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Interaction of Indole-3-Propionic Acid (IPA) and Surfactant Micelles: A Fluorescence Study.

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ABSTRACT

The study of interaction of indole-3-propionic acid (IPA) a biologically and analytically important molecule, with the micelles of non-ionic and ionic surfactants. IPA is a plant growth hormone in the auxin family and is an ingredient in many commercial horticulture plant rooting product. The studies have been conducted by fluorescence spectral technique at room temperature. IPA shows fluorescence excitation peak at 285 nm and emission peak at 370 nm. The non-ionic surfactants decreased the fluorescence intensity of IPA. The ionic surfactants enhanced the fluorescence intensity except CPC. Except few, all the surfactants caused a blue shift of 15 nm in the emission peak position. These spectral observations were supplemented by parallel absorption studies and some theoretically calculated spectral parameters. The fluorescence properties as well as the theoretically calculated spectral data have been used to characterize the heteroenvironments of the micelles in terms of their polarity, probe solubilization site and critical micelle concentration. This paper briefly discusses the importance of surfactants and biological system model as well as the use of micelles in pharmacy as an important tool that finds numerous applications.

Keywords: Fluorescence, IPA, Micelles, Surfactant.

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INTRODUCTION

The term micellization has been applied to several kinds of aggregation in solution, such as aggregation of surfactant molecules which contain large alkyl group (6-20 atoms) in dilute aqueous[1] and surfactant aggregation in polar solvents to form inverted micelles[2]. The capacity of water insoluble solute to get dissolved in micelles is a well-known phenomenon[3], often called solubilization. Different kinds of solubilizate molecules are thought to be solubilized in different regions of the micelles[3,4,5] and this has been verified by NMR measurements[6,7].

Indole -3-propionic acid (IPA) is widely employed in agriculture as a plant growth regulator (a phytoharmone or synthetic "auxin" from Greek auxin which means to growth and a promoter). It is a natural compound found in plants and animals, including humans. It has been found in mammalian plasma and cerebrospinal fluid. IPA is the most potent naturally occurring antioxidant known and is especially potent against hydroxyl radicals that are considered to be the most reactive and toxic of the oxygen derived free radicals. In contrast to other antioxidants known, IPA does not convert to reactive metabolites and thus may be a superior antioxidants choice compared to other known antioxidants. However, IPA is not water soluble, so has a poor dissolution rate and consequently is poorly absorbed. The issue of solubility in water and therefore increased bioavailability has remained problematic[8].

Micelles are dynamic microheterogenous structure containing surfactant molecules and constitute an important research subject[9-11]. It is possible within their internal environment to include some compounds that are insoluble in water, to perturb the kinetics of many photophysical processes and to provide structural mimicks of biological membrane[12-16]. Yau–Jan Chyan et.al[17] have studied the potent neuroprotective properties against the Alzheimer β-amyloid. Paul E. Bendheim et al. [18] have developed IPA as an inhibitor of β-amyloid fibril formation and a neuroprotectant against a variety of oxidotoxins. IPA has a great medicinal value as well as acts as a plant growth regulator which has poor solubility. The paper reports the solubilization of IPA molecules in micellar media of various surfactants which have a property, that is the improvement of the apparent solubility and the dissolution rate of poorly soluble IPA molecules. The measurements are being done by fluorescence and absorption spectral techniques.

MATERIALS AND METHODS

Analytically pure IPA used was a Merck sample. The following surfactants were employed : (A) Nonionic (i) TX-100 : Polyoxyethylene tert-octyl phenyl ether (ii) Tween-80 : Polyoxyethylene sorbitain monooleate (iii) Tween-20 : Polyoxyethylene sorbitain monolaurate (B) Anionic (i) SLS : Sodium lauryl sulphate (ii) DBSS : Dodecylbenzyl sodium sulphonate (iii) DSSS : Dioctyl sodium sulphosuccinate (C) Cationic (i) CPC : Cetylpyridinium chloride (ii) CTAB : Cetyltrimethyl ammonium bromide (iii) MTAB : Myristyltrimethyl ammonium bromide. All the surfactants used were either of Sigma (USA) or BDH (UK) products. The stock solution of IPA was prepared in distilled methanol. All the experiments were performed around 23-25^{0}C in aqueous medium containing 1% (v/v) methanol keeping the final concentration of IPA at 3 x 10^{-5} M for fluorescence studies. For absorption studies the concentration of IPA was kept at 3 x 10^{-5} M throughout the experiments.

All the fluorimetric experiments were carried out with Perkins Elmer Fluorescence Spectrophotometer (Model No. 204 A) with a synchronized strip chart recorder (Model no. 056). A Xenon lamp was used as a 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 diode array spectrophotometer. The purity of the surfactants was checked by determining their CMC values with the help of surface tension measurements, employing drop-weight method. The values obtained coincided with the recorded values. The absolute fluorescence quantum yield (Φ_f) of IPA was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of IPA. 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 ε). The Stokes' shift data have also been calculated and are expressed in nanometers.

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RESULTS AND DISCUSSION

The maximum excitation and emission wavelength of IPA was observed at 285nm & 370nm respectively. On adding TX-100, Tween-20, and Tween-80 nonionics the fluorescence intensity decreased significantly as a consequence of fluorescence quenching without any appreciable change in the shape of the emission band accompanied by a gradual blue shift in the range of 10-20nm. Maximum quenching in the emission intensity occurred in TX-100 micellar environment. At its higher concentration IPA became nonfluorescent. All the anionic surfactants enhanced the fluorescence emission intensity accompanied by a blue shift of 5-10nm in DBSS and SLS media. The cationic surfactant CTAB and MTAB on addition to IPA solution caused an enhancement in the fluorescence intensity with gradual blue shift upto 25nm in the peak position except with CPC which quenched the fluorescence of IPA completely at its higher concentration. The minimum and maximum fluorescence intensity in absence and presence of nonionic, anionic and cationic surfactant is given in Table-1. The fluorescence spectral changes in SLS micellar media are given in Fig 1. The absorbance of IPA was found to be maximum at 270 nm. The effect of all the three classes of surfactants on absorption spectra showed a similar trend of enhancement in peak height, with a small blue shift in λ_{max}.

Table 1: Fluorescence intensity of IPA in absence and presence of surfactant

Name of surfactant	Relative Fluorescence intensity in absence of surfactant	cmc's of surfactant (mM)	Max. Concentration of Surfactant used (mM)	Relative Fluorescence intensity	λ_{em} (nm)
TX-100	28	0.26	0.3	24	365
Tween-80	28	0.1	9.0	12	355
Tween-20	28	0.05	9.0	15	360
CPC	28	0.6	0.6	9	370
CTAB	28	0.90	9.0	41	355
MTAB	28	3.6	9.0	40	355
SLS	28	8.2	9.0	50	365
DSSS	28	0.91	9.0	44	370
DBSS	28	0.81	9.0	40	360

λ_{ex}=285nm λ_{em}=370nm P.M. Gain=2 Sensitivity Range=0.3

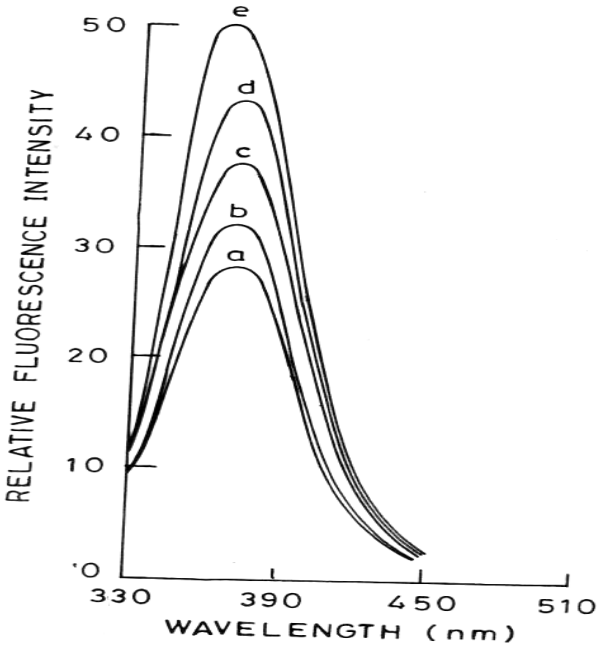

Figure 1: Influence of addition of SLS on fluorescence intensity of 3 x 10^{-5} M IPA solution (a) No Surfactant; (b) 0.07 mM SLS; (c) 0.5 mM SLS; (d) 1.5 mM SLS; (e) 9.0 mM SLS

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The fluorescence quantum yield values and empirical fluorescence coefficient values obtained showed parallel trends to emission intensity of IPA. Molar extinction coefficient values for all the classes of surfactants obtained are in increasing order. The Stokes' shift for IPA at room temperature was from 5326cm^{-1} to 8060 cm^{-1} on its dilution. All the theoretically calculated spectral parameters are listed in Table-2 and Table-3 respectively.

Table 2: Absorption maxima λ_{α} , fluorescence maxima λ_{em}, molar extinction coefficient (log ε) and quantum yield (Φ_f) of IPA at different concentration of SLS

S.No.	Concentration of SLS used (mM)	Absorption maxima λ_{α}	Molar extinction coefficient (log ε) (dm^3 mol^{-1}cm^{-1})	Fluorescence Maxima λ_{em} (nm)	Quantum yield Φ_f
1.	0.00	270	4.1471	370	0.32494
2.	0.07	270	4.1673	370	0.33605
3.	0.5	270	4.2031	370	0.35699
4.	1.5	270	4.2095	370	0.39876
5.	9.0	270	4.2600	365	0.41758

Table 3: Stokes'shift data of IPA at Room Temperature

S.No.	Concentration of IPA(M)	F.I	λ_{ex} (nm)	F.I	λ_{em} (nm)	Stokes' shift (cm^{-1})
1.	1×10^{-3}	9	295	32	350	5326
2.	7×10^{-4}	10	295	36	355	5729
3.	5×10^{-4}	11	295	36	360	6120
4.	3×10^{-4}	30	290	96	360	6704
5.	1×10^{-4}	21	285	58	370	8060
6.	7×10^{-5}	17	285	48	370	8060
7.	5×10^{-5}	15	285	37	370	8060
8.	3×10^{-5}	11	285	28	370	8060
9.	1×10^{-5}	4	285	10	370	8060

P.M. Gain = 2; Sensitivity range = 0.3

The results indicated that anionic surfactants had a stronger enhancement effect on the fluorescence absorption behaviour of IPA. These observations can be explained in a better manner on the basis of solubilization by microheterogenous environment of micelles present in the surfactant solution at or marginally above CMC. The enhancement in the fluorescence emission intensity of IPA in micellar media can be attributed to the increase in the quantum efficiency of fluorescence, which suggests that the surfactants have solubilized the suspended poorly soluble molecules of IPA very efficiently even at its low concentration.

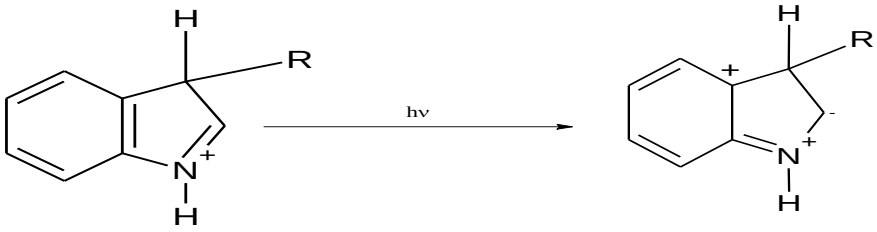
The nonionic surfactants have quenched fluorescence intensity due to formation of intramoleculer hydrogen bond in them. So, an effective hydrogen bonding does not take place between solubilizate IPA molecules and the nonionic surfactant micelles. The quenching also indicates that the compound prefers the hydrophobic core to the hydrophilic poly ethylene oxide, PEO shell particularly for TX-100. Evidently, the fluorescence is significantly weakened in the core like in nonaqueous solvents. This implies that the compound is embedded in the core is not hydrated around the aromatic rings[19]. Quenching can also be caused by non-radiation loss of energy from the excited molecules, CPC also caused fluorescence quenching which may be attributed to the interaction between the π- electron system of the excited state IPA fluorophore and quencher CPC molecule due to the presence of nucleophilic pyridine ring in its structure which makes it act as a quencher via hydrogen bond between the proton donor and acceptor. This will result in delocalization of the π-electrons of the excited state and hence loss of fluorescence[20].

Indoles are basic in nature which has been examined by many authors [21-25] using UV spectroscopy. It was also found that the ligand binding mode is influenced by substituent at the 3-position of the indole ring. The increase in fluorescence intensity and Φ_f values in ionic micellar media clearly indicate that the rates of non-radiative processes are less in these micellar systems in comparison to those in water, which could be due to the decrease in intersystem crossing rate as pointed out by Shizuka et. al.[26]. Another reason for the increase of Φ_f values could be due to the absorption of the fluorophore at the micellar surface which

decreases the rate of collisional deactivation of the fluorophore by water molecules. Greater quantum yields imply greater efficiency of the fluorescence pathway.

Absorption is less sensitive to its environment as compared to fluorescence, the absorption spectra are less affected in micellar media. However, the results obtained here, support those with fluorescence studies. The absorption spectra of indole 3-carboxy alkyl derivatives in non-polar and polar solvents consist of four bonds which are due to $\pi \rightarrow \pi^*$ transitions[25].

In the excited state, the IPA molecules get protonated due to the intramolecular charge transfer transitions from HOMO (H_B) of the phenylene ring which acts as an electron donor to LUMO (V_S) of the other part of the ion which serves as an electron acceptor these transitions are denoted HOMO→LUMO or $H_B \rightarrow$Vs. The spectra corresponding to the intramolecular charge transfer transition may be represented by the following scheme.



R=$(CH_2)_2$COOH

Studies have shown the protonation in the indole 3-carbon is the only reaction taking place. Large values of log ε are assigned to the $\pi \rightarrow \pi^*$ transitions. The large magnitude of Stokes' shift of IPA is due to hydrogen-bond formation, between solute and solvent in ground state. This bond breaks following excitation to S_1 but reform following proton transfer [27]. The hydrogen bonded excited state can be produced via two routes as shown by following scheme:

$$A + S \xrightarrow{h\upsilon} AS \rightarrow [AS]^*$$

$$A^* + S \rightarrow [AS]^*$$

Where S = solvent molecule

A= fluorophore

The blue shift may be attributed to the protic nature of solvent as here hydrogen-donor- solvent interaction takes place between IPA molecules and the solvent [28]. This shift may also be considered to be due to the difference in solvation energy of the solute in the ground state and excited state in different microheterogeneous micellar media. The values of empirical fluorescence coefficient K_f obtained may be assumed to be due to increased sensitivity of the fluorescence analysis of IPA molecules solubilization by surfactants which offer a protective microenvironment, leading to enhanced fluorescence of IPA by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution.

After interpreting and comparing the results obtained for IPA, it is found that the theoretical calculated spectral parameters and the experimental results are in good agreement. This proves the validity of the investigation that during solubilization of IPA solubilizate in micellar pseudophase, its incorporation influences the balance of favourable and unfavourable forces guiding micellization and structural changes occurring due to aggregation, dissociation and hydrogen-bonding.

CONCLUSION

As IPA has pharmaceutical applications as well as act as a phytoharmone, one can generalize the present physical understanding to study the phenomenon of drug solubilization. IPA being insoluble, the presence of micelles have enhanced its solubility also its activity through micellar solubilization and also the

rate of transportation to the site of action, a process which otherwise might have been a slow one. Hence solubilization increases the bioavailability of the antioxidant IPA molecules to the required site of the body.

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