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Research Article

STUDIES ON 5-SULPHOSALICYLIC ACID IN MICELLAR MEDIA BY SPECTROFLUORIMETRY

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ABSTRACT
5-Sulphosalicylic acid (5-SSA) is used in urine tests to determine urine protein content. 5-SSA is the most readily accessible plant growth regulators which are effective in other form of acetyl salicylic acid and methyl salicylate in plant as well. The fluorescence and absorption spectra of 5-SSA have been studied in presence of different surfactant solutions at room temperature with special reference to solubilization and micellization. The explanation for the result could be that non-ionic surfactants from micelles in aqueous solution above the critical micellar concentration (CMC) which increase the solubility of species with hydrophobic nature. Solubilization phenomenon is one of the important properties of micelles industrially as well as biologically. The dual nature of surfactant micelle is responsible for the occurrence of surface activity, solubilization and micellization. The maximum solubilization is attributed with the high absorbance as well as fluorescence intensity and it has also been confirmed by theoretically calculated spectral parameters, like empirical fluorescence coefficient (K_f), quantum yield (ϕ_f), molar extinction coefficient (log ε) and Stokes' shift.

Keywords: *Surfactants, 5-SSA, Fluorescence, Solubilization*

INTRODUCTION
Fluorescence spectroscopy is an extremely powerful analytical tool that has been used in areas ranging from biology to physics (Bright, 1988). In fluorescence spectrometry both an excitation spectrum and / or an emission spectrum can be measured. The concentration of the analyte is directly proportional with the intensity of the emission. Fluorescence spectroscopy is a widely used research tool in bio chemistry and molecular biology.
Fluorescence has also become the dominant method enabling the resolution in medical diagnostics, DNA sequencing, and genomics. To date all the fluorescence observables, including spectral shifts, anisotropies, quantum yields and lifetimes, have all been utilized in basic and applied uses of fluorescence (Lakowicz, 2001).
Micelles are dynamic micro heterogeneous structure containing surfactant molecules and constituent an important research subject. It is possible with in their internal environment to include some compounds that are insoluble in water perturb the kinetics of the many photo physical process to provide structural mimics of biological membranes (Maciejewski *et al.,* 2003).
5-Sulphosalicylic acid (5-SSA) is used in urine tests to determine urine protein content. The chemical causes the precipitation of dissolved proteins, which is measured from the degree of turbidity. 5-SSA is the most readily accessible plant growth regulators which are effective in other forms of acetyl salicylic acid and methyl salicylate in plant as well (Raskin, 1992).
SSA could induced the alternative oxidase enzyme activity in mitochondria which is involved in stress alteration mechanism and enhancing or reduction in species secondary metabolites of plants (Raskin, 1992; Kiddle *et al.,* 1994; Vanlerberghe and McIntosh, 1997; Prithviraj *et al.,* 2005; D'Onofrio *et al.,* 2009).
SSA has been documented to enhance flesh firmness of harvested peaches during storage and banana fruits during ripening (Srivastava and Dwivedi, 2000).
Acetylsalicylic acid, (aspirin) is an analgesic, anti-inflammatory, and antipyretic drug and is one of the most widely used anionic drugs in the world (Yamamoto *et al.,* 2007). The interaction between 5-SSA and

bovine serum albumin (BSA) at pH 7.40 was studied by fluorescence and UV-VIS absorption spectroscopy at different temperatures. The result revealed that SSA caused the fluorescence quenching of BSA through a static quenching procedure.

The binding constant K was measured by fluorescence quenching method. The thermodynamic parameters, ΔH and ΔS, were calculated to be 23.16 KJ/mols and 162.37 J/mol/K > 0, respectively, which suggested that the hydrophobic force played a major role in the reaction of SSA on BSA (Zhang *et al.,* 2013).

1% SSA in acetone has been used by a UV fluorescence procedure for detection of organic compounds on paper chromatogram (Yagni *et al.,* 2005).

The present study includes a study on the influence of various non-ionic, anionic and cationic surfactants on the fluorescence and absorption spectra of 5-SSA. The results have been interpreted from the calculations of molar extinction coefficient, empirical fluorescence coefficient, quantum yield of 5-SSA fluorescence in various micellar media and Stokes' shift calculations at various concentration of 5-SSA.

MATERIALS AND METHODS

Materials

Analytically pure 5-SSA was a Merck sample. The following surfactants were employed:

(A) Non-ionic (i) TX-100: Polyoxyethylene tert-octyl phenyl ether (ii) Tween-80: Polyoxyethylene sorbitain monolaurate (B) Anionic (i) SLS: Sodium lauryal sulphate (ii) DBSS: Dioctyl sodium sulphonate (iii) DSSS: Dioctyle sodium sulphosuccinate (c) Cationic: (i) CPC: Cetylpyridium chloride (ii) CTAB: Cetyltrimethyl ammonium bromide (ii) MTAB: Myristyl trimethyl ammonium bromide. All the surfactants were either of sigma (USA) or BDH (UK) products.

Methods

The stock solution of 5-SSA was prepared in distilled water. The final concentration of 5-SSA at $5X10^{-5}$ M for fluorescence studies. For absorption studies, the concentration of 5-SSA was kept at $1X10^{-4}$ M throughout the experiments.

All the fluorometric 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 10nm and a cell of 1cm path length was used. The absorption measurement was made with Hewlete Packerd (HP) 8452 and diode array spectrophotometer respectively.

The purity of the surfactants was checked by determining their CMC valued the help of surface tension measurements, employing drop-weight method. The values obtained coincide with the recorded values. The absolute fluorescence quantum yield (ϕ_f) of 5-SSA was calculated relative to anthracene solution as standard.

The total fluorescence emission intensity 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 nanometres.

RESULTS AND DISCUSSION

The aqueous solution of 5-SSA showed maximum emission peak at 295nm and the maximum emission peak at 400nm as shown in figure 1.

All the non-ionic surfactants, on addition to 5-SSA solution caused a continuous enhancement in its fluorescence emission intensity with increasing concentration. Among them Tween-20 exerted the maximum effect accompanied without shift in λ_{em}. The changes in the fluorescence spectra of 5-SSA on addition of Tween-20 are shown in figure 2.

Fluorescence intensity of 5-SSA in presence and absence of the surfactants are given in Table 1 and 2.

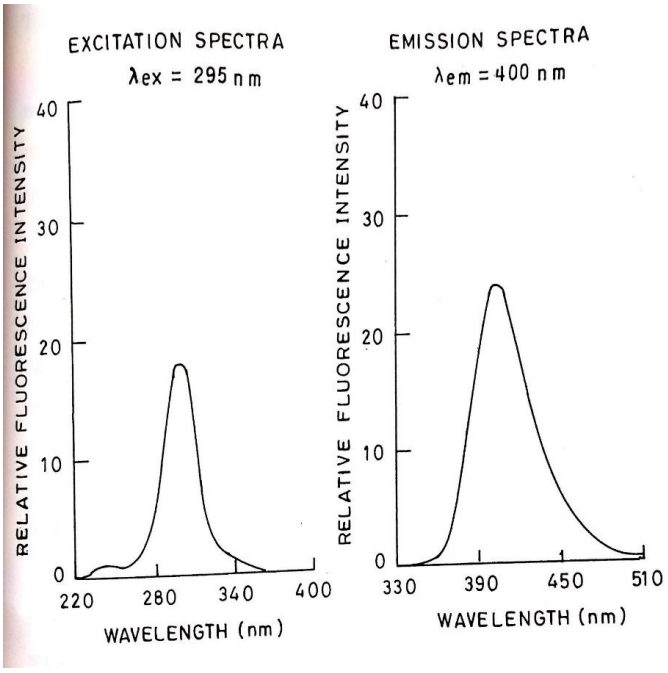

Figure 1: Excitation and Emission Spectra of 5-SSA

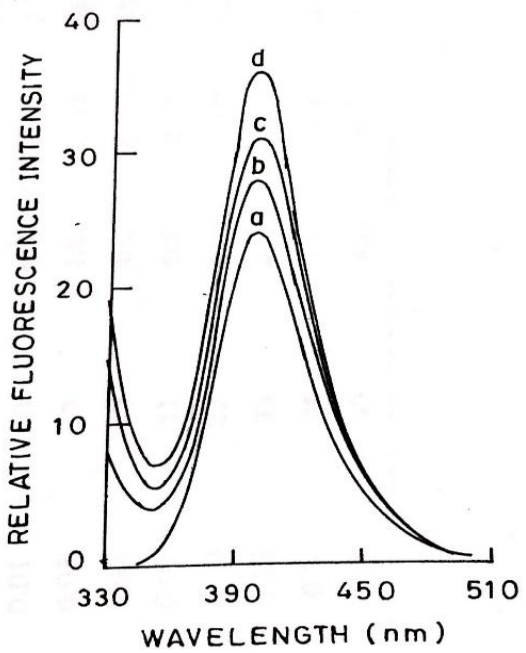

Figure 2: Spectra of 5-SSA with Tween-20

Table 1: Effect of Non-Ionic Surfactants on the Fluorescence Intensity (F.I.) of 5-SSA

S. No.	% of TX-100	F.I.	% of Tween-20	F.I.	% of Tween-80	F.I.
1.	0.000	24	0.000	24	0.000	24
2.	0.01	28	0.01	27	0.01	28
3.	0.10	32	0.10	32	0.10	32
4.	0.5	35	0.5	35	0.5	37

Table 2: Effect of Anionic Surfactants on the Fluorescence Intensity (F.I) of 5-SSA

S. No.	% of DBSS	F.I.	% of DSSS	F.I.	% of SLS	F.I.
1.	0.000	24	0.000	24	0.000	24
2.	0.01	20	0.01	18	0.01	20
3.	0.10	30	0.10	24	0.10	27
4.	0.5	Out of range	0.5	29	0.5	37

Table 3: Stokes' Shift Data Values of 5-SSA

S. No.	Concentration of Compound	λ_{ex}	F.I.	λ_{em}	F.I.	Stokes' Shift (cm^{-1})
1.	$1X10^{-3}$ M	295	47	405	76	9206
2.	$5X10^{-4}$ M	295	34	405	54	9206
3.	$3X10^{-4}$ M	295	22	405	35	9206
4.	$1X10^{-4}$ M	295	32	405	52	9206
5.	$7X10^{-4}$ M	295	23	400	38	8898
6.	$5X10^{-5}$ M	295	17	400	26	8898
7.	$3X10^{-5}$ M	295	10	400	16	8898
8.	$1X10^{-5}$ M	295	4	400	5	8898

There appeared an absorption peak at 300nm. All non-ionic surfactants absorbance peak enhanced with blue shift in peak except Tween-20. It did not show shift in λ_{max}. On addition of anionic surfactants, initially absorbance decreased and increased with 5nm blue shift in λ_{max}. Except SLS, both surfactants i.e. DBSS and DSSS caused blue shift in the peak position.

All cationic surfactants first decrease then increase in the absorption peak. CTAB showed 5nm red shift in absorbance peak, MTAB and CPC did not show any shift in peak position. The calculated fluorescence quantum yield data (ϕ_f) of the surfactant added 5-SSA solution showed parallelism with changes in fluorescence intensity. A decrease in ϕ_f values was obtained for the CPC added 5-SSA solution. Amongst all the surfactants ϕ_f values were highest for DBSS added 5-SSA solution. The molar extinction coefficient (log ε) values showed enhancement on raising the concentration of non-ionic surfactants. The calculated Stokes' shift values of 5-SSA gradually decreased on diluting the compound, illustrated in table 3. The results obtained can be explained based on solubilization by the micelles present in the surface solution at or marginally above CMC. The enhancement of fluorescence of 5-SSA in non-ionic micellar media can be attributed to the increase in quantum efficiency of fluorescence and TX-100 and Tween-80 showed blue shift in the absorbance peak. It may be because of the difference in solvation energy of the solubilization molecule in the ground state and excited state.

Both the anionic and cationic surfactants increased the emission intensity slowly. The ionic micelles have higher polarity which may be asserted to the loose fluctuating and disordered structure of these micelles. Here in ionic micellar media, the hydrophilic solubilizate must leave its aggregate and exclude water molecule inside the ionic micelle.

The molecules of 5-SSA have been subsequently solubilized by incorporation into the interior nonpolar core of the micelles. The presence of a nucleophilic pyridine ring in CPC causes a continuous decreasing effect in emission intensity and behaves differently from other cationic which have linear alkyl and aryl groups. These processes cause slow solubilization sufficiently large value of log ε in assigned to π-π* transitions. A relationship exists between the Stokes' shift and the fluorescence lifetime. The magnitude of both is dependent on the diameter of the quantum dot. Larger quantum dots have longer lived excitation because of the close spacing of the energy levels. The Stokes' shift meanwhile, decreases with increasing quantum dot size.

Conclusion

The present analysis and interpretation suggests that experimental results obtained and theoretically calculated spectral data are found to be in good agreement. This proves the validity of the investigation on

made. As 5-SSA is a pharmaceutically and biologically important, the present kind of study of micellar solubilization of 5-SSA molecules is of great important.

In the study made, the authors have put an effort to reveal on the most important application of the surfactant as micellar drug solubilisation (Yagni *et al.*, 2005). Thus, one can generalize the physical understanding to study the phenomenon of micellar solubilization and 5-SSA may be a micro environment probe.

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