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Fluorescence and Light Scattering Studies on Indole-3-Acetic Acid in Micellar Media

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Abstract —Indole-3-Acetic Acid (I-3-AA) is a phytochrome auxin found in tulip bulbs, in unripe pea seeds, in fungi and in rice cells. It is a plant growth hormone and is biologically and analytically an important molecule. Even small traces of I-3-AA can be assayed by using fluorimetric method. The enhanced fluorescence emission intensity as well as quantum yield values in microheterogeneous micellar environment prove that the suspending hydrophobic I-3-AA molecules are solubilized. The solubilizing action of surfactants on I-3-AA has been supplemented by light scattering studies and some theoretically calculated spectral parameters like empirical fluorescence coefficient (k_f), fluorescence quantum yield (σ_f), molar extinction coefficient (ϵ) and Stokes' shift values.

Keywords : *Surfactants, I-3-AA, Fluorescence, Solubilization.*

INTRODUCTION

Fluorescence technique is an important tool for the investigation of the interaction between micelles and biomolecules. Surfactants have been extensively used in areas related to detergency, emulsification, pharmaceuticals, agriculture, enhanced petroleum recovery, etc. as the surfactants exhibit pronounced interfacial properties [1-4]. Recently, polymer-surfactant systems are under extensive investigations [5-7]. The most striking feature of micelles is their ability to solubilize variety of compounds in different regions [8]. Micelles have been the subject of numerous investigations because of their importance as model system for mimicking biomembranes [9,10]. In recent years, extensive investigations have been made on the effects of the micelles formed by the surfactants, on diverse systems [11-13]. I-3-AA is a plant growth

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hormone and a very important biomolecule. Siddique et al. [14] have used I-3-AA for shoot bud formation and plant regeneration from cotyledonary node explants of *Capsicum annuum*. Seeni et al. [15] have used I-3-AA in vitrocloning and homostead cultivation of primitive *Musa* cultivars.

Borosarelli et al. [16] studied the exciplex type behaviour and partition of 3-substituted indole derivatives in reverse-micelles made with benzylhexadecyl dimethyl ammonium chloride, water and benzene. Sarpal and coworkers [17] studied the temperature dependence of fluorescence spectral characteristics of 2 [(*cp*-methylamino) phenyl]-3,3-dimethyl-5-carboethoxy-3H indole in aqueous micelles.

The present study is carried out to investigate the solubilization of I-3-AA in presence of various surfactants at their respective CMCs or marginally above the CMC's, employing fluorescence and absorption spectral studies and light scattering studies.

EXPERIMENTAL

Materials : Analytically pure I-3-AA was a Merck sample. The following surfactants were employed : (A) Nonionic (i) TX - 100 : Polyoxyethylene tert-octyl phenyl ether (ii) Tween-40 : Polyoxyethylene Sorbitan Monopalmitate (iii) Tween-20 : Polyoxyethylene Sorbitan monolaurate (B) Anionic (i) SDS : Sodium dodecyl sulphate (ii) SDBS : Sodium dodecylbenzene sulphonate (iii) Aerosol - OT (AOT) : Sodium bis(2-ethyl hexyl) sulpho succinate (C) Cationic (i) CPC : Cetylpyridinium chloride (ii) CDBAC : Cetyldimethylbenzyl ammonium chloride (iii) CTAB : Cetyltrimethyl ammonium bromide. All the surfactants were either of Sigma (USA) or BDH (UK) products.

Methods : The stock solution of I-3-AA was prepared in distilled methanol. All the experiments were performed around 23–25°C in aqueous medium containing 1% (v/v) methanol keeping the final concentration of I-3-AA at 10^{-5} M for fluorescence studies. For absorption studies the concentration of I-3-AA was kept at 10^{-4} M throughout the experiments.

All the fluorimetric experiments were carried out with Perkin Elmer fluorescence spectrophotometer (Model No. 204A) with a synchronized strip chart recorder (Model No. 056). A Xenon lamp was used as the light source. For recording the fluorescence excitation and emission spectra, its slit width was kept at 10 nm and a cell of 1 cm path length was used.

The absorption measurements were made with Hewlett Packard (HP) 8452, and a diode array spectrophotometer respectively. The light scattering studies were made with a Brice - Phoenix universal light scattering photometer, model no. 2000, in

conjunction with a multiflex galvanometer. Measurements are made at an angle of 90° to the incident beam and the scattering intensity was measured in terms of galvanometer deflection.

The purity of the surfactants was checked by determining their CMC values with the help of surface tension measurement, employing drop weight method. The values obtained coincided with the reported values. The absolute fluorescence quantum yield (σ_f) of I-3-AA was calculated relative to anthracene solution as standard [18]. Fluorescence emission of anthracene is in the same range as that of I-3-AA. Approximate corrections were made to compensate for different absorption of the compound and the standard. Each time the total intensity of fluorescence emission was measured for the standard and the sample from the area of the fluorescence spectrum recorded over the whole range of emission under identical conditions. Molar extinction coefficient data have been reported as its logarithm ($\log \epsilon$). The Stokes' shift [19] data have also been calculated in different micellar media and are expressed in nanometers.

RESULTS AND DISCUSSION

The methanolic solution of I-3-AA showed maximum excitation peak at 285 nm and maximum emission peak at ~ 360 nm. All the nonionic surfactants, on addition to I-3-AA solution caused a continuous enhancement in its fluorescence emission intensity with increasing concentration. Among them TX-100 at a concentration of 0.14 mM exerted the maximum effect accompanied with 50-55 nm blue shift in λ_{em} . At this concentration of TX-100, the increase in fluorescence emission intensity was about 6 times its original value. The changes in the fluorescence spectra of I-3-AA on addition of TX-100 are shown in Fig. 1. All the ionic surfactants, anionic as well as cationic caused initially a small increase in the intensity of the emission peak which then decreased on further addition of the surfactant and reached a constant minimum value except in the cases of CPC and CDBAC, where at higher concentrations I-3-AA solutions became non-fluorescent. The emission peak position got blue shifted by 5 nm, although the shape of the peak remained unchanged. Effect of solvent (methanol) was also studied. Upto 20% (v/v) methanol, the fluorescent intensity increased gradually while on further raising the concentration, the emission intensity started decreasing with a blue shift of 10-15 nm in the peak position. The fluorescence intensity of I-3-AA in presence and absence of the surfactants are given in Table 1.

There appeared an absorption peak at 280 nm. All the nonionic, anionic and cationic surfactants caused enhancement in the absorbance of I-3-AA with a small blue shift of 5-10 nm. TX-100 and CPC showed maximum effect. The changes in

the absorption spectra with CPC are shown in Fig. 2.

The light scattering studies of I-3-AA were made at an angle of 90° to the incident light. During addition of each surfactant, there occurred a sharp decrease in the galvanometer deflection with increase in surfactant concentration. The nonionic surfactants showed the maximum deflection. The concentration required to reach the minimum value of scattering flux was different for each surfactant. The results are given in Fig. 3.

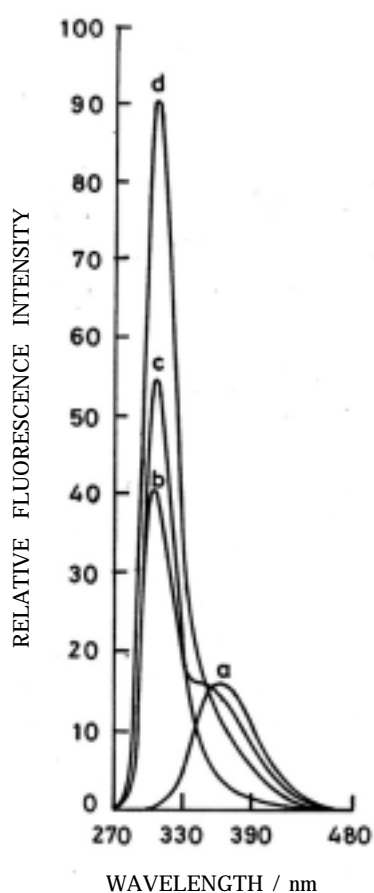


Fig. 1. Influence of addition of TX-100 on fluorescence intensity of 1×10^{-5} M I-3-AA solution. (a) No surfactant (b) 1.6 mM TX-100; (c) 4.8 mM TX-100; (d) 14.4 mM TX-100. $\lambda_{\text{ex}} = 285$ nm.

The calculated fluorescence quantum yield data (σ_f) of the surfactant added I-3-AA solution showed parallelism with changes in fluorescence intensity. Quantum yield values obtained show increasing trend with nonionic surfactants while with ionic surfactants, (σ_f) values initially increased and then decreased. Highest (σ_f) values obtained are for TX-100 added I-3-AA solution. The molar extinction coefficient ($\log \epsilon$) values showed a gradual increase on raising the concentration of nonionic as well as ionic surfactants. The calculated Stokes' shift values show that it becomes small for high concentration of I-3-AA solution. All the theoretically calculated spectral parameters are illustrated in Tables 2 and 3, respectively.

TABLE 1

Fluorescence Intensity of I-3-AA (1×10^{-5} M) in absence and presence of surfactants $\lambda_{ex} = 285$ nm $\lambda_{em} = 360-365$ nm

S. No.	Name of Surfactant	Relative fluorescence intensity in absence of surfactant	CMC's of surfactant* (mM)	Concentration of surfactant (mM)	Relative fluorescence intensity at maximum concentration of surfactant
1.	TX-100	16	0.14	0.14	91
2.	Tween - 20	15-16	0.06	0.07	29
3.	SDS	53	0.08	0.10	36
4.	Aerosol - OT	52	0.08	0.06	24
5.	CPC	52	0.07	0.08	1
6.	CTAB	53-54	0.14	0.09	25
7.	CDBAC	50	-	0.08	2

*These CMC's are in aqueous medium containing 1% (v/v) methanol in presence of 1×10^{-5} M I-3-AA.

The results obtained can be explained on the basis of solubilization by the micelles present in the surfactant solution at or marginally above CMC. The maximum fluorescence emission intensity enhancement of I-3-AA was obtained with TX-100, which has also been supported by absorbance, light scattering flux (σ_f) values and $\log \epsilon$ values. The enhancement of fluorescence of I-3-AA in TX-100 micellar media can be attributed to the increase in quantum efficiency of fluorescence. Furthermore, the quantum yield of fluorescence is higher in nonpolar medium because of the lesser

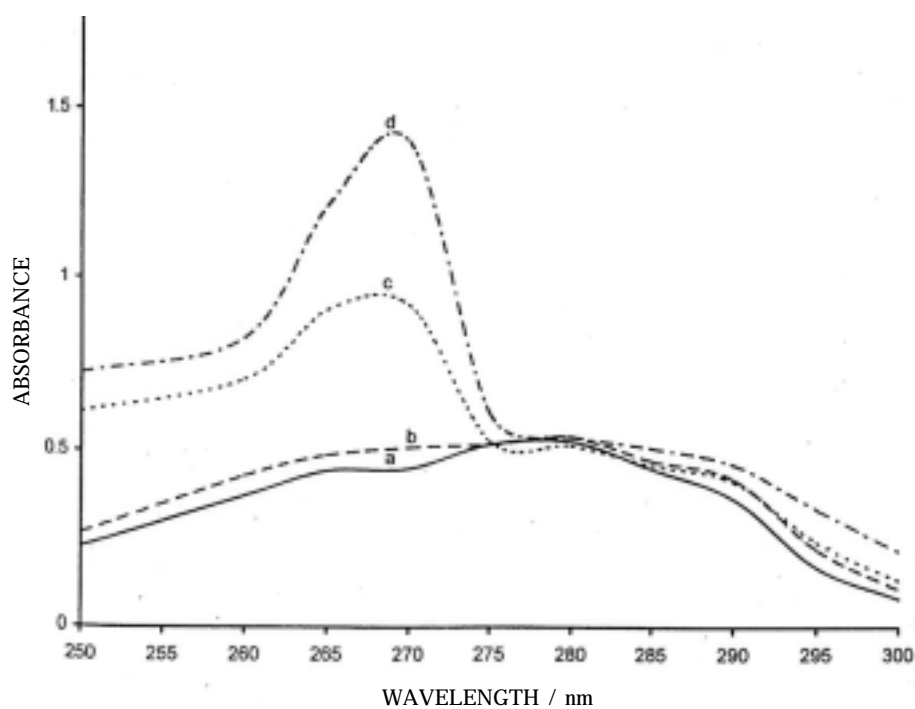
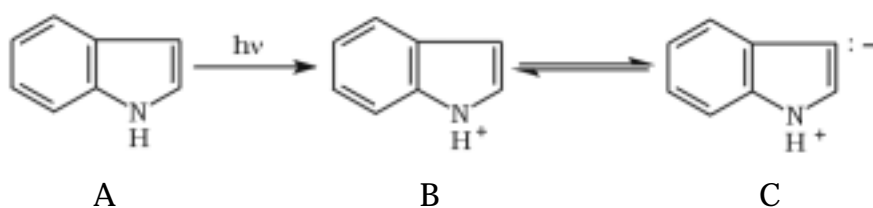


Fig. 2. Influence of addition of CPC on absorption spectrum of 1×10^{-5} M I-3-AA solution. (a) No CPC; (b) 0.028 mM CPC; (c) 1.397 mM CPC; (d) 2.793 mM CPC.

effect of other deactivation processes which compete with fluorescence [20]. Thus the increased (σ_f) values showed that the micelles have been possibly adsorbed on to the dispersed microcrystals of I-3-AA. The molecules of I-3-AA have been subsequently solubilized by incorporation into the interior nonpolar core of the micelles.



The quantum yield values (σ_f) of I-3-AA are higher in nonionic micellar medium because of lesser effect of other deactivation processes which compete with

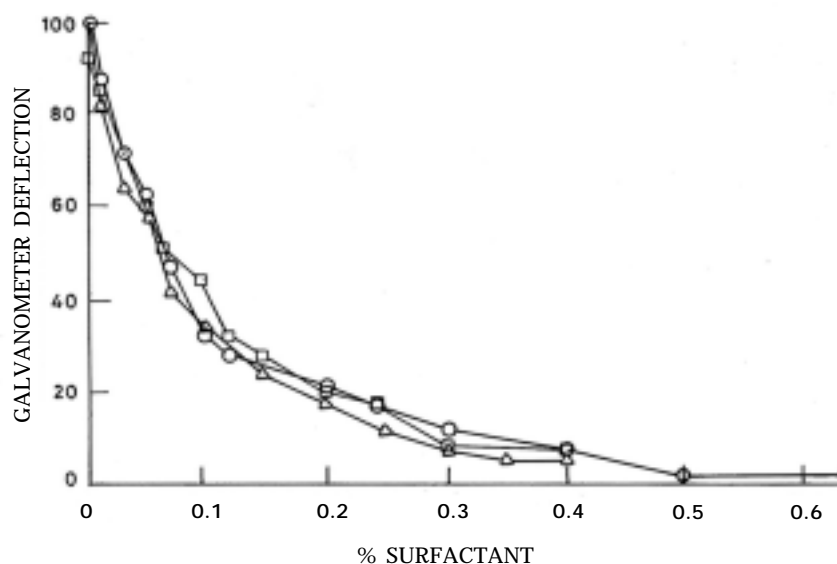


Fig. 3. Influence of surfactants on light scattering of 1×10^{-5} M I-3-AA solution. \circ TX-100, Δ CTAB, \square SDS. (0.1% Surfactant \equiv TX-100, 1.6 mM, CTAB, 2.75 mM and SDS, 3.48 mM).

TABLE 2.

Empirical Fluorescence Coefficient (κ_f) and Stokes' shift data for I-3-AA at $\sim 24^\circ\text{C}$.

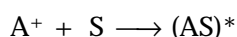
S. No.	Concentration of I-3-AA	Relative fluorescence intensity	λ_{ex} (nm)	λ_{em} (nm)	Stokes' shift (cm^{-1})	κ_f (conc. per mole)
1.	1×10^{-6} M	12	285	365	7690	12×10^6
2.	3×10^{-6} M	15	285	365	7690	5×10^6
3.	5×10^{-5} M	35	285	364	7615	7×10^5
4.	9×10^{-5} M	54	285	364	7615	6×10^5
5.	1×10^{-4} M	60	285	364	7615	6×10^5
6.	3×10^{-4} M	60	290	362	6858	2×10^5
7.	5×10^{-4} M	55	295	355	5729	11×10^4
8.	1×10^{-3} M	38	295	350	5326	38×10^3

TABLE 3.

Absorption Maxima (λ_{α}) Molar Absorptivity (represented as $\log \epsilon$), Fluorescence Maxima (λ_{F}) and Quantum Yield (σ_{F}) values of I-3-AA at different concentrations of Tween-20

Tween-20 Conc. (mM)	λ_{α} (nm)	$\log \epsilon$ (ϵ in $\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)	λ_{em} (nm)	(σ_{F}) I-3-AA
0.00	280	2.71	360–365	0.738
0.057	280	2.71	360–365	0.738
0.081	280	2.71	360–365	0.738
0.570	280	2.72	360–365	0.738
0.815	280	2.72	360–365	0.739
4.073	280	3.02	360–365	0.739
5.702	280	3.02	360–365	0.748

fluorescence [21]. Sufficiently large values of molar extinction coefficient ($\log \epsilon$) may be assigned to the π - π^* transitions. The large magnitude of Stokes' shift of I-3-AA are due to hydrogen-bond formation, between solute and solvent in ground state. This bond breaks following excitation to S_1 but reforms following proton transfer [22]. The hydrogen bonded excited state can be produced via two routes as shown by following scheme in which S represents the solvent molecule and A represents the fluorophore.



The absorption spectra of I-3-AA are very less affected on adding surfactants as compared to fluorescence spectra. This may be due to the fact that absorption is less sensitive to its environment as compared to fluorescence. The light scattering observations indicate that all the categories of surfactants (except CPC) when added to I-3-AA suspension results in a decrease in scattering flux which is indicated by a sharp decrease in galvanometer deflection readings.

The present analysis and interpretation suggests that experimental results observed and the theoretically calculated spectral data are found to be in good agreement. During micellar solubilization of I-3-AA the incorporation of solute influences the balance of favourable and unfavourable forces guiding micellization and structural changes occurring due to aggregation, dissociation and hydrogen bonding.

Aside from the presentation of the spectral and photophysical data, present kind

of study finds application in biochemical and agro-chemical analyses. The process of solubilization helps in transportation of this growth hormone I-3-AA to the various parts of the plant by the sap.

CONCLUSION

The present analysis indicate that during solubilization of solubilize into the surfactant system, the incorporation of the solubilize influence the balance of favourable and unfavourable forces guiding micellization and structural changes occurring due to aggregation, dissociation and hydrogen bonding. Thus, we can generalize the present understanding to study different kinds of solubilize. The solubilization process finds extensive application in the industrial, pharmaceutical and biochemical and agro-chemical fields.

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