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Research Article

STUDIES ON 1-NEPHTHALENEACETIC ACID IN MICELLAR MEDIA BY SPECTROFUOREMETRY

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ABSTRACT
1-Naphthaleneacetic acid (1-NAA) is a plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products. It has been employed for 50 years as a plant growth regulator for control of pre harvest fruit drop and flower induction or as a fruit thinning agent in different crops such as apples, potatoes, olives and citrus fruits. Micellar solubilization of 1-NAA in nonionic and ionic surfactants heteromicroenvironment is monitored by fluorescence and absorption spectral techniques has been reported by the authors. The influence of surfactant, concentration and working experimental conditions on the fluorescence spectra of 1-NAA is thoroughly evaluated and discussed. The increase in fluorescence intensity in micellar media can be attributed to the increase in quantum efficiency suggests that the suspended hydrophobic 1-NAA molecules have been solubilized. The solubilizing action has been supplemented and confirmed by few theoretically calculated spectral parameters like, empirical fluorescence coefficient (k_f), quantum yield (ϕ_f), molar extinction coefficient (ε) and Stokes shift values.

Keywords: *Surfactants, 1-NAA, Fluorescence, Solubilization*

INTRODUCTION
Today, fluorescence spectroscopy is an important tool of investigation in many areas in analytical sciences, its advantage is extremely high sensitivity and selectivity even single molecule can be detected and it achieves a high spatial resolution and time resolution in combination with microscopic techniques as laser techniques, respectively (Sharma and Schulman, 1999). In material sciences, this is used to study structure and dynamics of surfaces, particularly in this area of biochemistry and molecular genetics, fluorescence spectroscopy has become a dominating technique. Together with the latest imaging techniques, fluorescence spectroscopy allows a real time observation of the dynamics of intact biological system with an unprecedented resolution (Andreef and Pinkel, 1999).
Micelles are dynamic microheterogeneous structure containing surfactant molecules and constitute an important research subject. It is possible within their internal environment to include some compounds that are insoluble in water perturb the kinetics of many photophysical process to provide structural mimics of biological membrans (Maciejewski *et al.,* 2003) .
1-Naphthaleneacetic acid (1-NAA) is a plant growth hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products, it is a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting. It is also used for plant tissue culture and to improve seed quality and prevents shedding of grains in paddy and wheat; increases fruit size, induces flowering, uniform growth in pineapple and berry size and weight in grapes (Linan and Vicente, 1985). Several investigators have published methods for the determination of NAA by different techniques such as electron affinity (Bache *et al.,* 1964), ultraviolet absorption (Bache *et al.,* 1962; Young *et al.,* 1963; Randall, 1970; Munoz dela Pena *et al.,* 1993), HPLC (Cochrane and Lanouette, 1979; Moye and Wheaton, 1979) or GC with photometric detection, in potatoes (Zweig *et al.,* 1962) and olives (Zweig *et al.,* 1964), or with mass spectroscopy detection (Heberer and Stan, 1996). A sensitive and selective phosphormetric method for the determination of 1-NAA in water and apples based on a flow injection system have studied (Fernandez et al.2005). 1-NAA suppresses peroxidase activity during the induction of adventitious roots in soybean hypocotyls (Chen et al. 2002). A Simple and rapid high-performance liquid chromatographic method was developed for the separation and determination of small amount of 2-

NAA in 1-NAA (Husain et al 1991). The present study includes a study on the influence of various nonionic, anionic and cationic surfactants on the fluorescence and absorption spectra of 1-NAA. The results have been interpreted from the calculations of molar extinction coefficient, empirical fluorescence coefficient, quantum yields of 1-NAA fluorescence in various micellar media and Stokes' shift calculations at various concentration of 1-NAA.

MATERIALS AND METHODS

Materials

Analytically pure 1-NAA 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: Myristyl-trimethyl ammonium bromide. All the surfactants were either of Sigma (USA) or BDH (UK) products.

Methods

The stock solution of 1-NAA 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 1-NAA at 3 x 10-5 M for fluorescence studies. For absorption studies the concentration of 1-NAA was kept at 1 x 10-4 M throughout the experiments.

All the fluorimetric experiments were carried out with Perkin 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 Hewlet Packard (HP) 8452, and diode array spectrophotometer respectively.

The purity of the surfactants was checked by determining their CMC values 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 1-NAA was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of 1-NAA. 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 ($\log \varepsilon$). The Stokes' shift data have also been calculated and are expressed in nanometers.

RESULTS AND DISCUSSION

The metholic solution of 1-NAA showed maximum excitation peak at 275 nm and the maximum emission peak at 330 nm as shown in figure 1

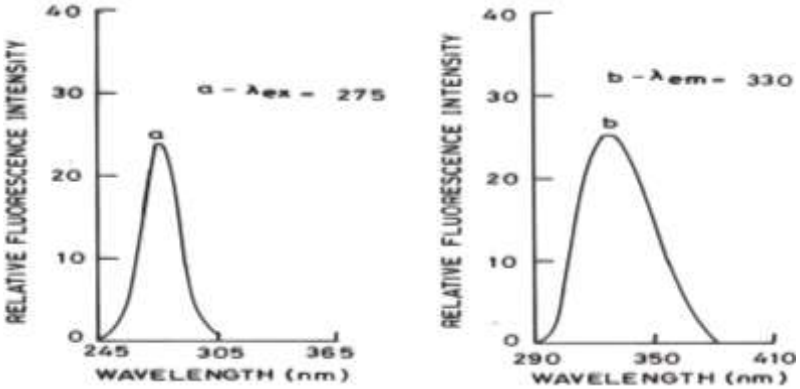

Figure 1: Absorption and emission spectra for 1-NAA

All the nonionic surfactants, on addition to 1-NAA solution caused a continuous enhancement in its fluorescence emission intensity with increasing concentration. Among them TX-100 exerted the maximum effect accompanied with 5 nm red shift in λ_{em}. The changes in the fluorescence spectra of 1-NAA on addition of TX-100 are shown in Figure 2.

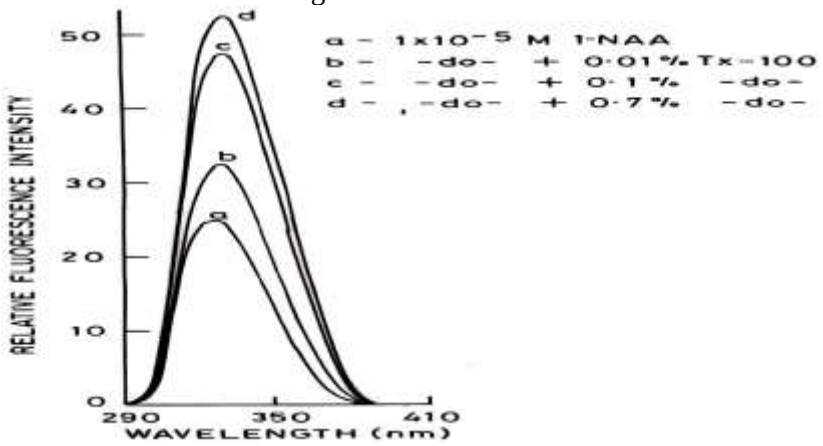

Figure 2: Influence of addition of TX-100 on fluorescence intensity of 1-NAA

All the anionic surfactants caused initially a small decrease in the intensity of the emission peak which then increased on further addition of the surfactant with 5 nm red shift while in case of cationic surfactant first increase and then decreases. Effect of solvent (methanol) was also studied upto 90% (v/v) methanol, the fluorescent intensity increased gradually.

Fluorescent intensity of 1-NAA in presence and absence of the surfactants are given in Table 1 and 2.

Table 1: Effect of nonionic surfactants on the fluorescence intensity (F.I.) of 1-NAA

S. No.	% of Tween-40	F.I.	% of Tween-80	F.I.	% of TX-100	F.I.
1.	0.000	25	0.000	25	0.000	25
2.	0.01	31	0.01	32	0.01	33
3.	0.1	36	0.1	39	0.1	48
4	0.7	40	0.7	48	0.7	53

Table 2: Effect of anionic surfactants on the fluorescence intensity (F.I.) of 1-NAA

S. No.	% of DBSS (w/v)	F.I.	% of SLS (w/v)	F.I.	% of DSSS (w/v)	F.I.
1.	0.000	25	0.000	25	0.000	25
2.	0.01	18	0.01	21	0.01	18
3.	0.1	28	0.1	31	0.1	29
4	0.7	35	0.7	33	0.7	37

Table 3: Stokes' shift data values of 1-NAA

S. No.	Concentration of compound	F.I.	λ_{ex} (nm)	λ_{em} (nm)	Stokes' Shift (cm^{-1})
1.	1 x 10^{-5} M	25	275	330	6060
2.	3 x 10^{-5} M	27	275	335	6512
3.	5 x 10^{-5} M	29	275	335	6512
4.	7 x 10^{-5} M	34	275	335	6512
5.	1 x 10^{-4} M	37	275	337	6690
6.	3 x 10^{-4} M	39	275	340	6951
7.	5 x 10^{-4} M	42	275	342	7123
8.	1 x 10^{-3} M	42	275	342	7123

There appeared an absorption peak at 260 nm. All the nonionic, anionic and cationic surfactants show almost parallelism with fluorescence spectra.. The calculated fluorescence quantum yield data (ϕ_f) of the surfactant added 1-NAA solution showed parallelism with changes in fluorescence intensity. Quantum yield values obtained show increasing trend with nonionic surfactants while with anionic surfactants, (ϕ_f) values initially decreased and then increased. Highest (ϕ_f) values obtained are for TX-100 added 1-NAA solution. The molar extinction coefficient ($\log \varepsilon$) values showed a gradual increase on raising the concentration of nonionic surfactants. The calculated Stokes' shift values show that it becomes larger for high concentration of 1-NAA solution illustrated in Table 3 . 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 1-NAA was obtained with TX-100, which has also been supported by absorbance values and $\log \varepsilon$ values. The enhancement of fluorescence of 1-NAA 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 effect of other deactivation processes which compete with fluorescence (Shizuka et al. 1985). Thus the increased (ϕ_f) values showed that the micelles have been possibly adsorbed on to the dispersed microcrystals of I-NAA. The molecules of I-NAA have been subsequently solubilized by incorporation into the interior nonpolar core of the micelles. Sufficiently large values of molar extinction coefficient ($\log \varepsilon$) is assigned to the π-π* transitions .The large magnitude of Stokes' shift of 1-NAA are due to hydrogen-bond formation, between solute and solvent in ground state. This bond breaks following excitation to S1 but reforms following proton transfer (Banerjee *et al.*, 1995). 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 (Parker 1968).

$$A + S \longrightarrow AS \xrightarrow{h\nu} [AS]^*$$

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

The absorption spectra of 1-NAA are very less affected on adding surfactants ascompared to fluorescence spectra. This may be due to the fact that absorption is less sensitive to its environment as compared to fluorescence.

Conclusion

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 1-NAA 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 1-NAA to the various parts of the plant by the sap.

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