Indian Journal of Chemistry
Vol. 14A, May 1976, pp. 302-305

Environment-induced Spectral Behaviour of Acridine Orange Dye

S. P. MOULIK & S. GHOSH
Department of Chemistry, Jadavpur University, Calcutta 700032
and
A. R. DAS
Department of Physical Chemistry, Indian Association for the Cultivation of Science, Calcutta 700032

Received 24 *March* 1975; *accepted* 10 *July* 1975

The protonation-deprotonation and monomer-dimer conversions of acridine orange dye in solution have been studied employing visible spectra under varied environments. In aqueous medium, the acid-base equilibrium operates in a *p*H range of 10·0 to 11·50. At higher *p*H, the basic form of the dye remains in solution as monomers, whereas at *p*H < 10·0 the monoprotonated form of the dye exists in solution in equilibrium with its dimer. This dimerization can be enhanced with the addition of salts, but it can be totally prevented by the addition of co-solvents. In the presence of a large excess of acid, the dye forms a triprotonated species which does not dimerize.

THE potentialities of some basic dyes as laser materials have led to a renewed interest in their general spectral behaviour[1-5]. In exploring the laser possibilities of acridine orange dye, Ferguson and Mau[6,7] have concluded recently that the long anticipated monomer-dimer interchange of this dye is actually an acid-base conversion process. These authors envisaged a vital role of protons, supposed to be liberated from the traces of water even when the dye base is present in a non-aqueous medium. Before rejecting the old concept of monomer-dimer interchange of this dye[8-11], the physico-chemical behaviours of the dye in various environments have to be thoroughly investigated. The results of a detailed study on the visible spectra of acridine orange under varied environments are reported in this paper.

Materials and Methods

Acridine orange (BDH) was purified following the procedure of Lamm and Neville[8], and preserved in a vacuum desiccator. Its homogeneity was checked by TLC and IR spectra. All other reagents and salts used were of BDH analar grade. The alcohol used was further purified adopting the standard procedures. Conductivity water was used throughout. The chloride content of the dye was determined by the conductometric titration using standardized $AgNO_3$ solution.

UV spectra of the dye solutions of concentrations of the order $10^{-5}M$ were recorded on a SF-4A spectrophotometer (USSR), which was checked against a standard Beckman DU spectrophotometer. Dye adsorption on the cell surfaces was guarded by prolonged storage of dilute solutions in them. The coated surfaces were found to be resistant to ordinary washing.

The *p*H measurements were taken on a *p*H meter (Elico, Hyderabad, India) using a glass-calomel electrode assembly. The instrument was calibrated using standard buffers of *p*H 7·00 and 9·14. The hydrogen ion concentrations of the dye solutions were varied by adding NaOH solution, and their *p*H values measured at $25 \pm 0{\cdot}1°$.

Results

The visible spectra of the dye at different concentrations ($2{\cdot}0\text{-}6{\cdot}0 \times 10^{-5}M$) in water showed a regular increase in absorbance on increasing the [dye]. At higher concentrations the spectra were characterized by a band at 490 nm and a shoulder at 465 nm. No isosbestic point in contrast to the earlier results of Lamm[8] and Robinson[9] could be observed in the absorption spectra. This is in agreement with the proposition of Cohen and Fischer[12] who showed that the isosbestic points should not normally occur in open systems. The effect of alcohol on the dye solution is shown in Fig. 1. In the presence of alcohol, for the same dye concentration in the range $2{\cdot}0\text{-}6{\cdot}0 \times 10^{-5}M$ as in the case of water, higher absorbance with a sharp peak at 490 nm was noticed. The basic form of the dye exhibits λ_{max} at 430 nm ($\epsilon = 1{\cdot}95 \pm 0{\cdot}05 \times 10^4$ mole^{-1} cm^{-1}) which remained unaffected by the addition of non-aqueous solvents. The colour of the dye was greenish yellow with fluorescence.

The effect of added salts on the absorption behaviour of the dye is illustrated in Fig. 2. The 490 nm band gradually became broad and ultimately developed into a definite band at 465 nm, indicating the salt-induced monomer-dimer conversion in the system.

Within the *p*H range of 10·00-11·50 the spectra of the acid and basic forms of the dye (overall concentration $1{\cdot}46 \times 10^{-5}M$) exhibited isosbestic point at 445 nm (Fig. 3). In mild acid solutions the colour of the dye was orange-red which turned yellow with a greenish fluorescence when basic.

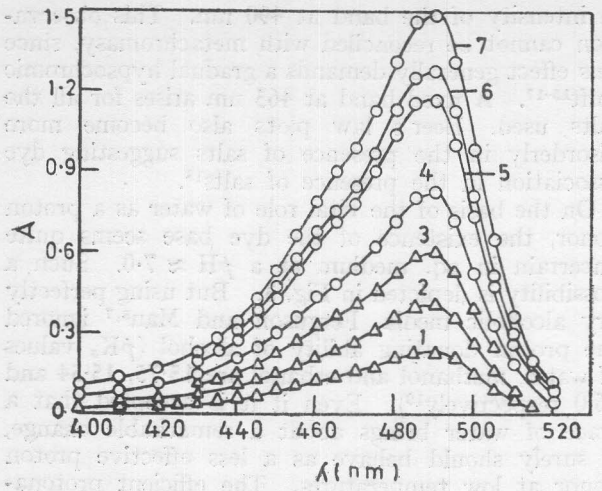


Fig. 1 — Visible spectra of acridine orange in 80% (v/v) ethanol {[Dye] = (1) 0·292, (2) 0·583, (3) 0·875, (4) 1·17, (5) 1·46, (6) 1·75, and (7) $2·03\times10^{-5}M$}

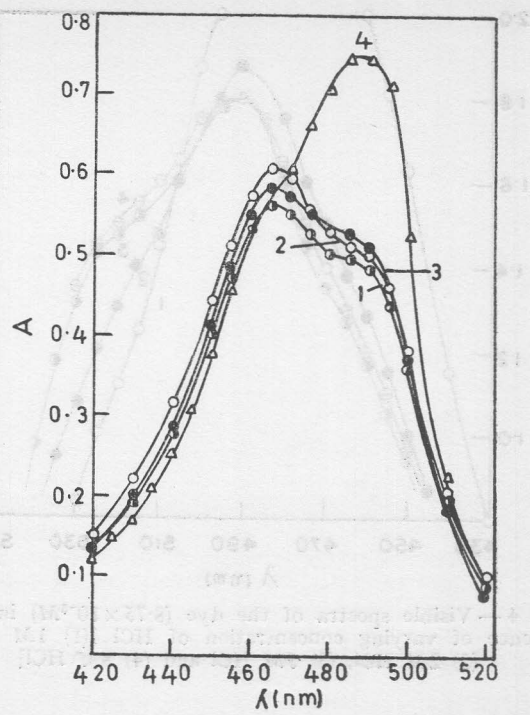


Fig. 2 — Effect of added salts on the visible spectra of acridine orange at [dye] = $1·46\times10^{-5}M$ [(1) $1M$ KCl, (2) $1M$ KBr, (3) $1M$ $MgCl_2$, and (4) no salt]

Under mildly acidic medium, unlike aq. environment, the 490 nm band was considerably suppressed with a red shift of both the band and shoulder (Fig. 4). At higher acid concentration ($>5M$), a band at 525 nm ($\epsilon = 2·68\pm0·1\times10^4$ mole^{-1} cm^{-1}) having an isosbestic point at 515 nm was formed, and the solution turned deep red. Multiprotonation phenomenon was anticipated to be operative in the present case[13,14]. The addition of non-aqueous solvents had no effect on the spectral characteristics of the dye when the acid present was in large excess.

Discussion

The protonation-deprotonation equilibrium of the acridine orange as shown in Scheme 1 is considered valid in aqueous medium. It is obvious from the experimental results that the absorption spectrum of the dye base undergoes a bathochromic shift at low [acid]. However, in the presence of a base ($\sim10^{-4}M$), an equilibrium between the monoprotonated (B) and its base (A) is supposed to exist, as a conspicuous acid-base interchange has been observed in the *p*H range 10·00-11·50. Excess acid brings about a further bathochromic shift of the absorption maximum to 525 nm which, however, is not

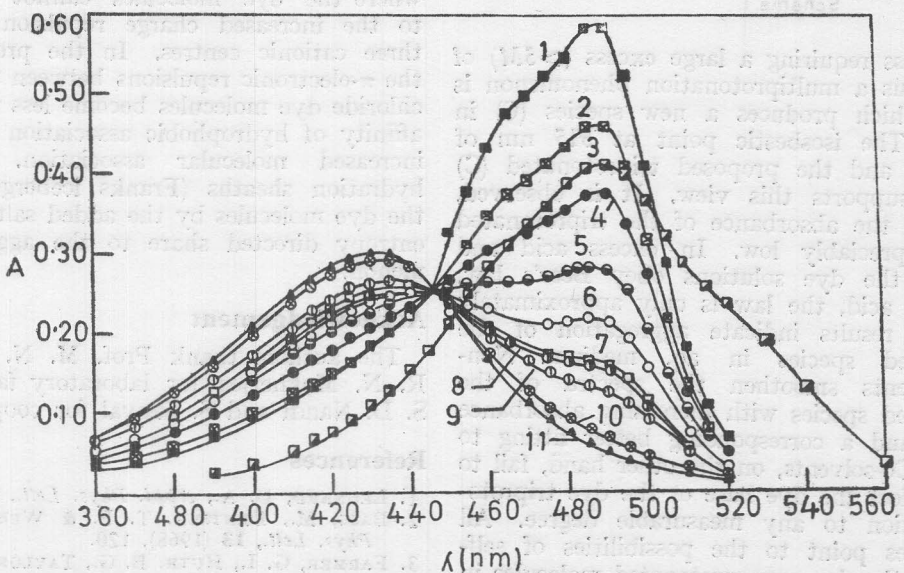


Fig. 3 — Effect of *p*H on the visible spectra of the dye at $1·46\times10^{-5}M$ (1) *p*H = 9·19, (2) *p*H = 9·81, (3) excess acid, (4) *p*H = 9·97, (5) *p*H = 10·11, (6) *p*H = 10·25, (7) *p*H = 10·58, (8) *p*H = 10·61, (9) *p*H = 10·80, (10) *p*H = 11·22, and (11) *p*H = 11·47

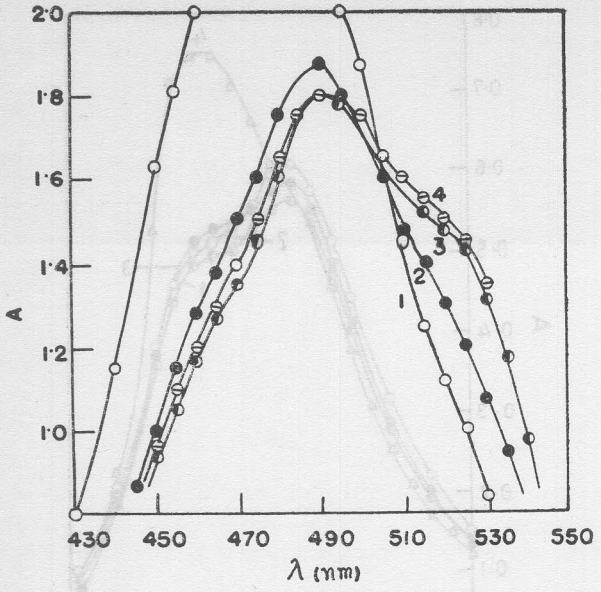
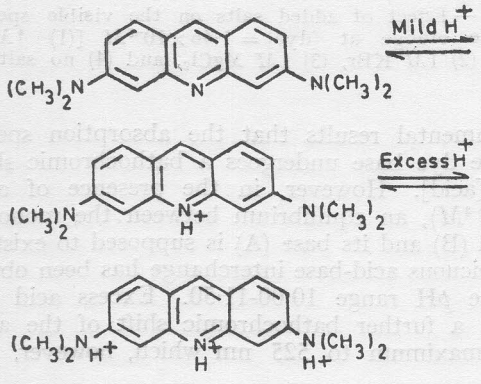


Fig. 4 — Visible spectra of the dye ($8{\cdot}75\times10^{-5}M$) in the presence of varying concentration of HCl [(1) $1M$ HCl, (2) $2M$ HCl, (3) $5M$ HCl and (4) $8M$ HCl]



Scheme 1

an easy process requiring a large excess ($>5M$) of the acid. Thus a multiprotonation phenomenon is anticipated which produces a new species (C) in solution[13,14]. The isosbestic point at 515 nm of the mono-(B) and the proposed triprotonated (C) species also supports this view. It is observed, however, that the absorbance of the triprotonated species is appreciably low. In excess acid and basic media, the dye solutions obey Beer's law, while in mild acid, the law is only approximately valid. These results indicate aggregation of the monoprotonated species in aq. medium. Non-aqueous solvents smoothen the spectra of the monoprotonated species with increasing absorbance at 490 nm, and a corresponding better fitting to Beer's law. Co-solvents, on the other hand, fail to improve or affect the dye base or the dye triprotonated absorption to any measurable degree. All these evidences point to the possibilities of self-association of the dye monoprotonated molecules in aq. environment. Addition of salts develops a new band at 465 nm with a concomittant decrease in intensity of the band at 490 nm. This observation cannot be reconciled with metachromasy, since this effect generally demands a gradual hypsochromic shift[15-17]. A fixed band at 465 nm arises for all the salts used. Beer's law plots also become more disorderly in the presence of salts suggesting dye association in the presence of salts[18].

On the basis of the vital role of water as a proton donor, the existence of the dye base seems quite uncertain in aq. medium at a pH $\approx$ 7·0. Such a possibility is depicted in Fig. 3. But using perfectly dry alcoholic media, Ferguson and Mau[6,7] ignored the proton donating ability of alcohol (pK$_a$ values of water, methanol and ethanol are 15·75, 15·54 and 16·0 respectively[19]). Even if it is accepted that a trace of water brings about a remarkable change, it surely should behave as a less effective proton donor at low temperatures. The efficient protonation suggested by the above authors at low temperatures is difficult to reconcile, because at low temperature pK$_a$ of water should increase and may become more than that of alcohol at an initial higher temperature. The non-protonation function of alcohol thus remains unaccountable. However, the present observation that the dye base fails to undergo association in aq. medium is in conformity with the findings of Ferguson and Mau in non-aqueous environments.

The monoprotonated dye does not even associate in the presence of appreciable proportion of a co-solvent of moderate polarity. Association of acid dye is, of course, possible in a medium of much low polarity, and definite solubility problems have been observed to arise in the present dye system using dioxane.

Since the monoprotonated form of the dye can only undergo aggregation, the acid-base equilibrium is considered entirely different from the monomer-dimer equilibrium, and this contention has been quantitatively estimated in a recent publication[20]. Multiprotonation is possible only in excess acid, where the dye molecules cannot associate owing to the increased charge repulsion as a result of three cationic centres. In the presence of a salt, the π-electronic repulsions between two monohydrochloride dye molecules become less compared to the affinity of hydrophobic association resulting in the increased molecular association. Dislocation of hydration sheaths (Franks icebergs)[21] surrounding the dye molecules by the added salts contributes an entropy directed share to the aggregation phenomenon.

Acknowledgement

The authors thank Prof. M. N. Das and Prof. R. N. Mukherjea for laboratory facilities and Drs S. D. Nandi and S. Sanyal for cooperation.

References

1. Leonard, D. A., *Appl. Phys. Lett.*, **7** (1965), 4.
2. Bass, M., Dentseh, T. F. & Weber, M. J., *Appl. Phys. Lett.*, **13** (1968), 120.
3. Farmer, G. I., Huth, B. G., Taylor, L. M. & Kegan, M. R., *Appl. Opt.*, **8** (1969), 363.
4. Samelson, R., *Electronics*, (1968), 142.
5. Keller, R. A., *IEEEJ, Quantum Electron.*, **6** (1970), 411.

6. Ferguson, J. & Mau, A. W. H., *Chem. Phys. Lett.*, **17** (1972), 543.
7. Ferguson, J. & Mau, A. W. H., *Aust. J. Chem.*, **26** (1973), 1617.
8. Lamm, M. E. & Neville, D. M., *J. phys. Chem.*, **69** (1965), 3872.
9. Robinson, B. H., Löffler, A. & Schwarz, G., *Faraday Trans. I*, (1973), 56.
10. Mataga, N., *Bull. chem. Soc. Japan*, **30** (1957), 375.
11. Chambers, R. W., Kijiwara, T. & Kearns, D. R., *J. phys. Chem.*, **78** (974), 380.
12. Cohen, N. D. & Fischer, E., *J. chem. Soc.*, (1962), 3044.
13. Ghosh, A. K., *J. Am. chem. Soc.*, **92** (1970), 6415.
14. Lewis, G. N. & Bigeleisen, J., *J. Am. chem. Soc.*, **65** (1943), 1144.
15. Bradley, D. F. & Wolf, M. K., *Proc. natl Acad. Sci.*, **45** (1959), 944.
16. Michaelis, L., *J. phys. Chem.*, **54** (1950), 1.
17. Pal, M. K. & Schubert, M., *J. phys. Chem.*, **67** (1963), 1821.
18. Krueser, T. & Straus, U. P., *J. phys. Chem.*, **74** (1970), 3081.
19. King, A. E., *Acid-base equilibria* (Pergamon Press, Oxford), 1965.
20. Moulik, S. P., Ghosh, S. & Das, A. R., *J. phys. Chem.*, (communicated).
21. Frank, H. S. & Evans, M. W., *J. chem. Phys.*, **13** (1945), 507.