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Micellar Solubilization of 3, 5-Dihydroxy Benzoic Acid (3, 5-DHBA) By Fluorimetry

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ABSTRACT

Phenolic acids seems to be universally distributed in the plant kingdom, essential for the reproduction of plants and are produced as a response to defence against pathogens of antioxidant activities of phenolic compounds and their possible usage in processed natural antioxidant has received attention in recent years. Here, solubilization of 3,5-dihydroxy benzoic acid (3,5-DHBA) in ionic and nonionic micellar media has been studied by fluorescence and absorption spectroscopy. Theoretical calculations of some spectral parameters like molar extinction coefficient values ($\log \varepsilon$), quantum yield (ϕ_f) and Stokes' shift values have been made. The fluorescence properties as well as the theoretically calculated spectral data have been used to characterize the hetero environment of the micelles in terms of their polarity, probe solubilization site and critical micelle concentration (CMC). Thus, an understanding of the process of solubilization of the otherwise insoluble such as selective drug inside the human body in the presence of micelles, which enhance their activity and their transport to the site of action, has been mimicked at the laboratory level.

Keywords : Micellization, 3,5-dihydroxy benzoic acid (3,5-DHBA), fluorescence, solubilization.

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INTRODUCTION

Phenolic acids seem to play vital role in many processes of interest in both fundamental and applied science. The formation of colloidal sized clusters in solutions, known as micelles are of particular significance in pharmacy because of their ability to increase the solubility of sparingly soluble substances in water.[1]

The most striking feature of micelles is the ability to solubilize a variety of compounds in its different regions [2]. 3,5-DHBA is a phenolic acid essential for reproduction of plants and of antioxidant activities of phenolic compounds and their possible usage in processed natural oxidant has received attention in recent years. S.M. Mandel et al. [3] studied first on combined HPLC and MALDI-TOF MS analysis of phenolic acid mixtures and showed a unique, individual pattern for phenolic acid. Dihydroxy benzoic acids are also used in formation of quinines. They exhibit antitumor and antimalarial activities [4].

A series of hyperbranched polyesters was produced by the condensation of 3,5-DHBA. Mansour S.H. et al. [5] studied these polyesters by ^{1}H NMR, thermal and photophysical properties and solutions exhibited intense fluorescence with a max of 430 nm.

Surfactants play a vital role in various drug delivery. They are pharmaceutically acceptable cosolvents and are employed to increase the solubility of compounds. Thus, increasing their bioavailability, stay in the blood long enough to provide a gradual accumulation in the required area. Moreover, specific legands can be attached to optimize the controlled release and specificity of pharamacological effect. 3,5-DHBA is antitumor and the utilization of aqueous solutions of 3,5-DHBA solubilization can be advantageous for drug delivery purposes, in enhancing permeability across the physiological barriers, substantial change in drug distribution and reducing toxicity and other side effects. We report here the investigations carried out on 3,5-DHBA of medicinally important molecule and discuss the importance of surfactant micelles to solubilize 3,5-DHBA molecules employing fluorescence and absorption spectral techniques.

EXPERIMENTAL

Materials:

Analytically pure 3,5-DHBA was a Merck sample. The following surfactants were employed :

Nonionic:

(1) Polyoxyethylene tert–octyl phenol (TX–100)
(2) Polyoxyethylene sorbitain monolaurate (Tween–20)
(3) Polyoxyethylene sorbitain monooleate (Tween–80)

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Anionic:

(1)Dodecylbenzene sodium sulphonate (DBSS)
(2) Dioctyl sodium sulphosuccinate (DSSS)
(3) Sodium lauryl sulphate (SLS)

Cationic:

(1) Cetylpyridinium chloride (CPC)
(2) Cetyltrimethyl ammonium bromide (CTAB)
(3) Myristyltrimethyl ammonium bromide (MTAB).

All the surfactants used were either of Sigma (USA) or BDH (UK) products.

Methods:

The fluorescence spectrum was taken with Perkin-Elmer Fluorescence Spectrophotometer model 204A with a synchronized model No. 056 strip chart recorder and absorption spectra were taken on Hewlett Packard (HP) 8452A diode array spectrophotometer.

The stock solution of analytically pure 3,5-DHBA was prepared in double distilled water. All experiments were made at room temperature (23-25°C) and final concentration of compound was kept at 9 x 10^{-6} M and 1 x 10^{-4} M for fluorescence and absorption spectra respectively.

The purity of the surfactants was checked by determining their CMC values with help of surface tension measurements, employing drop weight method. The values thus obtained coincided with the reported values. The absolute fluorescence quantum yield of the compound was calculated relative to anthracene solution as standard. Fluorescence emission obtained is in the same range as that of the compound. Approximate corrections were made to compensate for the different absorptions of the compounds 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.

RESULTS AND DISCUSSION

3,5-DHBA in its aqueous solution showed a maximum emission wavelength at 360 nm. The maximum excitation peak intensity appeared at 300 nm. All the nonionic surfactants caused an enhancement in peak value of fluorescence intensity with a gradual blue shift of 10-15 nm except Tween-20. Among these surfactants maximum effect was exerted by TX-100. Effect of nonionic surfactants viz., Tween-20 and Tween-80 given in Fig. 1 and Fig. 2. The fluorescence intensity of 3,5-DHBA increased on adding anionic surfactants to it. Out of all these maximum effect was exerted by DBSS. Effect of DBSS on fluorescence emission intensity was given in Fig. 3. Cationic surfactants caused initially a slight decrease in the fluorescence

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intensity and then increased it at their higher concentration except CPC which decreased the fluorescence intensity.

The absorption spectra gave a peak at 295 nm. For nonionic and anionic surfactants an increase in absorbance was observed with increase in concentration. TX-100 showed a blue shift of 5 nm. In cationic micellar media the absorbance decreased at low concentrations, but as the concentration increased the absorbance enhanced. CPC showed an exceptional behaviour by decreasing the absorbance gradually. The calculated fluorescence quantum yield (ϕ_f) of surfactant added 3,5-DHBA solution showed parallelism with changes in its fluorescence intensity in the micellar media. For the cationic surfactants added solution initially slightly decrease and then increase in (ϕ_f) values was observed. The calculated values are given in Table 2. The molar extinction coefficient values showed a gradual increase as the concentration of nonionic surfactant was increased. With cationic surfactants a decrease in log e values was observed for CTAB and MTAB at initial level and then on further addition an increase in ($\log \varepsilon$) values was observed except CPC which sharply decrease. The data are given in Table 2. The Stokes' shift values increase as the concentration of 3,5-DHBA solution was increased.

Table-1: Florescence intensity of 3,5-DHBA in presence and absence of surfactants.

λ_{ex} = 300 nm P.M. Gain = 3

λ_{em} = 360 nm Sensitivity Range = 3

S.N.	Name of surfactant	Relative fluorescence intensity in absence of surfactant	Max. conc. of surfactant used (g/L)	Relative fluorescence intensity	λ_{em} (nm)
1.	TX-100	27	0.07	Out of scale	350
2.	Tween-20	27	0.3	65	360
3.	Tween-80	27	0.3	65	345
4.	DBSS	27	0.1	Out of scale	360
5.	DSSS	27	0.5	37	360
6.	SLS	27	0.5	44	360
7.	CPC	27	0.5	04	360
8.	CTAB	27	0.07	37	360
9.	MTAB	27	0.5	39	360

The results indicate the surfactant added system has a stronger emission intensity enhancement than the system in which there was no surfactant present has been found. The non-polar environment of TX-100 micellar interior may be most preferable to incorporate the hydrophobic 3,5-DHBA than the ionic surfactant micelle. The fluorescence and absorption behaviour of 3,5-DHBA has been supported by ($\log \varepsilon$) and (ϕ_f) values.

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Table 2:Absorbance Maxima (λ_{max}), Molar Extinction Coefficient ($\log \varepsilon$), Fluorescence Emission (λ_{em}) and Quantum yield (ϕ_f) values of 3,5-DHBA at different concentrations of TX-100

S.N.	% of TX-100 (w/v)	λ_{max} (nm)	$\log \varepsilon$ ($dm^3\ mol^{-1}\ cm^{-1}$)	λ_{em} (nm)	ϕ_f
1.	0.0	295	4.3511	360	1.1621
2.	0.005	290	4.3679	360	1.2214
3.	0.01	290	4.3940	355	1.2295
4.	0.05	290	4.4093	350	1.3018

The results so obtained can be explained in a manner by considering the oblate ellipsoidal model for TX-100[6]. The octaphyenyl group and poly oxyethylene group of TX-100 can separate each other and each layer packs well. This model predicts the hydrophobic and less fluid interior of the TX-100 micelle. This fact has also been supported by Kano et al. [7] An initial decrease in fluorescence intensity of 3,5-DHBA in cationic micellar media and then an enhancement on raising the surfactant concentration may be attributed to the interactions, which are mainly electrostatic in nature between the OH^- group of the exocyclic substituents of 3,5-DHBA and the cationic head groups of the surfactant. At low ionic micellar concentration, below cmc, solubilizate 3,5-DHBA – cationic aggregate formation took place which accounts for initial decrease in fluorescence. But above cmc only the monomeric form of the compound exists exhibits which penetrated into the micellar interior and got solubilized, hence fluorescence enhanced. [8] The decreasing trend in Stokes' shift in very dilute solution of 3,5-DHBA is rationalizable in view of water-water hydrogen bonding interactions which lowers the overall hydrogen-donations to the solute 3,5-DHBA

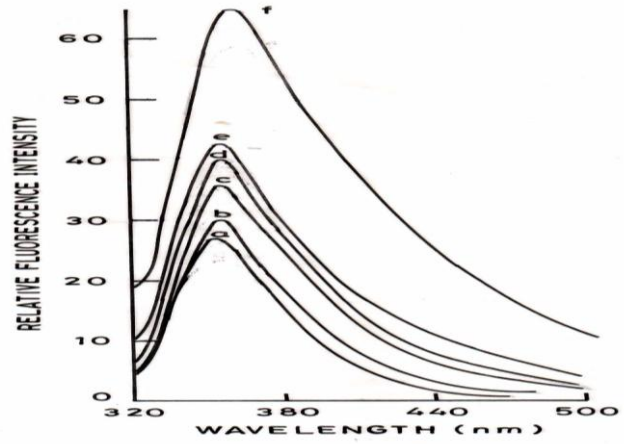

Fig 1: Influence of addition of Tween-20 on fluorescence intensity of 9 x 10^{-6} M, 3,5-DHBA solution

(a) No surfactant,
(b) 0.01% Tween-20
(c) 0.05% Tween-20
(d) 0.07% Tween-20
(e) 0.15% Tween-20
(f) 0.3% Tween-20

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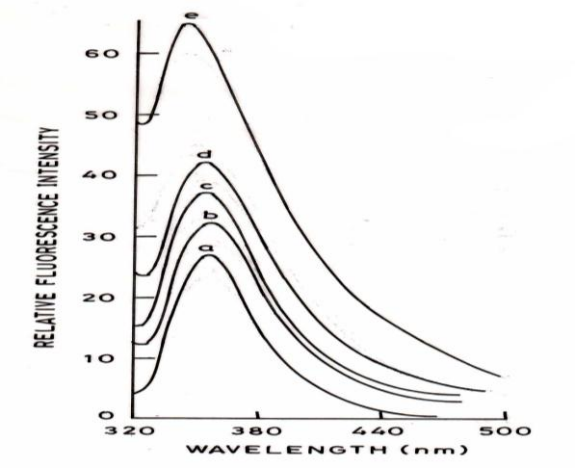

Fig 2: Influence of addition of Tween-80 on fluorescence intensity of 9 x 10^{-6} M, 3,5-DHBA solution

(a) No surfactant
(b) 0.03% Tween-80
(c) 0.07% Tween-80
(d) 0.1% Tween-80
(e) 0.3% Tween-80

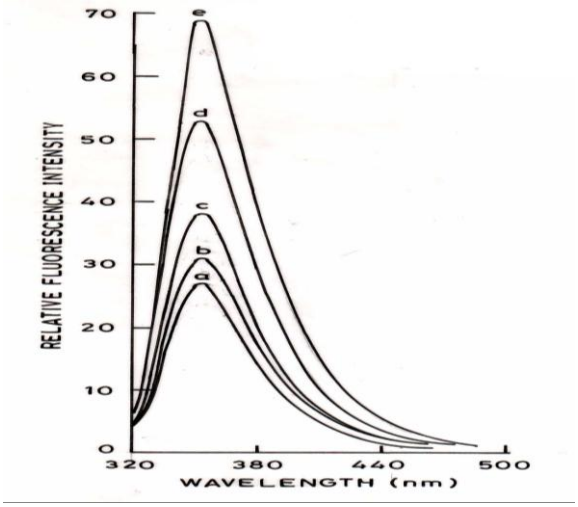

Fig 3: Influence of addition of DBSS on fluorescence intensity of 9 x 10^{-6} M, 3,5-DHBA solution

a) No surfactant
b) 0.005% DBSS
c) 0.01% DBSS
d) 0.03% DBSS
e) 0.05% DBSS

The absorption spectra are less affected on adding surfactants as absorption is less sensitive to its environment as compared to fluorescence sufficiently large value of ($\log \varepsilon$) is assigned to the π-π* transitions.

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The small blue shift in absorption maxima may be because of the difference in solvation energy of the solute in ground state and excited state. The high quantum yield (ϕ_f) value in micellar medium may be attributed to the rate of non radioactive processes lesser in the micellar medium [9] in comparison to those in aqueous medium. It may be due to the absorption of the fluorescence at the micellar surface, which decreases the rate of collision of fluorophore by water molecules. [10]

CONCLUSION

All theoretical calculated spectral parameters are in good agreement to the interpretation. This prove the validity of the investigations made. Thus, it can be generalized from the present fundamental study to the understanding of assimilation of some essentially important drugs in the human body by phospholipids, which acts as micellar in body fluid. The process of micellization followed by solubilization of 3,5-DHBA substrate would catalyze to drug delivery activities. The nonionic surfactants could be considered the best alternative for solubilization of 3,5-DHBA as well as other basic drugs. This class of surfactants provides a reasonable molar solubilization capacity combined in low cmc values, resulting in increased solubility. This leads to increase the bioavailability of the drug, stay in the body (blood) long enough to provide gradual accumulation in the required area and their size permit them to accumulate in areas and with leaky vasculature. This may serve better results in medical and analytical fields. Hence, micellar solubilization finds an extensive application in biochemical and biomedical fields. The present analysis is an effort to mimic this at laboratory level.

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