

Solubilization by β -Cyclodextrin: A Fluorescence Quenching Study

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Solubility of carbon tetrachloride (CCl_4) in water increases appreciably in presence of β -cyclodextrin (βCD). CCl_4 is a very good quencher of 1-naphthol (1ROH) fluorescence. By studying the quenching of fluorescence of 1ROH included in βCD cavity, it was found that there is an increase in the availability of CCl_4 around βCD in the aqueous medium. This could help to rationalize the enhanced solubility of CCl_4 .

key words: solubilization, fluorescence quenching, inclusion complex, quenching sphere of action

INTRODUCTION

A characteristic property of cyclodextrin is its ability to form inclusion complexes with various compounds of appropriate size called guests or substrates [1-6]. Its interior surface is hydrophobic and substrates included in the cavity not only exchange with free substrate molecules but also re-accommodate themselves by presenting several molecular orientations. Substituted naphthalenes such as 1- and 2-naphthol are known to be included into β -cyclodextrin (βCD) cavity. In some recent studies of fluorescence of naphthol- βCD complexes, it has been observed that for the inclusion complexes in aqueous medium, dual fluorescence from both the neutral and anionic forms occurs [7]. Though 1-naphthol (1ROH) is an extremely weak acid in its ground state ($\text{pK}_a=9.39$) [8], when excited at neutral pH, it loses one proton to water, its pK_a^* being 0.4 [9]. On excitation at 290 nm in water, it dissociates completely exhibiting an emission peak at 465 nm due to the anion (RO^-) only. Being a very good complexing agent forming water-soluble complexes with the non-polar compounds like 1ROH, βCD can also be used to improve the aqueous solubility of the compounds, which are sparingly soluble in water [10]. In the present work, we have studied the ability of aqueous βCD solutions to increase the solubilization of CCl_4 by using fluorescence quenching as a technique. It is well known in literature that carbon tetrachloride (CCl_4) is a very efficient quencher of 1-naphthol fluorescence [11,12]. Thus the solubilization behaviour of CCl_4 in water in presence of βCD is expected to reflect in fluorescence quenching. In this study, the quenching of fluorescence for 1ROH- βCD inclusion complex by CCl_4 has been monitored so as to understand the cyclodextrin-induced solubilization of CCl_4 . Comparison of fluorescence quenching by a water-soluble quencher copper sulfate (CuSO_4) has been made.

EXPERIMENTAL DETAILS

Materials

1-Naphthol (Sisco-Chem) was purified by subliming twice after crystallization from alcohol. Carbon tetrachloride (GR Grade, Merck) was purified before use [13]. Copper sulfate (Sigma) was crystallized from water. β -Cyclodextrin obtained from s.d. Fine Chemicals Ltd. was used as such. Freshly prepared solutions were used for all the experiments. The final mixture of 1ROH and βCD was equilibrated overnight to obtain the inclusion complex and then used for quenching studies. The quencher was added to the equilibrated solution just before taking the fluorescence spectrum. The solution was shaken well and spectrum was taken immediately to prevent the possibility of any photoreaction.

Spectral measurements

Absorption and fluorescence spectra were recorded by Hitachi 220A UV-Vis spectrophotometer and Hitachi F-4500 spectrofluorimeter respectively. Lifetime measurements were carried out using the IBH single photon counting fluorimeter. The concentrations of 1ROH and βCD were maintained at 1×10^{-4} M (0.05% methanol) and 1×10^{-3} M respectively. For quenching study, the concentrations were varied in the range 0-0.01 M and 0-0.05 M for CCl_4 and CuSO_4 respectively. The fluorescence intensities of 1ROH ($\lambda_{\text{ex}}=290$ nm, $\lambda_{\text{em}}=465$ nm in water; $\lambda_{\text{ex}}=290$ nm, $\lambda_{\text{em}}=362$ nm, 465 nm in βCD) were monitored at various concentrations of CCl_4 and Cu^{++} at 25°C.

Determination of Equilibrium constant of 1ROH- βCD inclusion complexes

The equilibrium constants of the inclusion complexes can be obtained by absorption and/or fluorescence spectrophotometric methods. There was no perceptible shift in the λ_{max} of the absorption spectra of 1-naphthol in βCD medium. However the variation of fluorescence intensity of a fixed concentration of 1-naphthol (1×10^{-4} M) with βCD concentration (concentration range 0-0.007 M) showed saturation behaviour. This was made use for the calculation of complexation constant. To obtain more accurate values, the fluorescence intensities of 1ROH^{*} peak was

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used instead of $1RO^-*$ peak since the variation in the fluorescence intensity of the neutral species was more pronounced. A method suggested by Hoshino *et al.* [14] for some benzene derivatives with β CD in aqueous solutions was used to calculate the equilibrium constant (K). The expression used is

$$\Delta\Phi_R^{-1} = ((\Phi_b/\Phi_a) - 1)^{-1} (1 + (1/\gamma)KC_\beta) \quad (1)$$

Here, $\Delta\Phi_R = (\Phi/\Phi_a) - 1$, Φ is the fluorescence quantum yield of a fluorophore in aqueous β CD solution. Subscripts a and b refers to the fluorophore and its inclusion complex respectively; $\gamma = \epsilon_b/\epsilon_a$, where ϵ is the molar extinction coefficient; C_β is the concentration of the β CD. Plot of $\Delta\Phi_R^{-1}$ versus C_β^{-1} was made. From the slope and intercept, the equilibrium constant was found to be 917 (± 20). This value agrees with the reported value of 1134 in pure water [6].

RESULTS AND DISCUSSION

Solubilization of CCl_4 in water by β CD

The solubility of CCl_4 in water with increasing concentration of cyclodextrin was determined by visual titration. It was observed that the solubility of CCl_4 goes on increasing with the β CD concentration and 0.082 M CCl_4 is soluble in (1×10^{-3} M) β CD, $[CCl_4]$ being more than 80 times to $[\beta$ CD]. Thus it can be said that the enhancement of solubilization of CCl_4 in β CD is not due to the formation of inclusion complexes, rather it is due to the solubilization CCl_4 in the aqueous phase.

Absorption and fluorescence spectra

The absorption and fluorescence spectra of 1ROH in water with and without β CD are given in Figure 1. The emission peak of 1ROH at 362 nm is due to the molecular species ($1ROH^*$), whereas the peak at around 465 nm is due to the dissociated $1RO^-*$ [15]. Thus 1ROH dissociates completely in water and partially in the β CD medium. Wu and Hurtubise [6] have also obtained the fluorescence emission from both the molecular and the anionic forms of 1ROH in alcohol:water solvents with β CD. They have noticed that the fraction of the molecular form of 1ROH in methanol:water (1:9) was increasing in a nonlinear fashion with the addition of β CD. They have suggested that the hydroxyl group was not buried in the β CD cavity and could undergo ionization with a sizeable amount of β CD present.

Lifetime of the excited fluorophore

In β CD, a bi-exponential decay has been observed indicating two localization sites for the excited 1ROH whereas there is a single exponential decay in water. The lifetime of the two species of the excited 1ROH in β CD were found to be 1.0 ns and 8.73 ns, which are due to the neutral and the dissociated species respectively. These values are comparable with those obtained by Behera *et al.* [16] in the SDS microemulsion

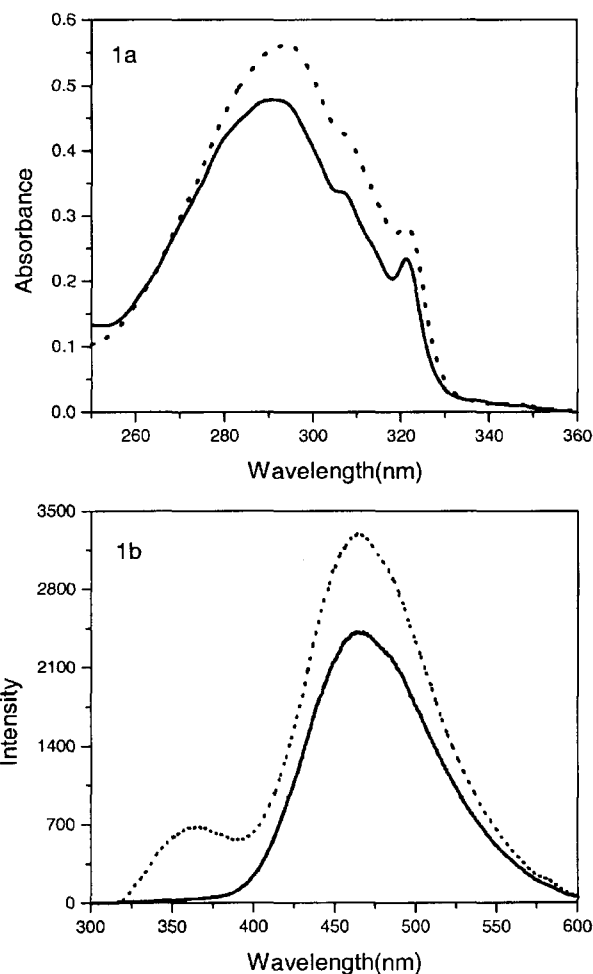


Figure 1 (a) Absorption Spectra of 1ROH in water (solid line) and in β CD (dotted line) (b) Fluorescence Spectra of 1ROH in water (solid line) and in β CD (dotted line).

system (neutral 1.65ns, anion 7.27ns). The lifetime value of $1RO^-*$ in the cyclodextrin corresponds to that in water (8.07ns) indicating that the photo-dissociated naphthol has its phenolic group exposed to water. Clark *et al.* [17] have also obtained the lifetime values 0.1ns and 8.0ns for the neutral and anionic species respectively in water. The excited state lifetime value for $1ROH^*$ obtained by us is 0.5ns which may be due to the presence of 0.05% methanol in water. By comparing the measured excited state lifetime for 1ROH in β CD with that in various aqueous mixture of ethanol [18], it can be said that the environment of the molecule corresponds to about 85% ethanol in water.

Fluorescence quenching by CCl_4

Stern-Volmer Plots Usually in the case of collisional quenching [19] the Stern-Volmer (SV) plot i.e. the plot of the ratio of fluorescence intensities in the absence and presence of quencher (I_0/I) against the quencher concentration [Q] is a straight line with an intercept 1 and slope as K_{SV} , the Stern-

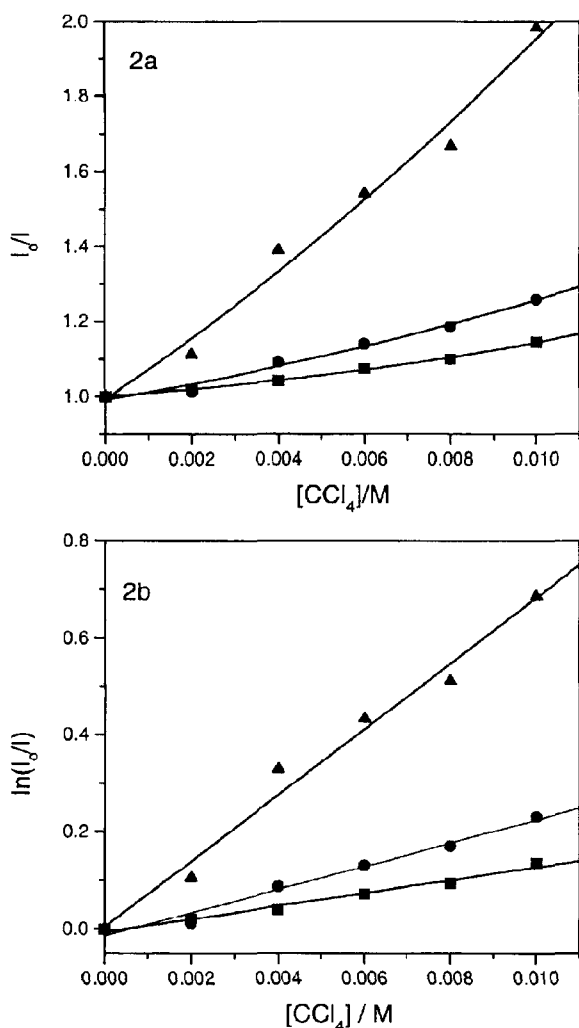


Figure 2 (a) Stern-Volmer plots for the fluorescence quenching of IRO^- in water (\blacksquare), IRO^- in βCD (\bullet) and IROH^+ in βCD (\blacktriangle) by CCl_4 . (b) Plots of $\ln(I_0/I)$ versus $[\text{CCl}_4]$ for IRO^- in water (\blacksquare), IRO^- in βCD (\bullet) and IROH^+ in βCD (\blacktriangle).

Volmer quenching constant. In the present study, as the data points in Figure 2a show, none of the systems followed a linear SV relation. Positive deviations from linearity were obtained in all the cases even at low concentration of CCl_4 , indicating that the quenching is not purely collisional. Similar observations have been obtained by Behera *et al.* [20,21] when they studied the fluorescence quenching behaviour of some naphthalene derivatives by a series of water and oil-soluble quenchers in the cationic and anionic microemulsions. In the narrow concentration range of 0-0.01 M of CCl_4 used, the change in the polarity of the medium would be negligible. Deviations from linearity in SV plots have often been explained satisfactorily in certain systems using a transient quenching mechanism involving a 'quenching sphere of action model'. According to this model, the fluorophore assumes a 'sphere of action' with varying dimensions, within which the probability

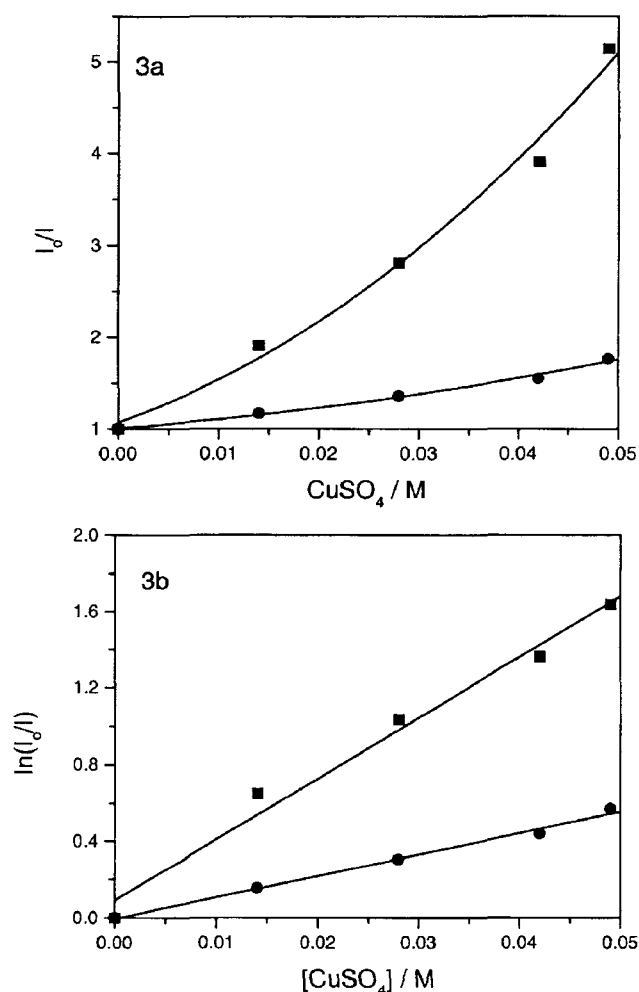


Figure 3 (a) Stern-Volmer plots of IRO^- (\blacksquare) and IROH^+ (\bullet) by CuSO_4 in βCD . (b) Plots of $\ln(I_0/I)$ versus $[\text{CuSO}_4]$ for IRO^- (\blacksquare) and IROH^+ (\bullet) in βCD .

of quenching is unity and outside the sphere it is assumed to be zero. Once the quencher is present in this sphere, quenching occurs before the fluorophore and quencher diffuse apart. The expression describing this quenching process is represented by eq. (2) [22-24].

$$I_0/I = \exp(K_T [\text{CCl}_4]) = \exp(v N_a [\text{CCl}_4]) \quad (2)$$

Here, K_T is the transient quenching constant, v is the volume of the quenching sphere of action and N_a is Avogadro's number.

The plots of $\ln(I_0/I)$ versus $[\text{CCl}_4]$ (Figure 2b) are found to be linear ($r \geq 0.99$). The K_T values (Table 1) for all the IROH^+ - CCl_4 systems under study are in the order: IROH^+ (βCD) > IRO^- (βCD) > IRO^- (water).

Probability distribution of CCl_4 in the quenching sphere
Assuming that the association of CCl_4 in forming a quenching

Table 1. Values of transient quenching constant (K_T), radius of the quenching sphere of action (R) and lifetime of the excited fluorophore (τ_0) in aqueous medium with and without β CD. $[1\text{-ROH}] = 1 \times 10^{-4} \text{ M}$ (0.05% methanol), $[\beta\text{CD}] = 1 \times 10^{-3} \text{ M}$.

Medium	Fluorophore	τ_0 (ns)	Quencher	K_T ($\text{mol}^{-1}\text{dm}^3$)	R (nm)	No. of bound quenchers in the quenching sphere calculated for $[Q]=0.1 \text{ M}$
Water	1RO^*	8.07	CCl_4	13.32	1.74	1
			Cu^{++}	31.72	2.33	3
	1ROH^*	0.5	—	—	—	—
β CD	1RO^*	8.73	CCl_4	23.86	2.11	1
			Cu^{++}	31.70	2.32	3
	1ROH^*	1.0	CCl_4	67.80	2.99	6
			Cu^{++}	11.18	1.64	1

sphere of action obeys Poisson statistics, the probability distribution (P_i) that a fluorophore is bound with 'i' number of CCl_4 is given by [25]

$$P_i = (m^i / i!) \exp(-m) \quad (3)$$

Here, m is the mean number of CCl_4 per volume v ; $m(1\text{ROH}^*) = [\text{CCl}_4]_b / [1\text{ROH}^*]_t$ and $m(1\text{RO}^*) = [\text{CCl}_4]_b / [1\text{RO}^*]_t$. Here $(1\text{ROH}^*)_t$ and $[1\text{RO}^*]_t$ denote the total concentrations of naphthol and naphtholate respectively and $[\text{CCl}_4]_b$ is the concentration of CCl_4 bound to the fluorophore within the quenching sphere.

The values of m has been calculated from the equation $m = K_T [\text{CCl}_4]$. The values of P_i were obtained for different i values (1,2,3,...) of CCl_4 at the concentration range 0-0.1 M and then theoretical plots of P_i versus the number of CCl_4 molecules incorporated in the sphere were made. Some representative plots are given in Figure 4. The maximum of each curve is taken as the number of the CCl_4 molecules incorporated in the sphere at a specific $[\text{CCl}_4]$. Calculated at the concentration 0.1 M, the 'i' values for the systems studied are in the order: 1ROH^* (β CD) ($i=6$) > 1RO^* (β CD) ($i=1$) \approx 1RO^* (water) ($i=1$).

The fluorescence of neutral 1ROH^* is not observed in aqueous medium in the absence of β CD. CCl_4 is sparingly soluble in water, and in a homogeneous aqueous medium 1ROH and CCl_4 molecules get distributed uniformly in the solution. Thus the probability for the CCl_4 molecule to reside inside the quenching sphere of action is less, which causes the lower quenching efficiency. However when a β CD molecule is present in water, the hydrophobic CCl_4 molecules would prefer to crowd around it, thereby increasing the value of 'i'. Thus the K_T value for 1ROH^* in β CD is highest amongst all the systems studied. The higher quenching efficiency of 1ROH^* as compared to the 1RO^* could be a due to a greater hydration of the charged species. The K_T of 1RO^* - β CD is much higher than that of 1RO^* in aqueous medium, although both show the same value of 'i'.

As mentioned in the introduction, the reasons for the choice of 1-naphthol as a fluorescent probe were the following: (i) 1-naphthol forms inclusion complex with β CD (ii) the neutral

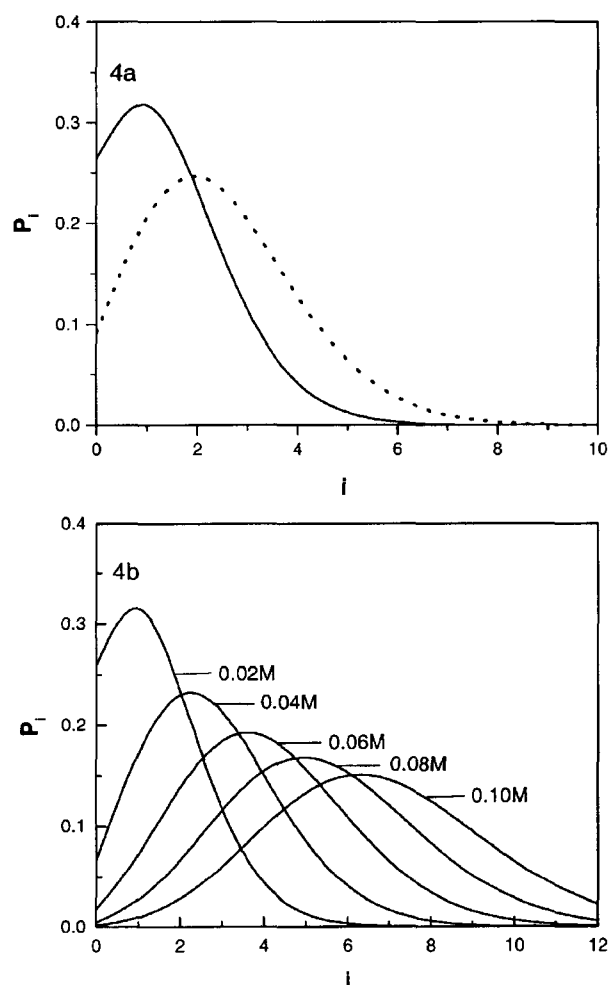


Figure 4. (a) Probability distribution curves for CCl_4 incorporation into the quenching sphere of 1RO^* in water (solid line) and in β CD (dotted line); ($[\text{CCl}_4] = 0.1 \text{ M}$). (b) Probability distribution curves for CCl_4 incorporation into the quenching sphere of 1ROH^* in β CD ($[\text{CCl}_4] = 0-0.1 \text{ M}$).

form fluorescence that appears as a result of this complexation is specifically attributable to the β CD environment and (iii) the fluorescence of 1-naphthol is efficiently quenched by CCl_4 ,

thus the quenching efficiency of the neutral form fluorescence can be related to the availability of CCl_4 in the βCD environment. The amount of 1-naphthol used being very small compared to the amount of quencher, any role of 1-naphthol in the solubilization effect can be safely discounted. To have a more clear idea about the solubilization of CCl_4 in aqueous solution of βCD , its quenching efficiency is compared with that of a water-soluble divalent quencher, copper ion (Cu^{++}).

Fluorescence quenching by Cu^{++}

Cu^{++} is known to be a very efficient quencher of fluorescence [26]. It has been reported earlier that its quenching efficiency for 1ROH in a confining system like microemulsion is less as compared to water [24]. Due to its hydrophilic nature, CCl_4 -like clustering of Cu^{++} is not expected; rather it is expected to be distributed uniformly in the aqueous region. The K_T values (Table 1) of 1ROH obey the order: $1\text{RO}^{-*}(\beta\text{CD}) \approx 1\text{RO}^{-*}(\text{water}) > 1\text{ROH}^*(\beta\text{CD})$. At the concentration 0.1 M, the number $\text{Cu}^{++}(i)$ available near the fluorophore are in the order: $1\text{RO}^{-*}(\beta\text{CD}) (i=3) \approx 1\text{RO}^{-*}(\text{water}) (i=3) > 1\text{ROH}^*(\beta\text{CD}) (i=1)$. As was expected, K_T of 1RO^{-*} is the same both in water and in presence of βCD . The electrostatic effect of 1RO^{-*} and Cu^{++} as well as hydrophilicity of both result in a higher K_T value as compared to that of 1ROH^* .

SUMMARY

Solubilization of carbon tetrachloride (CCl_4) in water increases appreciably in presence of β -cyclodextrin (βCD). This has been rationalized by a fluorescence quenching method.

CCl_4 is an efficient quencher of 1-naphthol (1ROH) fluorescence. Emission from both the neutral and anionic forms of 1ROH^{*} included in βCD cavity get quenched by CCl_4 by a mechanism involving 'quenching sphere of action' model. The presence of transient quenching indicates an increase in the availability of CCl_4 around the βCD cavity.

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REFERENCES

1. Turro, N. J., Okubo, T. and Chung, C. J. (1982) *J. Am. Chem.*

- Soc.* **104**, 1789.
2. Herkstroeter, W. G., Matric, P. A. and Farid, S. (1984) *J. Chem. Soc.*, Perkin Trans. II, 1453.
3. Hansen, J. E., Pines, E. and Fleming, G. R. (1992) *J. Phys. Chem.* **96**, 6904.
4. Park, H.-R., Mayer, B., Wolschann, P. and Kohler, G. (1994) *J. Phys. Chem.* **98**, 6158.
5. Stam, J., Feyter, S. D., De Schryver, F. C. and Evans, C. H. (1996) *J. Phys. Chem.* **100**, 19959.
6. Wu, D. and Hurtubise, R. J. (1993) *Talanta*, **40**, 901.
7. Agbaria, R. A. Uzan, B. and Gill, D. (1989) *J. Phys. Chem.* **93**, 3855.
8. Albert, A. and Serjeant, E. P. (1984) in "The Determination of Ionization Constants", Chapman and Hall, London, pp.145.
9. Harris, C. M. and Selinger, B. K. (1980) *J. Phys. Chem.* **84**, 891.
10. Kang, J., Kumar, V., Yang, D., Chowdhury, P. R., Hohl, R. J. (2002) *European J. Pharm. Sc.*, **15**, 163.
11. Behera, P. K. and Mishra, A. K. (1993) *J. Photochem. Photobiol. A:Chem.* **71**, 115.
12. Behera, P. K., Mukherjee, T. and Mishra, A. K. (1995) *J. Lumin.*, **65**, 131.
13. Riddick, J. A. and Bunger, W. B. (1970) *Organic Solvents*, Wiley, New York.
14. Hoshino, M., Imamura, M., Ikehara, K. and Hama, Y. (1981) *J. Phys. Chem.* **85**, 1820.
15. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1995) *Ind. J. Chem.* **34A**, 11.
16. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1996) *J. Lumin.* **69**, 95.
17. Webb, S. P., Yeh, S. W., Philips, L. A., Tolbert, M. A. and Clark, J. H. (1984) *J. Am. Chem. Soc.* **106**, 7286.
18. Behera, P. K. (1993) Ph. D. Thesis, Sambalpur University.
19. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1999) *Advances in Colloid and Interface Sc.*, **83**, 1.
20. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1998) *J. Photochem. Photobiol. A*, **113**, 73.
21. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1998) *Ind. J. Chem.* **37A**, 206.
22. Turro, N. J. (1978) *Modern Molecular Photochemistry*, Benjamin/Cummings, San Francisco, CA pp.318.
23. Kaneko, M., Hou, X.-H. and Yamada, A. (1986) *J. Chem. Soc.*, Faraday Trans. I, **82**, 1637.
24. Panda, M., Behera, P. K., Mishra, B. K. and Behera, G. B. (1995) *J. Photochem. Photobiol.* **90**, 69.
25. Kaneko, M. (1992) *Proc. Indian Acad. Sci. (Chem. Sci.)* **104**, 723.
26. Holmes, A. S., Brich, D. J. S., Suhling, K., Imhof, R. E., Salthammer, T. and Dreeskamp, H. (1991) *Chem. Phys. Lett.* **186**, 189.