

## INTERACTION OF 3-HYDROXY PYRIDINE AND SURFACTANT MICELLES: A FLUORESCENCE STUDIES

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## ABSTRACT

**Objective:** Micellar solubilization is a powerful alternative for dissolving hydrophobic compound in aqueous environment. 3-hydroxy pyridine (3-HP) derivatives are the potential endogenous photosensitizers. 3-HP derivatives show protective effect in clinical extreme condition such as hypoxia, hyperthermia, hypokinesia. Micellization of 3-HP followed by solubilization would catalyze its pharmaceutical activities which may serve better results in medicinal and analytical fields.

**Methods:** Fluorescence and absorption spectroscopy techniques are used to monitor the micellar solubilization studies of 3-HP. Solubilization studies of 3-HP with various anionic, cationic and nonionic surfactants have been performed in aqueous medium around 23–25°C temperature. The solubilization action of the surfactant has also been determined by theoretical calculated spectral parameters such as empirical fluorescence coefficient, quantum yield, stokes, shift and molar absorption coefficient.

**Results:** 3-HP shows fluorescence excitation peak at 315 nm and emission peak at 390 nm respectively while the absorbance of 3-HP has been found to be maximum at 305 nm. The fluorescence as well as the theoretically calculated spectral data has been used to characterize the hetero environment of the micelles in terms of their polarity, probe solubilization site and critical micelle concentration.

**Conclusion:** This article briefly discusses the importance of surfactants in biological system model as well as the use of micelles in pharmacy as an important tool that finds numerous applications.

**Keywords:** 3-hydroxy pyridine, Fluorescence, Micelles, Solubilization.

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## INTRODUCTION

Fluorescence spectroscopy is a well-established extensively used research and analytical tool in many disciplines [1]. Fluorescence spectroscopy can also serve as fantastic tool to study the micellization of surfactants due to its excellent sensitivity towards the environment surrounding the fluorophore which exhibits different fluorescence characteristics depending on the properties of the solubilizing medium. In recent years, a remarkable growth in the use of fluorescence in food analysis, biotechnology, drug delivery and design and clinical diagnostics of disease have been observed and several reports are given on its applications [2-6].

Micellization is an important phenomenon not only because a number of important interfacial phenomena, such as detergency and solubilization, depend on the existence of micelles in solution but also because it affects other interfacial phenomena, such as surface or interfacial tension reduction, that do not directly involve micelles. Micelles have been the subject to the numerous investigations due to their importance as model system for mimicking bio membranes [7-9]. Many characteristics of molecules, for example, absorption and fluorescence spectra, deprotonation and protonation equilibrium etc. are changed drastically in micellar media [10-13]. Conversely, changes observed in the absorption spectra of molecules have been utilized to study the properties of micelles, such as critical micelle concentration (CMC), viscosity, polarity of different sites [14]. Micelles also involve in drug delivery, to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bioavailability.

<sup>13</sup>C and <sup>1</sup>H NMR spectra of 3-hydroxy pyridine (3-HP) and its derivatives were analyzed [15]. The protective effect of 3-HP derivatives in various extreme conditions such as hypoxia, hypothermia, and hypokinesia

have been investigated [16]. Koval'chukova *et al.* [17] studied the physicochemical properties of some complex compounds of 3-HP and transition metals. Bromido and Chlorido complexes of Cu with 3-HP were prepared and the magnetic properties of complexes were analyzed by the infrared, ultraviolet/visible and electron paramagnetic resonance spectra [18]. Bridges *et al.* [19] were investigated that 2 and 4-HP were non-fluorescent at all p<sup>H</sup> values while 3-HP were fluorescent and studied the variations of the excitation and fluorescence wavelength and fluorescence intensity at different p<sup>H</sup> values. The anticancer properties of 3-HP and platinum complexes were studied and their activity against ovarian cancer cell lines have been determined [20].

## METHODS

Analytically pure 3-HP used was a Loba 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 3-HP was prepared in double distilled water. All the experiments were performed around 23–25°C in aqueous medium keeping the final concentration of 3-HP at 6\*10<sup>-5</sup>M for fluorescence studies. For absorption studies the concentration of 3-HP was kept at 2\*10<sup>-4</sup>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 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 8452, and diode array spectrophotometer.

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 ( $\Phi_f$ ) of 3-HP was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of 3-HP. 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 data have also been calculated and are expressed in nanometers.

## RESULTS

### Fluorescence studies

The aqueous solution of 3-HP has been showed maximum excitation peak at 315 nm and maximum emission peak at 390 nm. On addition of Tx-100 the fluorescence intensity decreased significantly as a consequence of fluorescence quenching without any appreciable change in the shape of the emission band. Tween-20 and Tween-80 increased the fluorescence intensity. Tween-20 caused maximum enhancement in fluorescence intensity with a red shift of 10 nm. The fluorescence spectral changes on addition of Tween-20 are as given in Fig. 1. The fluorescence intensity of 3-HP increased on adding anionic surfactants to it. All the anionic surfactants caused a blue shift of 10–30 nm in peak position. The cationic surfactants CTAB and MTAB caused an enhancement in fluorescence intensity with a blue shift of 5 nm and 30 nm respectively, while CPC decreased the fluorescence intensity. The fluorescence intensity in absence and presence of nonionic, anionic and cationic surfactants is given in Table 1.

### Absorption and quantitative studies

The absorbance of 3-HP was found to be maximum at 305 nm. Tween-20 and Tween-80 increased the absorbance with a red shift of 5–10 nm. TX-100 decreased the absorbance with a red shift of 5 nm. Anionic and cationic surfactants caused an enhancement in the absorbance with a blue shift of 5–15 nm. The fluorescence quantum yield ( $\Phi_f$ ) values of surfactant added 3-HP have been calculated. Data obtained from calculation show the increasing order in quantum yield values for all the nonionic, cationic and anionic surfactants except TX-100 and CPC. Molar extinction coefficient ( $\log \epsilon$ ) values for all surfactants showed an increasing trend except Tx-100. Empirical fluorescence coefficient ( $K_f$ ) values of 3-HP with different surfactants were found to be parallel with the fluorescence intensity of the compound. All the theoretically calculated spectral parameters are listed in Table 2.

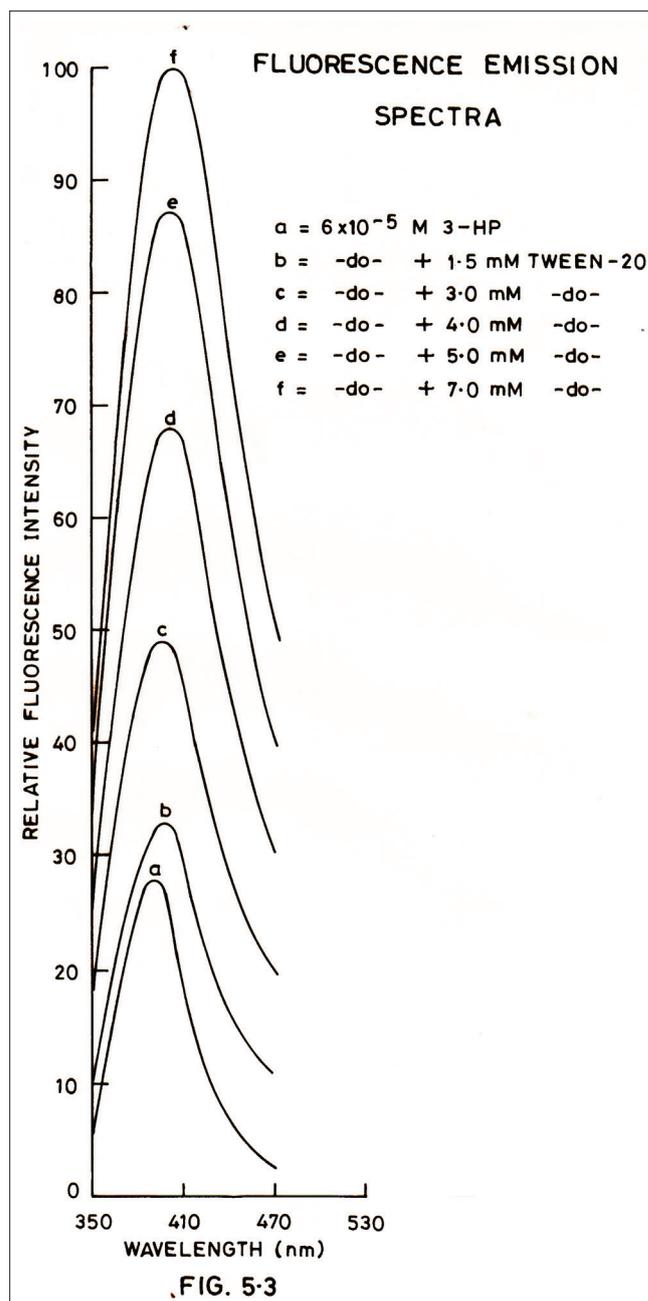


Fig. 1: Influence of addition of Tween-20 on fluorescence intensity of  $6 \times 10^{-5}$  M, 3-hydroxy pyridine solution. (a) No Surfactant; (b) 1.5 mM Tween-20; (c) 3.0 mM Tween-20; (d) 4.0 mM, Tween-20; (e) 5.0 mM Tween-20; (f) 7.0 mM Tween-20

Table 1: Fluorescence intensity of 3-HP in absence and presence of surfactant  $\lambda_{ex}=315$  nm,  $\lambda_{em}=390$  nm, P.M. Gain=3 Sensitivity Range=3

Name of surfactant	Relative fluorescence intensity in absence of surfactant	CMC'S of surfactant (mM)	Maximum Concentration of Surfactant used (mM)	Relative fluorescence intensity in presence of surfactant	$\lambda_{em}$ (nm)
Tx-100	28	0.26	1.5	10	390
Tween-80	28	0.1	6.0	94	390
Tween-20	28	0.05	7.0	100	400
CPC	28	0.6	9.0	22	390
CTAB	28	0.90	9.0	33	385
MTAB	28	3.6	7.0	85	360
SLS	28	8.2	7.0	39	380
DSSS	28	0.91	9.0	42	365
DBSS	28	0.81	0.8	65	360

3-HP: 3-Hydroxy pyridine, CMC: Critical micelle concentration, CPC: Cetylpyridinium chloride, CTAB: Cetyltrimethyl ammonium bromide, MTAB: Myristyltrimethyl ammonium bromide, SLS: Sodium lauryl sulphate, DSSS: Dioctyl sodium sulphosuccinate, DBSS: Dodecylbenzyl sodium sulphonate

**Table 2: Absorption maxima  $\lambda_{\alpha}$ , fluorescence maxima  $\lambda_{em}$ , molar extinction coefficient ( $\log \epsilon$ ) and quantum yield ( $\Phi_f$ ) of 3-HP at different concentration of Tween-20**

Concentration of Tween-20 (Mm)	Absorption maxima $\lambda_{\alpha}$	Molar extinction coefficient ( $\log \epsilon$ ) ( $dm^3 mol^{-1}cm^{-1}$ )	Fluorescence Maxima $\lambda_{em}$ (nm)	Quantum yield $\Phi_f$
0.00	305	3.3607	390	0.2418
1.5	305	3.4608	395	0.2968
3.0	305	3.5409	395	0.3920
4.0	310	3.5693	400	0.5084
5.0	310	3.6159	400	0.5914
7.0	315	3.6776	400	0.6055

3-HP: 3-hydroxy pyridine

**Stokes' shift**

The Stokes' shift values of 3-HP were determined for different concentrations at room temperature. The Stokes' shift values increased on diluting the 3-HP solution. The calculated values are given in Table 3.

**DISCUSSION**

Tx-100 quenched fluorescence intensity because effective hydrogen bonding does not take place between solubilize and surfactant micelles. The quenching also indicates that these compounds prefer the hydrophobic core to the hydrophilic poly(ethylene oxide) shell, particularly for Tx-100. Evidently the fluorescence of compounds is significantly weakened in the core such as in non-aqueous solvents. This implies that compound is embedded in the core is not hydrated around the aromatic rings [21]. Quenching can also be caused by non-radiation loss of energy from the excited molecules. Fluorescence quenching was also observed by the addition of CPC, which may be attributed to the electrostatic preferential interaction between the polar substituent of 3-HP molecules and the cationic head group of the surfactant which may result in change in the geometry of the solubilize molecule, where it loses the coplanarity. The quenching may also be due to interaction between the  $\pi$ -electron system of the excited state fluorophore and quencher molecule CPC due to the presence of nucleophilic pyridine ring in the structure which makes it act as a quencher through hydrogen bond between the proton donor and acceptor. This will result in delocalization of the  $\pi$ -electrons of the excited state and hence loss of fluorescence [22].

On addition of anionic surfactants, a continuous enhancement in the fluorescence intensity was observed for 3-HP. The increase in fluorescence intensity and quantum yield suggests that they have solubilized suspended molecules, which are dispersed as microcrystals in water which collide with anionic micelles to penetrate into the micellar core interior. Here the anionic micelles have formed 1:1 complex with the protonated solubilized molecules. This complex is called ion association complex [23].

Absorption spectra of 3-HP are very less affected in micellar media as compared to the fluorescence spectra. This may be because absorption is less sensitive to its environment as compared to fluorescence. No major change in the nature of absorption spectrum indicates no structural changes due to complex formation or dissociation or hydrogen bonding between 3-HP in the ground state and the surfactant. Blue shift obtained in maxima may be due to the difference in salvation energy of the excited state. The sufficiently large value of  $\log \epsilon$  are assigned to the  $\pi \rightarrow \pi^*$  transitions.

The increase in fluorescence intensity and quantum yield values in ionic micellar media clearly indicate that the rates of non-radiative processes are less in micellar system in comparison to those in water. This could be due to the decrease in intersystem crossing rate [24]. Another reason for the increase in values could be due to absorption of fluorophore at the micellar surface, which decrease the rate of collisional deactivation

**Table 3: Stokes' shift data of 3-HP at room temperature**

Concentration of 3-HP (M)	F.I	$\lambda_{ex}$ (nm)	F.I	$\lambda_{em}$ (nm)	Stokes' shift ( $cm^{-1}$ )
$1 \times 10^{-3}$	22	330	37	390	4662
$7 \times 10^{-4}$	20	330	36	390	4662
$5 \times 10^{-4}$	19	325	33	390	5128
$3 \times 10^{-4}$	51	320	89	390	5608
$1 \times 10^{-4}$	28	315	47	390	6105
$7 \times 10^{-5}$	20	315	30	390	6105
$6 \times 10^{-5}$	17	315	28	390	6105
$5 \times 10^{-5}$	15	315	23	390	6105
$3 \times 10^{-5}$	12	315	17	390	6105

3-HP: 3-hydroxy pyridine

of fluorophore by water molecule. Greater quantum yields imply greater efficiency of fluorescence pathway.

Large Magnitude of Stokes' shift data obtained in the solubilize molecules of 3-HP suggests the preferential solvation of the solute in the protic solvent to increase hydrogen bonding interaction. Structural transitions can be induced in charged micelles by increasing ionic strength of the medium. These large Stokes' shift values are due to hydrogen bond formation between the solute and the solvent in ground state. This bond breaks following excitation to  $S_1$  but reforms following proton transfer [25].

**CONCLUSION**

The present analysis indicates that during solubilization of solubilize 3-HP into the surfactant system, the incorporation of the solubilize influence the balance of favorable and unfavorable forces guiding micellization and structural changes occurring due to aggregation, dissociation and hydrogen bonding. Hence the process of micellization followed by solubilization of 3-HP would catalyze its pharmaceutical activities which may serve better results in medicinal and analytical fields. Thus one can generalize the physical understanding to study the phenomenon of micellar solubilization

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**AUTHOR'S CONTRIBUTION**

Anshu Mahlawat has performed the Solubilization, Fluorescence and absorption spectral study of the compound. Arun Goyal has been contributed in spectral study and data analysis. Both the authors draft the final manuscript.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interests.

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