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A Fluorescence study of Solubilization of 4-Hydroxy-3-Methoxy Benzoic Acid (4-H-3-MBA) in Surfactant Micelles.

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ABSTRACT

The fluorescence and absorption study was carried out of 4-Hydroxy-3-Methoxy Benzoic Acid (4-H-3-MBA) molecule which is a dietary phenolic compound found in plants and fruits. It can exhibit antioxidant, antimicrobial and antimalarial activity. It is a pharmaceutically and analytically important molecule. The solubilization of 4-H-3-MBA is significantly enhanced in micellar media of ionic and nonionic surfactants. The solubilizing action of the surfactant has been supplemented by theoretically calculated spectral parameters like, empirical fluorescence coefficient, molar extinction coefficient, quantum yield and stokes' shift. An attempt has been made to provide a unique format for analytical and medicinal application of 4-H-3-MBA based on micellization and solubilization process.

Keywords: Micelles, Solubilization, Fluorescence, 4-Hydroxy-3-Methoxy Benzoic Acid (4-H-3-MBA).

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INTRODUCTION

The fluorescent measurements are powerful tools in the study of range of processes that can lead to potential applications to surface science, medical and biological fields where chemistry, physics and biology meet together. The high sensitivity of fluorescence technique and its great selectivity has been well justified by the study made at very low concentrations of the reactions.

Phenolic acids seem to play vital role in many processes of interest in both fundamental and applied science. 4-Hydroxy-3-Methoxy Benzoic Acid (4-H-3-MBA) is a dietary phenolic compound found in plants and fruits[1]. It is a metabolic product of caffeic acid[2]. The antioxidant, antimicrobial and antimalarial activity of 4-H-3-MBA has been studied by Rechner et al.[3]. Merve et al.[4] studied the genotoxic and Anti-genotoxic effects of 4-H-3-MBA in human lymphocytes. A study on spectroscopy and photophysics of 4-H-3-MBA in different solvents, pH and β- cyclodextrin has been made by Stalin and Rajendiran[5]. Addanki et al.[6] studied the role of methyl ester derivative of 4-H-3-MBA in prostate cancer management. The beneficial effects of 4-H-3-MBA against DSS-induced ulcerative colitis have been studied by Kim et al.[7]. 4-H-3-MBA has been used as a preservative and antiseptic agent in the food, pharmaceutical and cosmetic industries[8]. Photophysics of fluorescent molecules is a field of constant interest because a better understanding of the excited state properties helps in design of new molecules offering the best performances for a given application.

Surfactants play a vital role in various drug delivery. To formulate compounds springly soluble in water, pharmaceutically acceptable cosolvents or surfactants are typically employed to increase solubility. Polymeric micelles made by surfactants have a whole set of unique characteristics, which make them a very promising drug carriers for a wide range of drugs. The low solubility in biological fluids displayed by about 50% of the drugs still remains the main limitation in oral, parental and transdermal administration. To overcome these drawbacks, inclusion of hydrophobic drugs into polymeric micelles, which are composed of surfactants are one of the most effective alternatives[9]. Analytical methods which rely on the use of surfactants are becoming more and more numerous, since addition of surfactants provides an increase in selectivity and sensitivity[11, 12]. From an analytical view point, the use of surfactants increases the solubility of organic substances in water, through shallow or deep penetration of the micelles or simply by surface adsorption[13], and can also catalyse specific reactions by modification of the microenvironment which these reactions take place[14].

This paper includes study of the influence of various nonionic and ionic surfactants on the fluorescence and absorption spectra of 4-H-3-MBA. Some spectral parameters have also been calculated theoretically and interpreted which help in understanding the paramount importance of surfactants in pharmacy specially with respect to their ability of solubilizing hydrophobic drugs.

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 4-H-3-MBA (Sigma chemical) was prepared in distilled methanol. All the experiments were made at room temperature (23-25^{0}C) and 1% (v/v) methanolic medium keeping the final concentration of 4-H-3-MBA at 3 x 10^{-4} M. All the surfactants used were either of sigma (USA) or BDH product. The following surfactants were employed.

Nonionic: (i) Polyoxyethylene tertoctyl phenol (TX-100), (ii) Polyoxyehylene sorbitain monolaurate (Tween-20) and (iii) Polyoxyethylene sorbitain monooleate (Tween-80).

Cationic: (i) Cetyltrimethyl ammonium bromide (CTAB), (ii) Myristyltrimethyl ammonium bromide (MTAB) and (iii) Cetylpyridinium chloride (CPC)

Anionic: (i) Dodecylbenzyl sodium sulphonate (DBSS), (ii) Dioctyl sodium sulphosuccinate (DSSS) and (iii) Sodium lauryl sulphate (SLS)

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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 4-H-3-MBA was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of 4-H-3-MBA. 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.

RESULTS AND DISCUSSION

The methanolic solution of 4-H-3-MBA showed maximum excitation peak at 300nm and the maximum emission peak at 355nm. All the nonionic surfactants caused an enhancement in peak value of fluorescence intensity with a gradual blue shift of 10nm. Among these surfactants maximum effect was exerted by Tween-80. On addition of anionic surfactants to the 4-H-3-MBA solution an increase in fluorescence with all the three surfactants was observed accompanied by 15-20 nm blue shift. On addition of cationic surfactants to the solution of 4-H-3-MBA the fluorescence intensity increased with 5-10 nm blue shift except with CPC. For CPC, the emission intensity decreased. The minimum and maximum fluorescence intensity in absence and presence of nonionic, anionic and cationic surfactants is given in Table-1. The fluorescence spectral changes in Tween-80 micellar media are given in Fig.1.

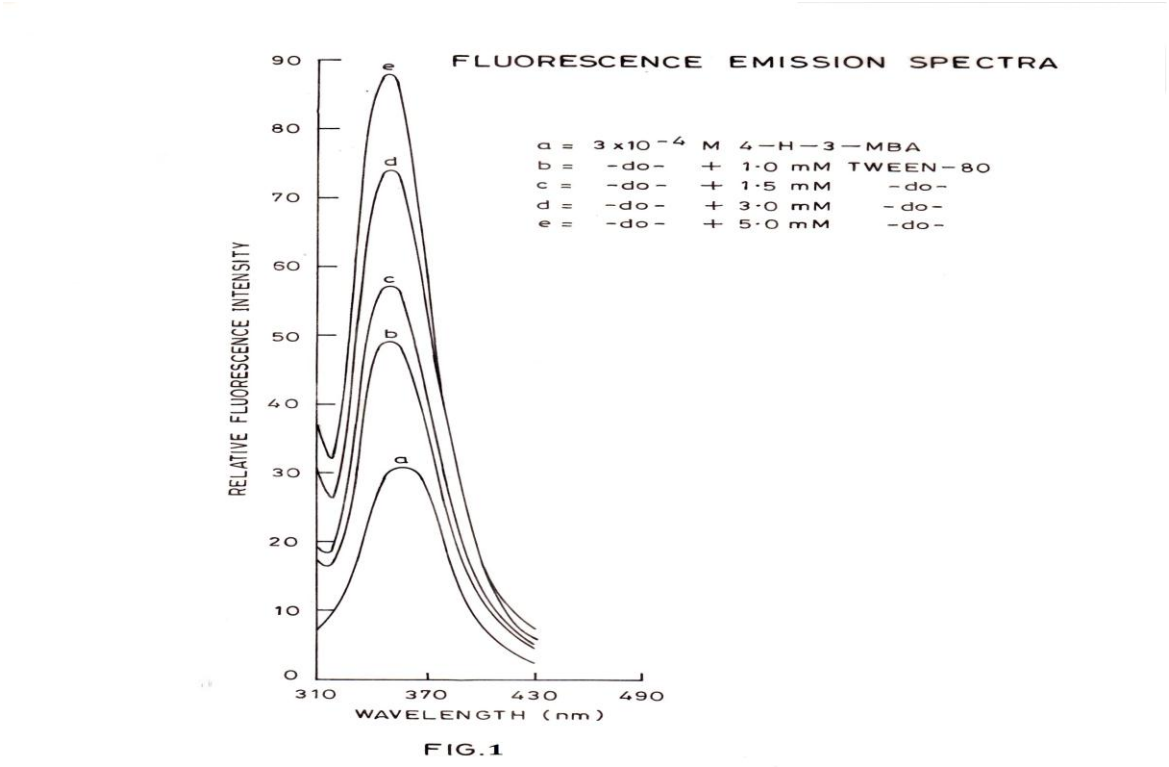

Figure 1. Influence of addition of Tween-80 on fluorescence intensity of 3 x 10^{-4} M 4-H-3-MBA solution (a) No Surfactant; (b) 1.0 mM Tween-80; (c) 1.5 mM Tween-80; (d) 3.0 mM Tween 80; (e) 5.0 mM Tween-80

In absence of surfactant, peak of absorption spectra was found at 290 nm. For nonionic and anionic surfactants, an enhancement in the absorbance was observed with a blue shift of 10-15 nm. Cationic surfactants showed enhancement in absorbance without any shift in peak position except CPC. CPC showed an exceptional behavior by decreasing the absorbance.

The fluorescence quantum yield values and empirical fluorescence coefficient values obtained showed parallel trends to emission intensity of 4-H-3-MBA. Molar extinction coefficient values for all the

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classes of surfactants obtained are in increasing order except CPC. The Stokes' shift for 4-H-3-MBA at room temperature was from 3164 cm^{-1} to 5174 cm^{-1} on its dilution. All the theoretically calculated spectral parameters are listed in Table-2 and Table-3 respectively.

Table 1: Fluorescence intensity of 4-H-3-MBA in absence and presence 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	31	0.26	3.0	61	345
Tween-80	31	0.1	5.0	88	345
Tween-20	31	0.05	5.0	66	345
CPC	31	0.6	9.0	10	345
CTAB	31	0.90	7.0	56	350
MTAB	31	3.6	7.0	66	355
SLS	31	8.2	7.0	46	335
DSSS	31	0.91	5.0	39	340
DBSS	31	0.81	1.5	61	340

λ_{ex} =300nm λ_{em}=355nm P.M. Gain=3 Sensitivity Range=1

Table 2: Absorption maxima λ_{α}, fluorescence maxima λ_{em}, molar extinction coefficient (log ε) and quantum yield (Φ_f) of 4-H-3-MBA at different concentration of Tween-80

S.No.	Concentration of Tween-80 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	290	4.7853	355	0.22619
2.	1.0	285	4.8750	350	0.28800
3.	1.5	280	4.9063	350	0.31369
4.	3.0	280	4.9898	350	0.33754
5.	5.0	280	5.0358	345	0.34415

Table 3: Stokes' shift data of 4-H-3-MBA 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^{-2}	5	315	26	350	3174
2.	7×10^{-3}	3	315	13	355	3577
3.	5×10^{-3}	8	315	33	355	3577
4.	3×10^{-3}	18	315	63	355	3577
5.	2×10^{-3}	15	310	50	355	4089
6.	1×10^{-3}	11	305	40	355	4617
7.	7×10^{-4}	10	305	36	355	4617
8.	5×10^{-4}	9	300	32	355	5164
9.	3×10^{-4}	8	300	31	355	5164

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

The results obtained can be explained on the basis of solubilization by microheterogenous environment of micelles present in the surfactant solution at or marginally above CMC. Enhancement in the fluorescence intensity of the compound on adding surfactant can be attributed to the increase in the quantum efficiency of fluorescence. Furthermore, the quantum yield of fluorescence was higher in nonionic nonpolar medium, because of the lesser effect of other deactivation processes which compete with fluorescence.

The increase in fluorescence intensity and Φ_f values in ionic micellar media clearly indicates that the rate of non-radiative processes are less in micellar system in comparison to those in aqueous media, which could be due to the decrease in intersystem crossing rate. Quenching of fluorescence by CPC may be attributed to the interaction between the π-electron system of the excited state 4-H-3-MBA fluorophore and

the quencher CPC molecule due to the presence of nucleophilic pyridine ring in its structure which forms H-bond between the proton donor and accepter which results in delocalization of the π-electrons of the excited state and hence loss of fluorescence [15].

On adding the surfactants to the methanolic solution of the compound, the surfactant micelles get absorbed at the interfaces and remove the hydrophobic groups from contact with water, thereby reducing the free energy of the system. But on transferring the hydrophobic group from solution, to the micelle in the solvent, may experience some loss of freedom confined to the micelle and, in the case of ionic surfactants, from electrostatic repulsion from other similarly charged surfactant molecules in the micelle. These forces increase the free energy of the system and thus oppose micellization. Whether micellization occurs in a particular case, if so, at what concentration of monomeric surfactant, therefore depends on the balance between the factors promoting micellization and opposing it. Thus the increase in quantum yield suggests that all the surfactants except CPC have solubilized the suspended solubilizate molecules (4-H-3-MBA).

Sufficient large value of log ε are assigned to the $\pi \rightarrow \pi^*$ transitions. The large magnitude of stokes' shift of 4-H-3-MBA is due to hydrogen-bond formation, between solute and solute in ground state. This bond breaks following excitation to S but reform following proton transfer. The hydrogen bonded excited state can be produced via two routes as shown by following scheme in which S_1 represents the solvent molecule and A represents the flurophore.

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

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

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 the solubilizate and solvent. This shift may also be considered to be because of the difference in solvation energy of the solute in the ground state and excited state in different microheterogenous micellar media.

Absorption is less sensitive to its environment as compared to fluorescence, thus absorption spectra are less affected on addition of surfactants. On addition of all the three kinds of surfactants a gradual enhancement in the absorbance occurred. The values of empirical fluorescence coefficient (K_f) obtained may be attributed to the increased sensitivity of the fluorescence analysis of the solubilization of organic molecules by surfactants which offer a protective microenvironment, leading to enhanced fluorescence of the solubilizate by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution.

CONCLUSION

The present analysis and interpretation suggest that experimental results observed and the theoretically calculated spectral data are found to be in good agreement. This proves the validity of the investigations made. Hence the process of micellization followed by solubilization of 4-H-3-MBA solubilizate would catalyse its drug delivery activities. This study has revealed one of the important applications of surfactants in micellar drug solubilization[16]. Solubilization increases the bioavailability of the drug to the required site of the body. This may serve better results in medical and analytical fields.

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