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Research Article

STUDIES ON THE PLANT GROWTH REGULATOR 1-NAPHTHALENEACETAMIDE IN MICELLAR SURFACTANT SOLUTION BY FLUORESCENCE SPECTROSCOPY

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ABSTRACT
This study focuses on the spectrofluorimetric behaviour of an important plant growth regulator 1-naphthaleneacetamide (1-NAD) in the presence of various surfactant solutions. Micellar solubilization is a powerful alternative for dissolving hydrophobic compounds in aqueous environment. Fluorescence and absorption spectroscopy are the two techniques used to monitor the micellar solubilization studies of plant growth regulator 1-naphthaleneacetamide. The influence of surfactant, concentration and working experimental conditions on the fluorescence spectra of 1-naphthaleneacetamide 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 plant growth regulator 1-naphthaleneacetamide 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-naphthaleneacetamide, Fluorescence, Solubilization*

INTRODUCTION
Today, fluorescence spectroscopy is an important tool of investigation in many areas in analytical sciences. During the past 35 years there has been a remarkable growth in the use of fluorescence in the biological sciences (Lakowicz, 1999). Fluorescence spectroscopy and time-resolved fluorescence are considered to be primarily research tools in biochemistry and biophysics (Valeur, 2000). This emphasis has changed, and the use of fluorescence has expanded. Fluorescence is now a dominant methodology used extensively in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, forensics, agriculture and genetic analysis, to name a few. Fluorescence detection is highly sensitive, and there is no longer the need for the expense and difficulties of handling radioactive tracers for most biochemical measurements. There has been dramatic growth in the use of fluorescence for cellular and molecular imaging (Sharma and Schulman, 1999). Fluorescence imaging can reveal the localization and measurements of intracellular molecules, sometimes at the level of single-molecule detection (Andreeff and Pinkel, 1999).

Micelles are biologically important aggregates that are formed in aqueous solution by surfactants, which are compounds possessing a water soluble moiety (often an ionic group) and a water insoluble portion (a long hydrocarbon chain). Micelles are spherical aggregates of 20-200 molecules, containing hydrocarbon interiors and ionic surfaces, which have particular significance in various fields because of their ability to increase the solubility of sparingly soluble substances in water (Moroi, 1992). It is possible within their internal environment to include some compounds that are insoluble in water, to perturb their kinetics of many photophysical processes and to provide structural mimics of biological membrane (Fendler, 1982). The mode of preparation of a thermodynamically stable isotropic solution of a substance normally insoluble or very slightly soluble in a given solvent by the introduction of surfactant micelles is well known as solubilization. This process caused by incorporation of hydrophobic organic substances into micelles, play a very important role not only in industrial processes, but also in biological processes, such as adsorption and transfer of materials in living tissue. Physiochemical studies of aqueous surfactant solution are important from fundamental as well as from application point of view. Such studies have importance in pharmacy and agriculture.

1-Naphthaleneacetamide (1-NAD) is a plant growth regulator, a natural plant growth hormone that is important for seed and root development. 1-NAD has been widely used in agriculture for more than 60 years as a component in many commercial plant rooting and horticultural formulations (Gardner, 1941; Clarke *et al.*,, 1941). It is used as fruit thinning agent for a variety of fruits such as apple, pear, peach, grape, for root cuttings and to prevent fruit drop shortly before harvest (Untiedt and Blanke, 2000; Link, 2000; Shrestha, 1986). Its action mainly involves inducing abscission of flower buds. It is often mixed with carbaryl and is slightly less toxic than 1-naphthaleneacetic acid (1-NAA). Fluorescence techniques (Sigrist *et al.*, 1974; Valero-Navarro *et al.*, 2009) and several other methods have been proposed for the determination of 1-naphthaleneacetamide residues in food, plants, vegetables and water samples, including phosphorescence (Segura *et al.*, 1997; Munozdela *et al.*, 1999) and chromatography (Cochrane *et al.*, 1980; Martonez *et al.*, 2004).

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

MATERIALS AND METHODS

All the fluorimetric and absorption experiments were carried out with Perkin- Elmer fluorescence spectrophotometer model no. 204 A with a synchronized model no. 056 strip chart recorder and Hewlet Packard (HP) 8452 A diode array spectrophotometer, respectively. The stock solution of analytically pure 1-naphthaleneacetamide (Sigma Chemicals) was prepared in distilled methanol. All the experiments were made at room temperature (23^0-25^0C) and 1% methanolic medium keeping the final concentration of 1-naphthaleneacetamide 1 X 10^{-5} M. All the surfactants used were either of sigma (USA) or BDH product. The following surfactants were employed.

(A) Nonionic: Polyoxyethylene tertoctyl phenol (TX-100) , Polyoxyethylene sorbitan monolauriate (Tween-80) and Polyoxyethylene sorbitan monopalmitate (Tween-40)

(B) Cationic : Cetyltrimethyl ammonium Bromide (CTAB) , Cetylpyridinium chloride (CPC) and Cetylpyridinium bromide (CPB)

(C) Anionic : Dodecylbenzene sodium sulphonate (DBSS) , Dioctylsodium sulphosuccinate (DSSS) and Sodiumlauryl sulphate (SLS)

The purity of surfactant was checked by determining their CMC values with the help of surface tension measurement, employing drop weight method. The absolute fluorescence quantum yield (Φ_f) of the compound was calculated relative to anthracene solution as 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 (ε) data have been reported in term of its logarithm log ε, the Stokes' shift data been calculated in different micellar media and are expressed in term of nanometers.

RESULTS AND DISCUSSION

The metholic solution of 1-naphthaleneacetamide showed maximum excitation peak at 290 nm and the maximum emission peak at 340 nm. The nonionic surfactants caused an enhancement in the fluorescence intensity with 5–10 nm gradual red shifts. Among these surfactants TX-100 exerted maximum effect. The changes in fluorescence intensity of 1-NAD on addition of TX-100 are showed in Figure 1.

On addition of any of the anionic surfactant, initially the fluorescence intensity decreased the further addition of the surfactant showed enhancement in the fluorescence intensity of 1-NAD. On addition of the cationic surfactant, initially it caused a small enhancement in fluorescence intensity with a 5 nm gradual blue shift while its further addition showed a gradual decrease in the fluorescence intensity at the shifted wavelength. The changes observed in fluorescence emission intensity in presence of surfactants are as given in table 1.

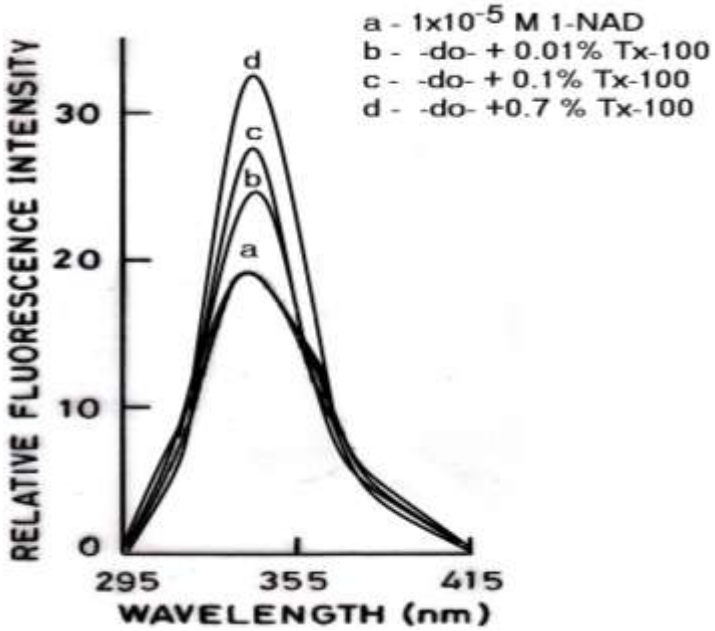

Figure 1: Influence of addition of TX-100 on fluorescence intensity of 1-NAD

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

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

There appeared an absorption peak at 280 nm for the metholic solution of 1-naphthaleneacetamide. All the nonionic, anionic and cationic surfactants show almost parallelism with fluorescence spectra. Molar extinction coefficient (log ε) calculations showed a gradual increase in log ε values with increase in nonionic surfactant concentration. With anionic surfactants, the log ε values initially decrease and at their higher concentration an increase in log ε value is obtained. For cationics, log ε values increase first than decease. The empirical fluorescence coefficient (k_f) values showed a similar trend to the fluorescence emission intensity. The value of (k_f) confirms this observation and attributes to the increased sensitivity of fluorimetric analysis of the organic molecule by solubilization. This was attributed to the fact that surfactants offer protective microenvironment, leading to enhanced fluorescence of the guest molecule (solubilizate) by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution. The calculated fluorescence quantum yield data (ϕ_f) of the surfactant added 1-naphthaleneacetamide 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-naphthaleneacetamide solution.

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-naphthaleneacetamide was obtained with TX-100, which has also been supported by absorbance values and log ε values. The enhancement of fluorescence of 1-naphthaleneacetamide 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). Fluorescence intensity of

the compound on adding surfactants can be attributed to the increase in the quantum yield. Initially the hydrophilic part of nonionic surfactants interact with the solubilizate molecules and tend to break it into monomeric form. This causes an enhancement, in the fluorescence emission intensity. According to the observed results, the nonpolar environment of TX-100 micelle interior and similarly of other nonionic micelles may be preferable to incorporate the hydrophobic solubilizate molecule then the ionic surfactant micelle. Thus the increased (ϕ_f) values showed that the micelles have been possibly adsorbed on to the dispersed microcrystals of 1-naphthaleneacetamide. The molecules of 1-naphthaleneacetamide 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 (Parker, 1968). The large magnitude of Stokes' shift of 1-naphthaleneacetamide are due to hydrogen-bond formation, between solute and solvent in ground state (Banerjee *et al.,* 1995). Quenching can also be caused by non-radiation loss of energy from the excited molecules. Fluorescence quenching was also observed by the addition of cationic surfactants, which may be attributed to the electrostatic preferential interaction between the polar substituent of 1-naphthaleneacetamide molecules where it loses the coplanarity. The quenching may also be due to interaction between the π-electron system of the excited state fluorophore and quencher molecule due to the presence of nucleophilic pyridine ring in the structure which make 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. The absorption spectra of 1-naphthaleneacetamide 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. In micellar media many characteristics of organic molecules e.g. absorption and fluorescence spectra are changed drastically. Thus the above observations can be explained by the solubilizing action of surfactant micelles.

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-naphthaleneacetamide 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, forensic and agriculture science. The present analysis is an effort to mimic this at laboratory level.

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