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Photophysical Properties of Lumazine in Micellar Media

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The electronic spectral studies include the effect of different micellar heteromicroenvironment on the fluorescence and absorption spectral behaviour of lumazine. It is a medicinally and analytically important biomolecule. The dual nature of surfactant micelle is responsible for the occurrence of surface activity, solubilization and micellization. The increase in fluorescence intensity on addition of surfactant can be attributed to the increase in quantum efficiency. The solubilization phenomenon has also been confirmed by quantitative calculations like quantum yield, molar extinction coefficient (log ε) and Stokes' shift values and fluorescence coefficient. The fluorescence properties as well as the theoretically calculated spectral data have been used to characterize the microenvironment of the micelles in terms of their micropolarity, probe solubilization site and critical micelle concentration.

Key Words: Fluorescence, Micelles, Lumazine, Solubilization.

INTRODUCTION

Absorption and fluorescence spectral deprotonation and protonation equilibria of molecules have been changed drastically in micellar media. Micelles have been the subject of numerous investigations because of their importance as model system for mimicking biomembranes[1,2]. The most striking feature of micelles is the ability to solubilize variety of compounds in its different regions[3]. In recent years extensive investigations have been made on micellar effects of the surfactants on polynuclear heterocyclic compounds[4]. Lumazine is a yellow fluorescent heterocyclic compound of biological importance. When it is bound to proteins they become more strongly fluorescent with emission characteristics similar to the bioluminescence of certain bacteria. Lee *et al.*[5] have established that lumazine protein is a spherical monomeric novel protein and is one of the several major proteins produced by the bioluminescent bacteria. Gibson *et al.*[6] studied the fluorescence dynamics measurement of energy transfer in the bacterial luciferase and lumazine protein interactions. Studies have been made[7] on the stereospecifity for binding the lumazine protein. The lumazine when bound to protein has been proved to be of great importance in microbiology of bioluminescent bacteria[8,9].

The present study is carried out to investigate the solubilization of lumazine which is an essential yellow pigment with great binding property and of biological importance in various surfactant micelles, employing fluorescence and absorption spectral studies.

EXPERIMENTAL

A Perkin-Elmer 204A fluorescence spectrophotometer was used. All experiments were carried out at room temperature. Absprotion spectra of lumazine were taken on a double beam specord UV-Vis spectrophotometer. The stock solution of analytical pure grade lumazine (Sigma) was prepared in double distilled water. The concentration of compound for various experiments was kept at 1×10^{-5} M for fluorescence measurements.

Surfactants employed for the study were as follows:

(A) Non-ionic surfactants: (1) Polyoxyethylene teroctyl phenol (Tx-100), (2) polyoxyethylene sorbitain monopalmitate (Tween-40), (3) polyoxyethylene sorbitain monolaurate (Tween-20). **(B)** Anionic surfactants: (1) Dioctyl sodium sulpho succinate (DSSS), (2) sodium lauryl sulphate (SLS). **(C)** Cationic surfactants: (1) Cetyl pyridinium chloride (CPC), (2) cetyldimethyl benzyl ammonium chloride (CDBAC). All surfactants were sigma product and were used as such.

RESULTS AND DISCUSSION

The maximum excitation wavelength was at 335 nm. The emission spectrum of lumazine in 1 % ethanolic medium showed a peak at 470 nm. All the non-ionic and anionic surfactants caused an enhancement in fluorescence intensity. The change in the fluorescence intensity of lumazine on varying concentration of TX-100 and Tween-40 are illustrated in Fig. 1. The cationic surfactants showed first an increase and then a decrease in fluorescence intensity with a red-shift of 2-5 nm in peak position. The results of presence and absence of surfactants on emission peak are given in Table-1.

There appeared an absorption peak at 325 nm. On addition of non-ionic and cationic surfactants first an increase and then a decrease in absorbance was observed except cetyl pyridinium chloride, which showed a continuous increase in the absorbance with a blue-shift of 5 nm. The anionic surfactants first showed a decreasing and then an increasing effect on absorbance.

Enhancement in the fluorescence intensity was also observed on adding ethanol to the solution of lumazine. The enhancement in the fluorescence intensity was accompanied by blue-shift of 10 nm in the emission wavelength at 40 % ethanolic medium. On further increasing the concentration of ethanol decrease in fluorescence intensity was observed.

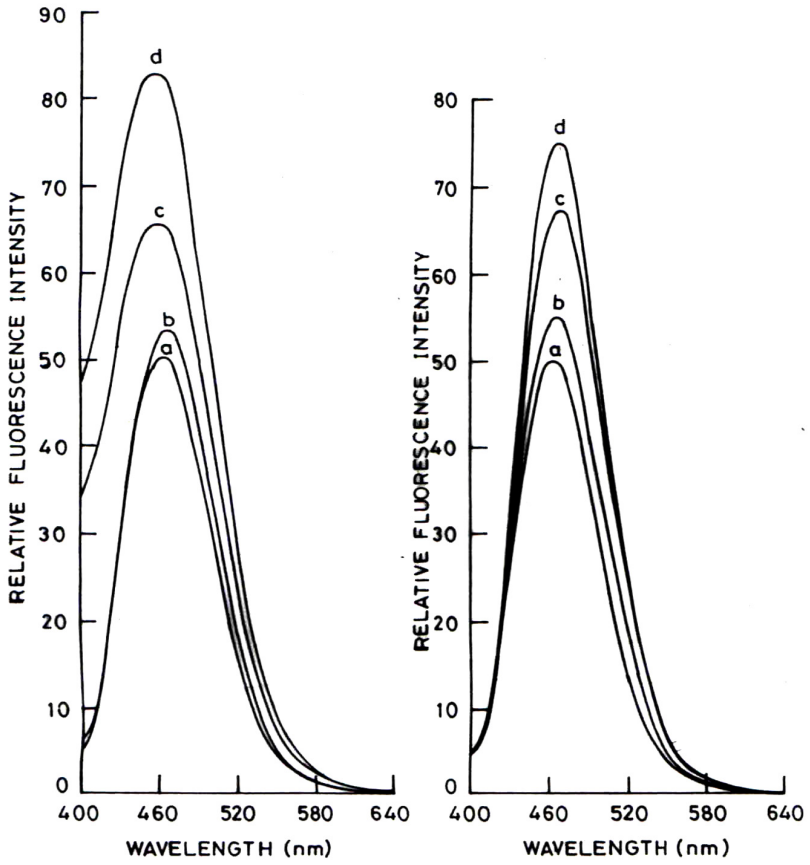

Fig. 1. Influence of addition of Tx-100 and Tween-40 on fluorescence intensity of 1×10^{-5} M, lumazine solution (A) Tx-100: (a) 0.00 % (b) 0.05 % (c) 0.5 % (d) 0.7 % (B) Tween-40: (a) 0.00 % (b) 0.003 % (c) 0.007 % (d) 0.1 %

TABLE-1
FLUORESCENCE INTENSITY OF LUMAZINE IN ABSENCE AND PRESENCE OF SURFACTANTS
λ_{em} = 470 nm, λ_{ex} = 335 nm, P.M. Gain = 3, Sensitivity range = 0.3

Name of surfactant	Fluorescence intensity in absence of surfactant (nm)	Concentration of surfactant used (%)	Maximum fluorescence intensity (nm)
Tx-100	50	0.90	87
Tween-40	50	0.90	79
Tween-20	51	0.90	67
DSSS	51	0.05	108
SLS	52	0.30	95
CPC	52	0.30	38
CDBAC	55	0.30	48

The results indicate that the surfactant added system has a stronger enhancement than the system in which there was no surfactant present. The maximum fluorescence enhancement[10] was obtained by adding TX-100. An oblate ellipsoid model has been postulated for TX-100.

A spherical model requires mixing of the hydrophobic part and the hydrophilic part. But in oblate ellipsoid model, the octylphenyl group and the polyoxyethylene group of TX-100 can separate each other and each layer packs well. This model therefore, predicts less fluid interior of TX-100 micelle. The interior of the TX-100 micelle is more hydrophobic than those of ionic micelles. Therefore, the non-polar environment of the TX-100 micellar interior and similarly, also of other non-ionic micelles may be preferable to incorporate the hydrophobic solubilizate molecules[11]. On adding ethanol to solubilizate, the fluorescence intensity attained a limiting value. Then it decreased on further increasing the concentration of ethanol accompanied by a blue-shift. This may be attributed to the protic nature of the solvent. Here hydrogen-donor solvent-interactions take place between the solute and solvent. The absorption spectra of the compound are less affected on adding surfactants as compared to the fluorescence spectra. A small blue-shift occured in the absorption maxima of lumazine, on adding surfactants. This may be because of the difference in solvation energy of the solute in the ground state and the excited state.

Quantitative aspects

The magnitude of Stokes' shift depends on several factors. The large Stokes' shift values of lumazine are due to hydrogen bond formation between the solute and the solvent in the ground state. This bond breaks following excitation to S_1 but reforms following proton transfer. The calculated Stokes' shift data are given in Table-2.

TABLE-2
STOKES' SHIFT DATA FOR LUMAZINE AT ROOM TEMPERATURE
λ_{em} = 470 nm, λ_{ex} = 335 nm, P.M. Gain = 3, Sensitivity range = 0.3

Conc. (M)	Relative fluorescence intensity	λ_{ex} (nm)	λ_{em} (nm)	P.M. Gain	μ	Stokes' shift (cm^{-1})	$k_f \times 10^3$ conc./mol
5×10^{-6}	53	366	462	3	0.3	5677	10600
1×10^{-5}	46	330	471	3	0.3	9071	4600
5×10^{-5}	63	330	471	3	0.1	9071	1260
1×10^{-4}	36	370	460	2	0.1	9075	360
1×10^{-3}	96	370	460	3	0.1	9098	96

The calculated quantum yield values (ϕ_f) are in good agreement with the fluorescence intensity. The quantum yield values increased on increasing the surfactant concentration and was found to be highest for DSSS added solubilizate solution (Table-3).

TABLE-3
ABSORPTION MAXIMA (λ_a), MOLAR ABSORPTIVITY (log ε), FLUORESCENCE MAXIMA (λ_f) AND QUANTUM YIELD (ϕ_f) VALUES OF LUMAZINE AT DIFFERENT CONCENTRATIONS OF DSSS
λ_{em} = 470 nm, λ_{ex} = 335 nm, P.M. Gain = 3, Sensitivity range = 0.3

Conc. DSSS used (M)	λ_a (nm)	log ε (dm^3 mol^{-1} cm^{-1})	λ_{em} (nm)	ϕ_f Lumazine
0.000	325	2.44	470	0.521
0.005	325	2.44	470	0.525
0.030	325	2.44	470	0.528
0.050	325	2.49	460	0.558

These high quantum yield values in micellar medium may be attributed to: (i) lesser effects of other deactivation process which compete with fluorescence, (ii) that the rate of non-radiative processes are less in micellar medium in comparison to those in aqueous medium, (iii) due to the absorption of the fluorophore at the micellar surface, which decreases, the rate of collision of the fluorophore by water molecules.

Conclusion

It is apparent that lumazine is not fully solubilized in 1 % ethanolic medium. The suspended particles of lumazine solubilize on addition of surfactants. Thus there appears to be straight forward correlation between solubilizing action and enhancement in fluorescence emission. Thus, the present analysis indicate that during solubilization of a solubilizate into the surfactant system, the incorporation of the solubilizate influences the balance of the favourable and unfavourable conditions guiding micellization. The solubilization process finds extensive application in the industrial, pharmaceutical and biochemical fields. The present kind of analysis is expected to be useful in these fields.

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