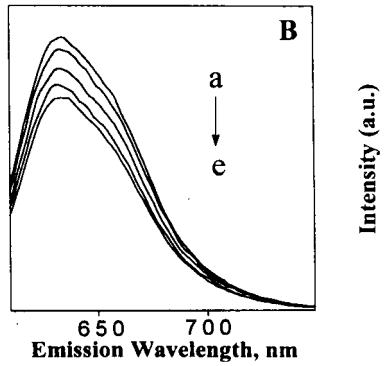

Figure 3B. Emission spectra of TBO (3.42×10^{-6} mol dm^{-3}) at various γ-CD concentrations. [γ-CD]: (a) 0, (b) 7.70×10^{-5}, (c) 1.54×10^{-4}, (d) 3.08×10^{-4} and (e) 6.16×10^{-4} mol dm^{-3}.

CD concentrations show that the excitation intensity decreased upon the addition of γ-CD as observed in the absorption spectra.

The emission spectra recorded at different concentrations of TBO in the absence of β-CD shows that the emission intensity increased with increasing concentration of dye and at higher concentration the emission intensity decreased. The decrease in the emission intensity at higher concentration is due to the formation of non-emittive dimer. The 3D emission spectra recorded for TBO in the absence and presence of β-CD show that the contour intensity was increased in the presence of β-CD (Figures 4A and B). The increase in the contour intensity was due to the deaggregation of non-emittive dimer dye by the selective inclusion of monomer dye molecule (Figure 4B) as described earlier [16]. However, in the presence of γ-CD the contour intensity decreased due to the formation of non-emittive dimer. These results confirms that the β-CD complexation increases the monomer dye

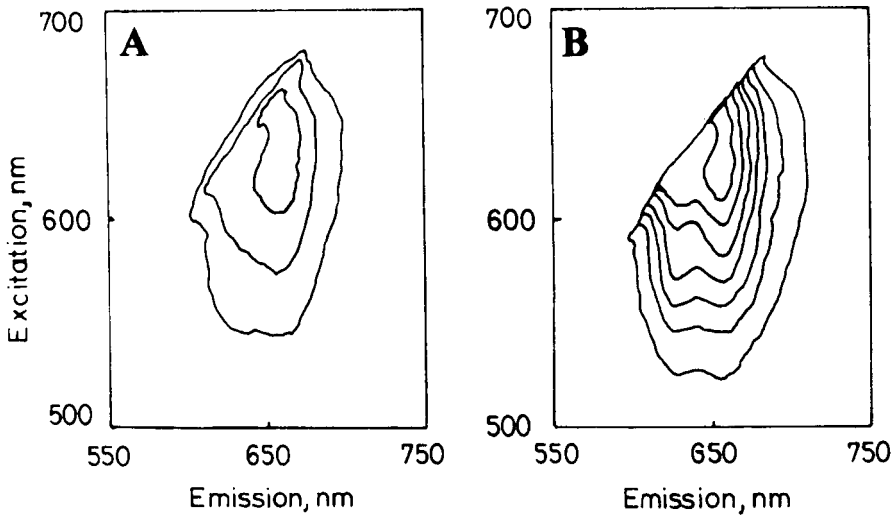

Figure 4. 3D Emission spectra of TBO in the absence (A) and presence of β-CD (B). [TBO] = 1.4×10^{-5} mol dm^{-3}; [β-CD] = 4.23×10^{-3} mol dm^{-3}.

concentration and γ-CD complexation decreases the monomer dye concentration in aqueous solution.

3.2. SPECTRAL STUDIES ON CD INCLUSION COMPLEXATION OF MDB

The absorption spectra of MDB show an absorption band at 567 nm and the addition of β-CD does not significantly influence the absorption spectra. However, the CD complexation of MDB shows significant changes in the emission spectra. The emission spectra of MDB at different concentrations of β-CD are shown in Figure 5. An observable enhancement in the emission intensity and a 5 nm blue shift in the emission band was noticed in the presence of β-CD. It has been observed that the oxazine dyes exist in dimer-monomer equilibrium in aqueous solution [18–19]. The increase in the emission intensity in the presence of β-CD is due to the inclusion of monomer MDB molecule into the cavity of β-CD. The emission spectra recorded for MDB at different concentrations in the absence of β-CD show similar results as observed for TBO (emission intensity increased with increasing the dye concentration and decreased at higher concentration). In the present investigation, the observed increase in the emission intensity in the presence of β-CD is due to the deaggregation of dimer dye by the selective inclusion of monomer and also due to the hydrophobic microenvironment provided by the β-CD cavity. When β-CD was added the monomer MDB molecule associated into the cavity of β-CD and the concentration of free MDB dye in solution decreased and the equilibrium shifted towards the monomeric form. Cosequently the monomer concentration increased in solution which leads to the enhancement in the emission intensity.

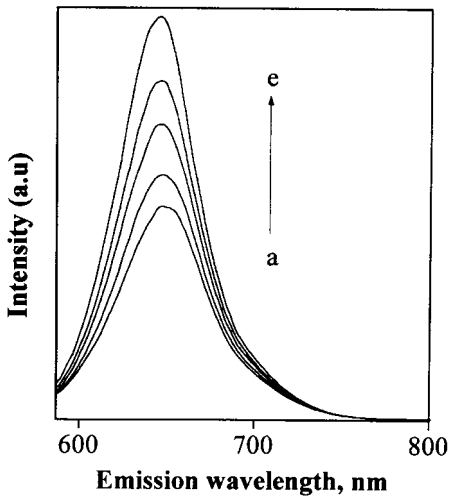

Figure 5. Emission spectra of MDB (1.54×10^{-5} mol dm^{-3}) at various β-CD concentrations. [β-CD]: (a) 0, (b) 7.04×10^{-5}, (c) 1.76×10^{-4}, (d) 3.50×10^{-4} and (e) 8.80×10^{-4} mol dm^{-3}.

The absorption spectra of MDB at different concentrations of γ-CD show a very small decrease in the absorbance at 567 nm. However, the emission spectra obtained for MDB at various concentrations of γ-CD show an observable decrease in the emission intensity and a red shift in the emission band (Figure 6). The observed decrease in the emission intensity was due to the self-quenching of the excited state aggregate. γ-CD forms a 1 : 2 γ-CD-dye inclusion complex and the complexation induces the aggregation of emittive monomer into non-emittive dimer and that leads to the decrease in the emission intensity. Since the γ-CD cavity is large enough to accommodate two MDB molecules into the cavity, the formation of dimer in the presence of γ-CD is feasible as described earlier.

The 3D emission spectra of MDB in the absence and presence of β-CD confirm the enhancement of emission intensity in the presence of β-CD. In the case of γ-CD the contour intensity decreased due to the formation of non-emittive dimer as observed for TBO. All these results confirm that the aggregation and deaggregation of dyes occur in the presence of β-CD and γ-CD, respectively. The influence of CD complexation on the dimer-monomer equilibrium of the dyes can be represented as given in Equations (1) and (2). When β-CD is added, it selectively includes the monomer dye which leads to the deaggregation of the nonemittive dimer and the equilibrium is shifted towards the monomer (Equation 1). In the case of γ-CD, since the γ-CD can accommodate the dimer dye, aggregation of emittive monomer occurs and the equilibrium is shifted towards the dimer (Equation 2).

$$\mathrm{D} \rightleftharpoons \mathrm{M} \overset{\beta\text{-CD}}{\rightleftharpoons} \text{M-}\beta\text{-CD}, \tag{1}$$

$$\mathrm{M} \rightleftharpoons \mathrm{D} \overset{\gamma\text{-CD}}{\rightleftharpoons} \text{D-}\gamma\text{-CD}, \tag{2}$$

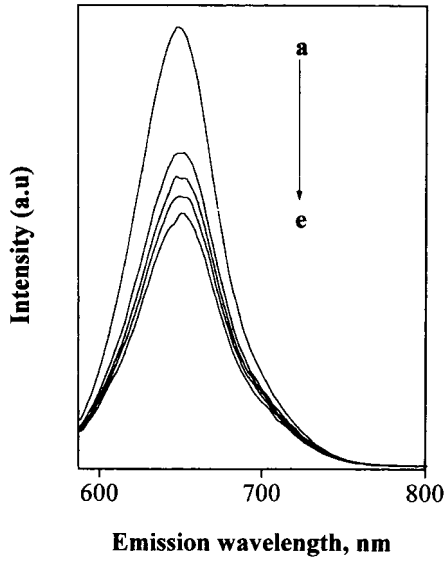

Figure 6. Emission spectra of MDB (1.54×10^{-5} mol dm^{-3}) at various γ-CD concentrations. [γ-CD]: (a) 0, (b) 7.70×10^{-4}, (c) 1.54×10^{-4}, (d) 4.60×10^{-4} and (e) 10.80×10^{-4} mol dm^{-3}.

where D is the non-emittive dimer dye and M is the emittive monomer dye.

3.3. CALCULATION OF ASSOCIATION CONSTANT

The stability of the inclusion complex can be measured by calculating the association constant (K_{ass}). The association constant for the inclusion of dye (TBO/MDB) into the cavity of β-CD has been calculated from the emission spectral data by using the Benesi–Hildebrand equation (Equation 3) [20].

$$1/\Delta I_f = 1/(I'_f - I^0_f) + 1/K_{ass}(I'_f - I^0_f)[\beta\text{-CD}], \tag{3}$$

where $\Delta I_f = (I_f - I^0_f)$, I^0_f is the emission intensity of the dye in the absence of β-CD, I_f is the emission intensity of TBO at a given concentration of β-CD and I'_f is the emission intensity of the complex. The double reciprocal plot obtained for the complexation of TBO with β-CD is shown in Figure 7. The K_{ass} values calculated from the emission spectral data are given in Table I. A similar method has been used to calculate the association constant of MDB with β-CD and the value is given in Table I. The association constant for the 1 : 2 CD-dye inclusion complex was calculated by using Equation (4) [21].

$$I^0_f/\Delta I_f = (1/I^a_f)K_{ass}[\gamma\text{-CD}] + (1/I^a_f), \tag{4}$$

where I^0_f is the emission intensity of the dye in the absence of γ-CD, ΔI_f is the difference in emission intensities of TBO in the absence and presence of γ-CD

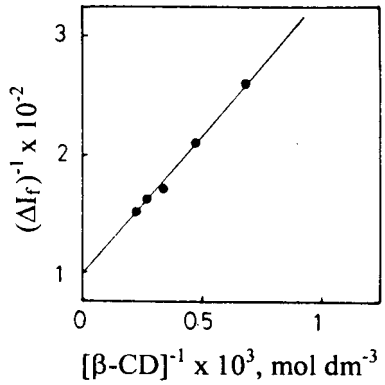

Figure 7. Double reciprocal plot for the calculation of the association constant of TBO with β-CD.

Table I. Association constants (K_{ass}) calculated for the complexation of TBO and MDB with CDs.

K_{ass}, dm^3 mol^{-1}		
CD	TBO	MDB
β[a]	442	1022
γ[b]	2527	10435

Average of three experimental values.
Error limit: ± 10%.
[a] 1 : 1 CD-dye complexation.
[b] 1 : 2 CD-dye complexation.

($\Delta I_f = (I_f^0 - I_f)$, I_f is the emission intensity of TBO at a given concentration of γ-CD, [γ-CD] is the concentration of γ-CD, I_f^a is the fraction of the initial fluorescence which is accessible to quencher and K_{ass} is the association constant. The plot of $I_f^0/\Delta I_f$ vs [γ-CD] gave a straight line as shown in Figure 8 and the K_{ass} values were calculated from the slope and intercept. The K_{ass} values calculated for γ-CD-TBO and γ-CD-MDB complexation (1 : 2 CD-dye complex) are given in Table I. The K_{ass} values calculated for TBO and MDB show that the association of MDB with β-CD and γ-CD is stronger than that of TBO. The higher association constant observed for MDB can be due to the more hydrophobic nature of the MDB. The MDB dye forms a more stable complex than the TBO dye.

α-CD has no influence on the absorption and emission spectra of MDB or TBO. Since the α-CD cavity diameter is too small (4.5 Å) to accommodate the MDB/TBO molecule in its cavity, the inclusion is not feasible. The absorption and emission spectral studies were carried out in the presence of α-D-glucose and it was found that α-D-glucose has no influence on the spectral behavior of TBO and

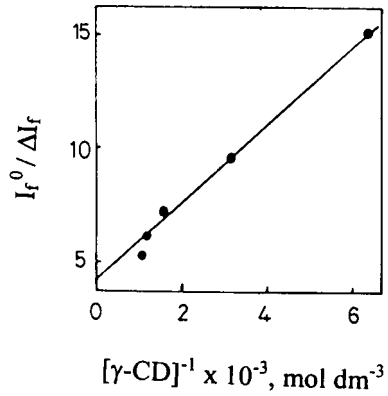

Figure 8. Plot of $I_f^0/\Delta I_f$ vs 1/[γ-CD] for the calculation of K_{ass} of TBO with γ-CD.

MDB. This clearly shows that the changes observed in the presence of CDs were due to the formation of an inclusion complex.

4. Conclusions

The present study reveals the influence of CD complexation on the spectral properties of TBO and MDB dye molecules. β-CD forms an emittive 1 : 1 (CD-dye) inclusion complex and γ-CD forms a non-emittive 1 : 2 (CD-dye) inclusion complex. The inclusion complexes are comparatively stable as evidenced from the K_{ass} values. The MDB dye forms a more stable inclusion complex than TBO. This study provides a basic understanding about the non-covalent interaction of dyes with CDs and the possible use of CDs to study the monomer-dimer equilibria of dye molecules. The selective inclusion of the TBO/MDB molecule in the β-CD cavity may bring about catalytic applications in the field of NADH oxidation and in photogalvanic cells.

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References

1. M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer Verlag, Berlin (1978).
2. J. Szejtli: *Cyclodextrins and their Inclusion Complexes*, Akademiai Kiado, Budapest (1982).
3. V. Ramamurthy: *Tetrahedron* **42**, 3753 (1986).
4. T. Yorozu, M. Hoshino and M. Imamura: *J. Phys. Chem.* **86**, 4426 (1982).
5. K. Kalyanasundaram: *Photochemistry in Microheterogeneous System*, Academic Press, New York (1987).
6. E. Rabinowitch: *J. Chem. Phys.* **8**, 551 (1940).

7. W. J. Albery, A. W. Foulds, K. J. Hall, A. R. Hillman, R. G. Egdell and A. F. Orchard: *Nature* **282**, 20 (1979).
8. R. Tamilarasan and P. Natarajan: *Nature* **224,** 292 (1981).
9. L. Gorton, A. Torstensson, H. Jaegfeldt and G. Johansson: *J. Electroanal. Chem.* **161**, 103 (1984).
10. L. T. Kubota, F. Gouvea, A. N. Andrade, B. G. Milagres and G. D. O. Neto: *Electrochim. Acta* **41**, 1465 (1996).
11. B. Persson: *J. Electroanal. Chem.* **287**, 61 (1990).
12. W. J. Albery, P. N. Bartlett, A. W. Foulds and W. Robert: *J. Chem. Soc., Perkin Trans. 2*, 794 (1981).
13. C. Retna Raj and R. Ramaraj: *J. Electroanal. Chem.* **405**, 141 (1996).
14. K. R. Gopidas and P. V. Kamat: *J. Phys. Chem.* **94**, 4723 (1990).
15. A. R. Guadalupe, K. E. Liu and H. D. Abruna: *Electrochim. Acta* **36,** 881 (1991).
16. C. Retna Raj and R. Ramaraj: *Chem. Phys. Lett.* **273,** 285 (1997).
17. M. S. Chan and J. R. Bolton: *Solar Energy* **24**, 561 (1980).
18. D. Liu and P. V. Kamat: *J. Electrochem. Soc.* **142**, 835 (1995).
19. W. G. Herkstroeter, P. A. Martic and S. Farid: *J. Am. Chem. Soc.* **112**, 3583 (1990).
20. S. Nigam and G. Durocher: *J. Phys. Chem.* **100**, 7135 (1996).
21. J. R. Lakowicz: *Principles of Fluorescence Spectroscopy*, Plenum Press, New York (1983).