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Absorption and emission spectroscopic studies of fluorescein dye in alkanol, micellar and microemulsion media

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Abstract

The photophysical behaviours of the dye fluorescein have been studied in alkanols (methanol to decanol), in micellar solutions of SDS, CTAB and Triton X-100 and in the w/o microemulsion system of water/AOT/heptane. The alkanols are found to affect the absorption and fluorescence spectra of the dye. On the basis of the solvent adsorption model the binding constants of the dye with the alkanols have been estimated. The absorption and emission spectra of the dye are observed to be influenced by the ionic surfactants SDS, CTAB and not influenced by the non-ionic representative Triton X-100. The results confirm formation of a 1 : 1 complex of the dye with SDS and CTAB micelles. In the w/o microemulsion medium, the photophysical behaviours of the dye are found to be different from non-aqueous and micellar media.

Keywords: Fluorescein; Absorption; Emission; Alkanol; Micelles (SDS, CTAB, Triton X-100); Microemulsion (water/AOT/heptane)

1. Introduction

The photophysics and photochemistry of dyes in general are of considerable interest in the appreciations of various phenomena in pico to micro-second range, viz, fluorescence, phosphorescence, long- and short-range excitation energy transfer, electron transfer and various modes of quenching. Dyes in general have planar hydrophobic centres with extended systems of single and double bonds and hydrophilic groups in the peripheral regions. The dye molecules may be sequestered in solvents and organised in the micellar interior or at the interface. Interaction of a dye with a solvent at the molecular level is reflected in its visible and fluorescence spectra [1–8]. Interesting features of such phenomena may occur in surfactant and micellar solutions which are of general and particular interest in view of the special role of surfaces in guiding and modifying physicochemical processes [9,10].

Fluorescein (FL) is an anionic dye. Both ground and excited state spectra of the dye are affected by solvents and micelles. We have studied the spectroscopic behaviour of the dye in different alkanols from methanol to decanol and aqueous micellar solutions of sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and octylphenyl polyoxyethylene ether (Triton X-100) as well as in water/AOT/heptane microemulsion (w/o type) medium. A comparative analysis of the results realized in the three categories of solvent media has been presented. Although allied studies on the physicochemical behaviours of dyes in non-aqueous solvents and self-organising systems are found in literature [3,4,20,21], similar investigations on FL are rare.

2. Experimental

FL was carefully purified by passing its alkaline solution through alumina column followed by precipitation with HCl [11]. The process of dissolution in alkali and precipitation with HCl was repeated thrice. The alkanols (methanol, MeOH; ethanol, EtOH; propanol, PrOH; butanol, BuOH; pentanol, PnOH; hexanol, HxOH; heptanol, HpOH; octanol, OcOH and decanol, DcOH) used were spectroscopic grade products of E. Merck, Germany. They were dried following the standard procedure [12] and purified by fractional distillation. The presence of photoactive impurities was checked by emission measurements and was found to be absent. The surfactants SDS, CTAB, Triton X-100, represented as TX-100 were BDH products. The surfactant AOT (2-bis ethylhexyl sulfosuccinate) was a 99% pure product of Sigma, USA.

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Absorption spectra were recorded using a Shimadzu (Japan) 160A UV-visible Spectrophotometer with a matched pair of silica cuvettes. Fluorescence spectra were taken in a Fluorolog F-III A Spectrofluorimeter (Spex, INC, NJ, USA) with a slit width of 2.5 nm, Duplicate spectral measurements were taken at a constant temperature, 25 ± 0.10°C, and the mean values were used for data analysis. Doubly distilled water was used for solution preparation.

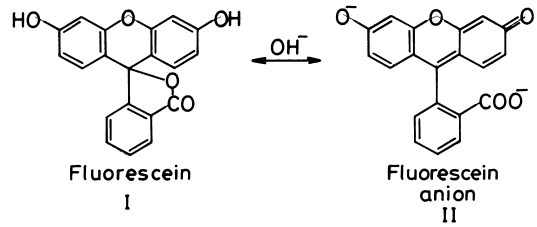

Fig. 2. Structure of fluorescein.

3. Results and discussion

3.1. Absorption and emission of FL in alkanols

The absorption spectra of FL in H_2O, MeOH, EtOH, PrOH, BuOH, PnOH, HxOH, HpOH, OcOH and DcOH are presented in Fig. 1. In the alkanols, the visible spectral transitions occur in the range of 440–454 nm, and the emission maxima occur in the range of 510–517 nm. According to Davies [12], the classical representations of FL in Structure I and II (Fig. 2) compare the structure of FL in alkaline medium. Fig. 3 (A) and (B) represent the absorption spectra of FL in acidic medium and alkaline medium, respectively. The observed spectral shifts and the associated intensities can be used for quantitative assessment of the interaction of the dye with its surrounding environments.

On gradual addition of water to the solution of FL in methanol both the absorbance and fluorescence intensities increase with a red shift of the λ_{max}. The mixed aquo-EtOH and aquo-PrOH have also shown similar behaviours with FL. Fig. 4 represents the variation of absorbance of FL with different combinations of methanol and water. Appreciable initial drop in fluorescence intensity and absorbance are observed which afterwards tend to level off at higher fraction of alcohol. This effect can be rationalized on the basis of solvent adsorption model [13], where primary solvent occupies fixed sites on the surface of the solute molecule, assuming that the Langmuir's adsorption isotherm is applicable, where γ represents the fraction of sites occupied.

According to Lippert, [14]

$$\gamma = \frac{n}{n_{max}} = \frac{\text{number of sites occupied}}{\text{maximum number of sites available}} = \frac{(F_0 - F)}{(F_0 - F_{max})} \quad (1)$$

where F_0 is the fluorescence intensity in aqueous solution, F is the fluorescence intensity in presence of water and alcohol and F_{max} is the fluorescence intensity when all the sites of the molecules are occupied by alcohol i.e. in pure alcohol.

For a specific case let us consider H_2O–MeOH system. On gradual addition of water molecules to alcoholic solution of FL, water molecules displace alcohol molecules initially

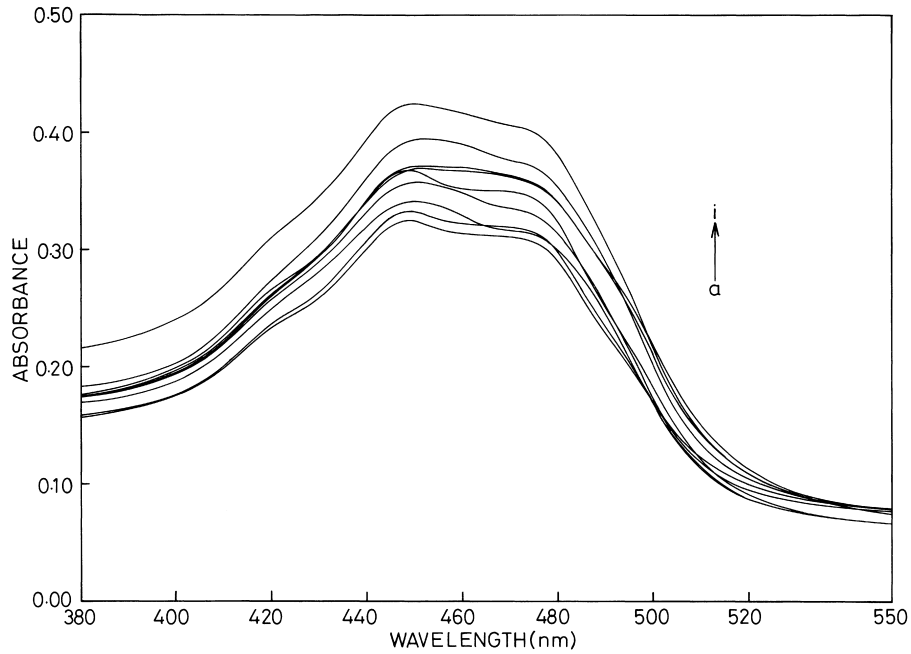

Fig. 1. Absorption spectra of fluorescein in different solvents: (a) MeOH, (b) EtOH, (c) PrOH, (d) BuOH, (e) PnOH, (f) HxOH, (g) HpOH, (h) OcOH, (i) DcOH at 303 K.

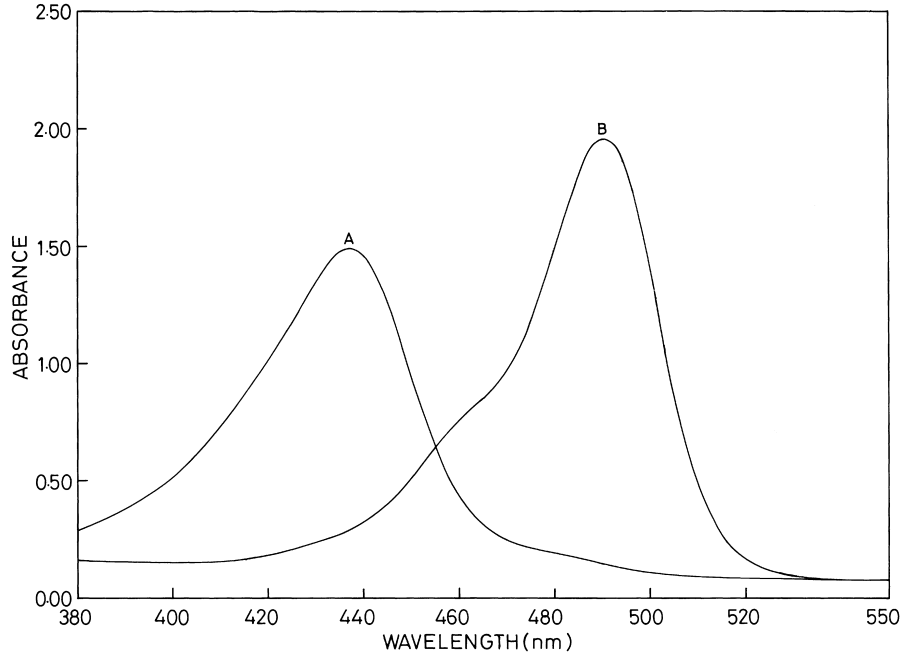

Fig. 3. Absorption spectra of fluorescein (5.3×10^{-5} mol dm^{-3}) in acidic medium (A), alkaline medium (B) at 303 K.

adsorbed on a definite number of sites available on the dye molecule following the equilibrium

$$M_L + S \overset{K}{\rightleftharpoons} M_A \quad (2)$$

where M_L is the free solvent molecules in solution phase, S is the number of free (available) sites on the solute surface, M_A is the number of solvent molecules adsorbed on the surface of solute molecules i.e. number of sites occupied by the solvent molecules, and K is the equilibrium constant for the binding of solvent (alkanol) with the solute (dye, FL).

If C_A and C_L are the concentrations of alcohol in the adsorbed and the free state, respectively such that the total

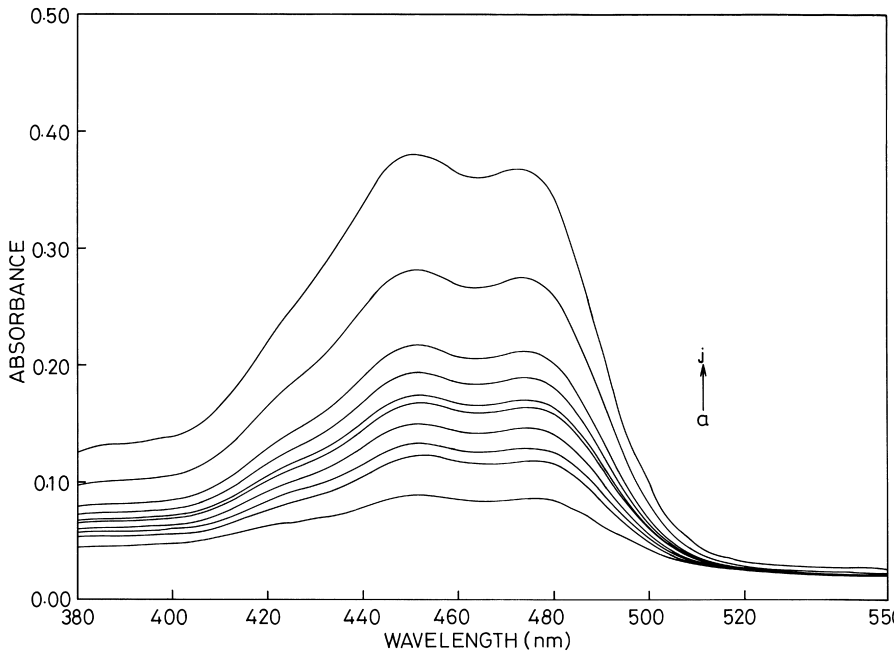

Fig. 4. Absorption spectra of Fluorescein (5.3 × 10−5 mol dm−3) in water–methanol medium at 303 K. a—j represent V/V compositions of MeOH : Water as 10 : 0, 4 : 1, 3 : 1, 2.3 : 1, 1.9 : 1, 1.5 : 1, 1 : 1, 0.82 : 1, 0.67 : 1, 0 : 10.

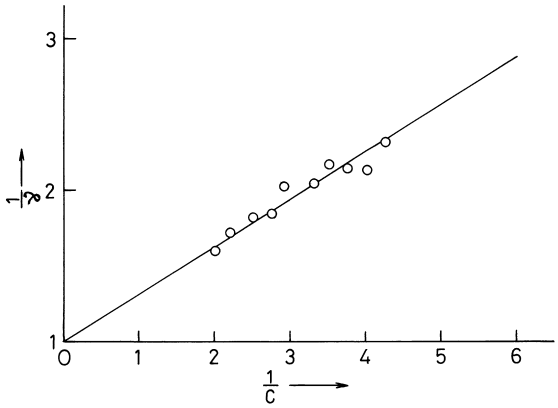

Fig. 5. Plot of $1/\gamma$ vs. $1/C$ according to the Eq. (5). C is expressed in V/V ratio.

concentration C is given by

$$C = C_A + C_L \equiv C_L \tag{3}$$

since, $C_L \gg C_A$.

The equilibrium constant

$$K = \frac{[M_A]}{[M_L][S]} = [C_A]/[C_L][C_S] = \frac{[C_A}{[C][C_S]} \tag{4}$$

where the new term C_S is the concentration of sites available for adsorption. With the help of Eqs. (1) and (4) can be written as

$$K[C] = \frac{[C_A]}{[C_S]} = \frac{n}{(n_{max} - n)} = \frac{\gamma}{(1-\gamma)}$$
$$\frac{1}{\gamma} = 1 + \frac{1}{K[C]} \tag{5}$$

Table 1
The FL–alkanol binding constant K obtained from absorption and emission studies at 298 K

System	From emission (K/mol^{-1} dm^3)	From absorption (K/mol^{-1} dm^3)	Mean (K/mol^{-1} dm^3)
MeOH : H_2O	3.2	2.2	2.7
EtOH : H_2O	3.4	2.8	3.1
PrOH : H_2O	3.6	3.5	3.6

From the straight line plots between $1/\gamma$ and $1/[C]$ depicted in Fig. 5, the equilibrium constant K for the binding process can be obtained. Similar K values can be also obtained from absorption measurements. The derived K values from absorption and emission measurements are presented in Table 1.

The evaluation of K for the interaction of higher alkanols (than PrOH) with the FL is not tenable due to their much reduced solubility in water.

The absorption and emission spectra of FL in alkanols produce distinct Stoke's shifts ($\Delta\bar{\nu}$). These shifts bear a linear relation with the dielectric constant (D) of the alkanols. A relation of the type Eq. (6) given below has been observed.

$$\Delta\bar{\nu} = 2616 + 4.62D \tag{6}$$

An inverse relation between Stoke's shift and number of C atoms in alkanols (n') also holds

$$\Delta\bar{\nu} = 2790 - 23.7n' \tag{7}$$

The 2790 cm^{-1} indicates the Stoke's shift in aqueous solution which is identical with the experimental observation.

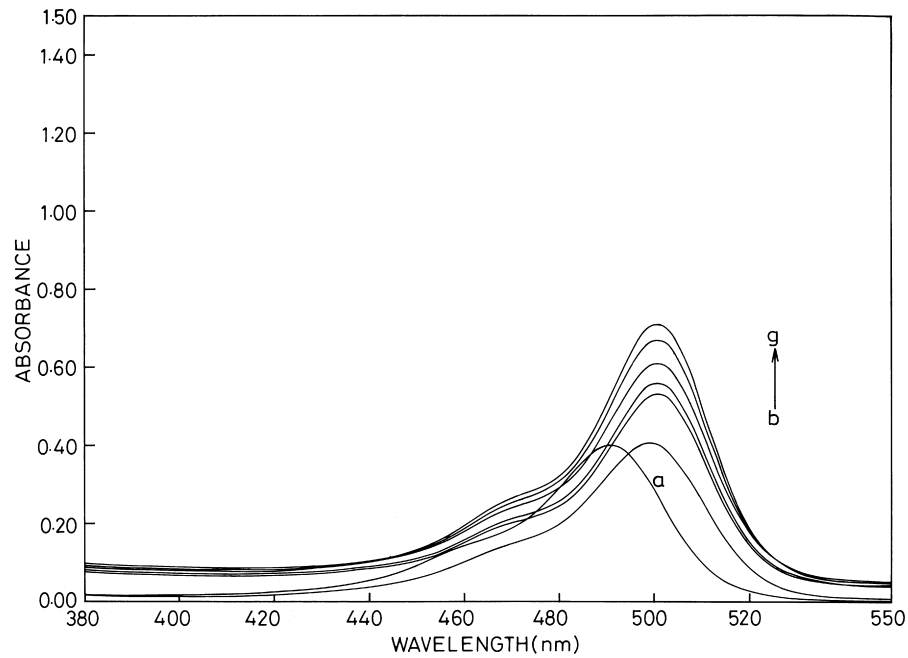

Fig. 6. Absorption spectra of fluorescein (3.9×10^{-5} mol dm^{-3}) in aqueous micellar solution of CTAB at 303 K. [CTAB]: a–g; 0–6 m mol dm^{-3}.

3.2. Spectroscopic behaviour in micellars solutions

The absorption spectra of FL anion in aqueous micellar solution of SDS and CTAB are given in Fig. 6. In micellar solution of the cationic CTAB, the absorption maximum of FL shifts from 494 nm and a new band develops at 500 nm. The emission maximum also shifts from 513 to 523 nm. In aqueous micellar solution of SDS, the shift in absorption maximum is negligible but the absorbance of FL increases with increasing [SDS]. Fluorescence also increases with increasing [SDS] without shift in the wavelength of the fluorescence maximum.

From the results one can assume that a complex (1 : 1 as dye : micelle) is formed between the ionic surfactants and FL. The equilibrium constant, K values are evaluated using the Scott Eq. (8).

$$\frac{[F][D]}{(A-A_0)} = \frac{[D]}{(\varepsilon_c-\varepsilon_0)} + \frac{1}{K(\varepsilon_c-\varepsilon_0)} \quad (8)$$

where $[F]$ and $[D]$ = ([Surfactant]/Aggregation number) represent the concentrations of FL and surfactant micelle, respectively, ε_c, ε_0, A and A_0 represent the molar extinction coefficient of the complex, aqueous solution of the pure dye, the absorbance of the solution and the pure dye, respectively. In the calculation of $[D]$ the aggregation numbers of SDS [20] and CTAB [20] of 50 and 55 were used. The K values obtained by the equation of Benesi and Hildebrand also agree with the results derived from Eq. (8). These results are presented in Table 2.

In presence of 0.5 M NaCl, the FL also forms 1 : 1 complex with aqueous solution of CTAB and SDS. In absence of salt the complexation with CTAB is stronger than with SDS. This is much enhanced in presence of the salt NaCl. For ionic micelles, the binding constants and other thermodynamic parameters represent a composite of hydrophobic and electrostatic interactions. The dye FL–CTAB complex has higher binding strength because of the opposite charges of the components. The hydrophobic interaction plays the major role in the formation of FL–SDS complex. Since the dye micelle complex is formed above the CMC, the miceller surface plays a crucial role in the complex formation. In presence of NaCl (0.5 M) the size and shape of the micelles are altered, relatively to different extents. The hydrophobic interaction is relatively prominent in salt environment than the electrostatic interaction. How all these factors guide the complexation process to yield the results given in Table 2 is not straightforwardly understood at present. Further experimentations are necessary in this direction.

Table 2
The binding constant K of Fluorescein with SDS and CTAB Micelles and in salt Environments at 298 K

System	10^{-2} K_C/mol^{-1} dm^3
CTAB	4.92
	36.8 [a]
SDS	2.25
	0.89 [a]

[a] In presence of 0.5 mol dm^{-3} NaCl.

The surfactant TX-100 has not affected the spectra of FL, even in its micellar solution. We cannot rule out hydrophobic interaction of FL with the neutral micelle of TX-100. But this could not be evaluated due to the lack of spectral manifestation as in SDS and CTAB.

3.3. Spectroscopic behaviours in microemulsion medium

In water/oil (w/o) microemulsion medium (water/AOT/heptane system) at pH = 12 the absorption and emission spectra of FL have been studied Fig. 7. Two absorption peaks at 455 and 480 nm are obtained. The [water]/[AOT] mole ratio, ω has been varied between 4 and 20 at a fixed [FL] and the corresponding absorption spectra measured. From the plot (Fig. 8) of $A_R = Abs_I/Abs_{II}$ (Abs_I and Abs_{II} are absorbances at the peaks, 455 and 480 nm, respectively) vs. ω it is observed that there is a change of the initial trend of the curve at $\omega \geq 6$. The ratio of the peak heights (A_R) also exhibits a break at a composition, where free water starts existing in the micro water pool (Fig. 8(b)). In the calculation of combined and free water, six water molecules per AOT anion has been considered according to literature reports [15,16]. The break at $\omega = 6$ or zero free water state indicates internal environmental change responsible for the manifestation of striking spectral behaviour. From the absorption peak ratio (A_R), change of water pool environment of AOT microemulsion at $\omega > 6$ has been reported in recent literature [16–19].

The beginning of free water in the aqueous pool demarcates microemulsion from reverse micelles. In the case of AOT the demarcating composition is $\omega = 6$. The spectral probing using the FL dye is, thus, a convenient method of elucidation of the distinction between reverse micelles and w/o microemulsion.

3.4. Acid–base behaviour of FL in different environments

The dye FL exhibits two distinct bands at 455 and 481 nm in acidic and basic medium, respectively. In AOT micellar medium the basic peak at 481 nm gets prominence with increasing [AOT] upto CMC similar to the observations reported on the dye [20,21] Safranine T.

At [AOT] > CMC, interchange between the two species (i.e, growth of one at the expense of the other) does not occur, same spectral pattern appears with reduced intensity. As if the [FL] decreases by a reaction with AOT. In Figs. 7 and 9, the absorption and fluorescence spectra of FL in AOT microemulsion medium (water/AOT/heptane at different ω values), and absorption spectra of FL in AOT micellar medium are presented, respectively. In the microemulsion medium, the acidic peak is more prominent than the basic peak. An intensity change similar to that in AOT micellar medium is observed. An onward decrease in absorbance

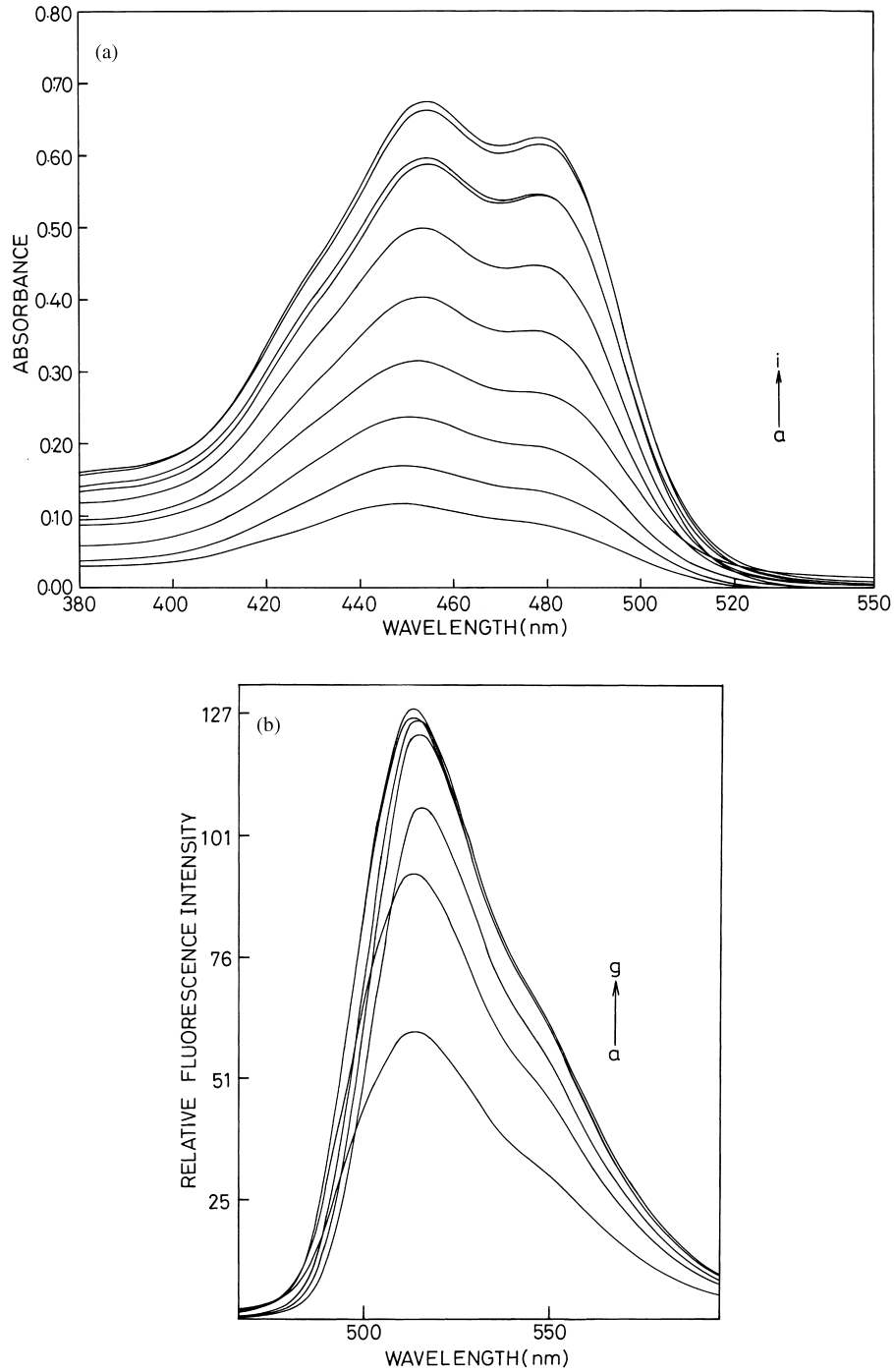

Fig. 7. Spectra of fluorescein in microemulsion medium. A: absorption spectra; a–i, $\omega = 4, 6, 8, 10, 12, 14, 16, 18, 20$. B: fluorescence spectra; a–g; ω same as above.

with decreasing ω (i.e. the water pool size) has been observed.

We have attempted to understand the dissociation of the hydroxyl group of FL in aqueous, AOT micellar solution and in w/o microemulsion medium but have encountered with a difficulty. The dye has shown insolubility in buffered micellar and microemulsion media. The solubilization can be only achieved at a high pH. Therefore, we have adjusted the $[H^+]$ of the aqueous dye solutions by adding acid (HCl) and alkali (NaOH) and have measured the pH with a pH meter. The solutions were prepared and handled as far as practicable in closed containers to minimize contact with air. The graphical representation of the processing of data has not been presented to save space. Equivalent measurements in unbuffered condition (as mentioned above) have been also taken. The respective pK values in buffered and unbuffered conditions obtained for FL are 7.8 and 8.0. The mean value of 7.90 is taken to be the pK of FL. We have been unable to find

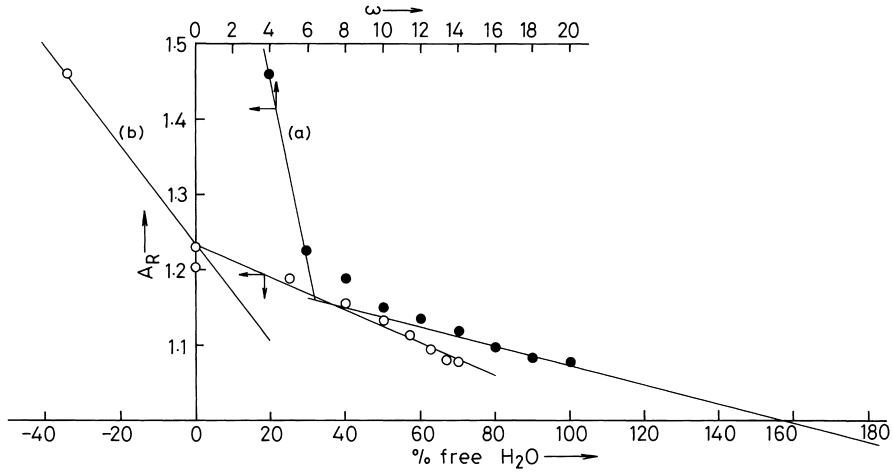

Fig. 8. Dependence of A_R on ω (a) and free water (b) in microemulsion at 303 K.

out the pK of FL in literature. In AOT miceller solution (three times its CMC), the pK value obtained in unbuffered condition is 7.0. Buffered medium could not be used for reasons mentioned above. The lowering of pH by one unit in AOT micellar solution may not be unreal. The acid–base equilibrium of dyes has been reported to be appreciably altered in micellar and microemulsion media [22–24]. But ineqality between the local (microscopic) pH and the bulk pH (used in calculation) remains a point of criticism in the data processing procedure. Further studies are required to resolve the point of criticism.

In aqueous buffered medium, pK of the dye has been determined from the spectral measurements at 490 nm (λ_{max} of FL) in terms of the relation,

$$\log \frac{(A_{buffer} - A_b)}{(A_a - A_{buffer})} = pK + pH \quad (9)$$

where A_a, A_b and A_{buffer} are the absorbances of FL in excess acid, excess base and buffered media, respectively.

4. Conclusions

1. The absorption and emission spectra of FL in alkanol and aquo-alkanol media have provided quantitative informations on the competitive binding of the alkanols with the dye.
2. FL also binds with SDS and CTAB micelles but not with TX-100.

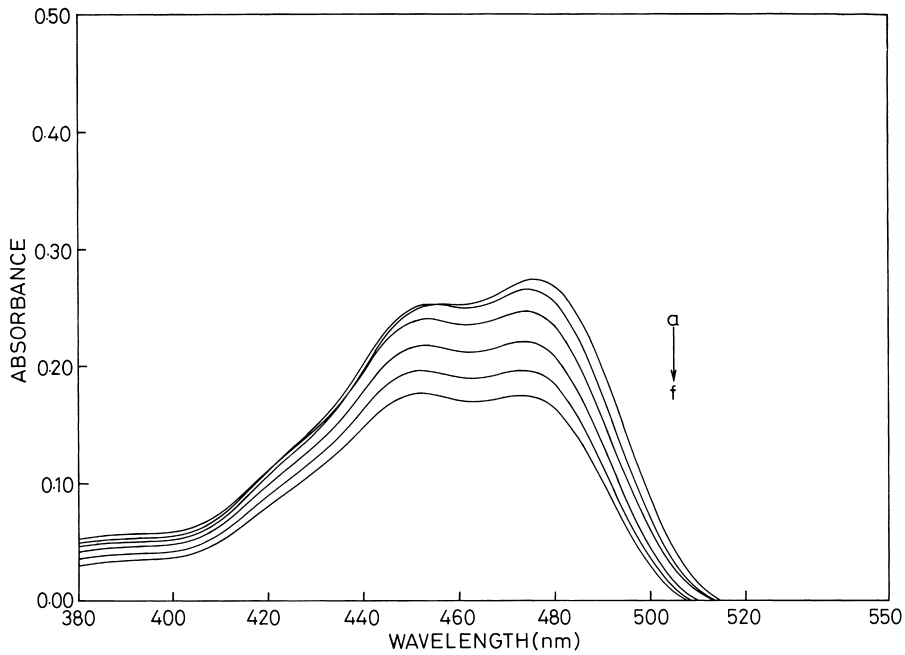

Fig. 9. Absorption spectra of fluorescein (3.9×10^{-5} mol dm^{-3}) in AOT micellar medium. [AOT] = (a) 0, (b–f) 1–5 m mol dm^{-3}.

3. In the water/AOT/heptane (w/o) microemulsion medium the spectral behaviour of FL can help identify the threshold composition between bound and free water in the microwater pool which is at $\omega = 6$.
4. The pK of FL is decreased by one unit in AOT micellar medium three times its CMC.

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References

[1] S.C. Bhattacharya, P. Roy, S.P. Moulik, J. Photochem. Photobiol. A: Chem. 88 (1995) 139.
[2] S.C. Bhattacharya, H. Das, S.P. Moulik, J. Photochem. Photobiol. A: Chem. 79 (1994) 109.
[3] M. Mukhopadhyay, B.B. Bhowmik, Colloid Polymer Sci. 266 (1988) 672.
[4] M. Mukhopadhyay, B.B. Bhowmik, Colloid Polymer Sci. 268 (1990) 447.
[5] S.K. Brahma, C. Bandopadhyay, A.K. Chakraborty, Indian J. Chem. 29A (1990) 1165.
[6] G. Cabor, E. Fischer, J. Phys. Chem. 75 (1971) 581.
[7] J. Griffitus, Chem. Soc. Rev. 1 (1972) 481.
[8] N. Mataga, V. Toritashi, K. Ezumi, Theor., Chim. Acta 2 (1964) 158.
[9] K. Shinoda, B. Tamamushi, T. Nakagawa, T. Isemura, Colloidal Surfactants, Academic Press, New York, 1963, p. 155.
[10] P. Roy, S.C. Bhattacharya, S.P. Moulik, J. Photochem. Photobiol A: Chem. 108 (1997) 267.
[11] K.K. Rohatgi, A.K. Mukhopadhyay, Photochem. Photobiol. 14 (1971) 551.
[12] I.L. Finar, Organic Chemistry I, ELBS, 6th ed. 1973, p. 890.
[13] S.C. Bhattacharya, S. Basu, K.K. Rohatgi Mukherjee, J. Indian Chem. Soc. 70 (1993) 425.
[14] E. Lippert, in: Organic Molecular Photophysics, vol. 2, J.B. Birks (Ed.), Wiley, New York, 1975.
[15] M. Wong, J.K. Thomas, T. Nowak, J. Am. Chem. Soc. 79 (1977) 4730.
[16] M. Moran, G.A. Bowmaker, R.P. Cooney, Langmuir 11 (1995) 738.
[17] N. Wittouck, R.M. Neghi, M. Ameloot, F.C. Deschyriber, J. Am. Chem. Soc 116 (1994) 10601.
[18] J. Nishimoto, E. Iwamoto, T. Fujiwara, T. Kumamaru, J. Chem. Soc., Faraday Trans. 89 (1993) 535.
[19] M. Hasegawa, T. Sugimura, Y. Shind, A. Kitahara, Colloids Surface A 109 (1996) 305.
[20] S.C. Bhattacharya, H. Das, S.P. Moulik, J. Photocem. Photobiol A: Chem. 74 (1993) 239.
[21] S.C. Bhattacharya, H. Das, S.P. Moulik, J. Photocem. Photobiol A: Chem. 71 (1993) 257.
[22] S.P. Moulik, B.K. Paul, D.C. Mukherjee, J. Colloid Interface Sci. 161 (1993) 72.
[23] C. Oldfield, B.H. Robinson, R.B. Freedman, J. Chem. Soc., Faraday Trans. 86 (1990) 883.
[24] C.J. Drummond, F. Grieser, T.W. Healy, J. Chem. Soc., Fraday Trans. 85 (1989) 55.