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Fluorescence behavior of ethidium bromide in homogeneous solvents and in presence of bile acid hosts

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ABSTRACT

Photoluminescence behavior of ethidium bromide (EB) was studied in homogeneous solvents as well as in presence of bile acid surfactants. A quantitative analysis based on Kamlet–Taft equation toward the contribution of different solvatochromic parameters like solvent polarizability (π^*), hydrogen bond donor (α) and hydrogen bond acceptor (β) ability, on EB spectral properties reveals that though fluorescence quantum yield and lifetime do not show any straight forward correlation individually, the substantial decrease in these quantities is due to the increase in total nonradiative decay rate in polar protic medium. The interaction of EB with bile acid hosts gives rise to large increase in fluorescence intensity due to shielding of the probe in less hydrogen bonding environment. Global analysis of the fluorescence decay behavior indicates that more than 50% of the fluorophores get sequestered inside the micellar sub-domain with an apparent binding constant of $\sim 10^3\ M^{-1}$.

1. Introduction

Ethidium bromide (3,8-diamino-5-ethyl-6-phenyl phenanthridiniumbromide, EB) is a cationic dye and an antiviral drug that interacts strongly and specifically with both double stranded DNA and RNAs. They bind DNA and RNA via intercalative mode between adjacent base pairs and elongate of double helical structure [1–8]. Because of the appreciable change in fluorescence intensity upon binding, EB is widely used as fluorescence bio-marker. More than 10 fold increase in the emission intensity and decay time is observed when EB binds to DNA in comparison with the bulk water [9]. There are numerous studies on EB with polynucleotide; however, surprisingly, reports on the investigation of EB fluorescence enhancement upon binding with DNA as well as possibility to use it as fluorescence probe in microenvironments other than DNA are relatively scarce. To understand the solvent dependent photophysics and fluorescence intensity change in EB, Olmstead and Kearns [10] concluded that the amino proton of the ethidium ion becomes quite acidic in one of its quinoid structures (see Chart 1), even in the ground state, which further increases in the excited state so that EB donates one of the amino protons to the surrounding solvent environment. This excited state proton donation process is the most dominant relaxation pathway and responsible for the low fluorescence yield in polar/protic solvents. Based on these findings and further investigation, Pal et al. [11] recently suggested that the nonradiative pathway of EB strongly depends on the hydrogen bond accepting (HBA) basicity of the solvent rather than the polarity.

Research in the field of bile acids (BAs) has received considerable interest in recent times both from their biochemistry and physiology [12–14]. BAs are steroid acids found predominantly in the bile of mammals. Bile acids aid in fat absorption and modulate cholesterol levels. They are produced from cholesterol in the liver and are stored in the gall bladder. Gall bladder contraction with feeding releases bile acids into the intestine. Bile acids undergo enterohepatic circulation, i.e. they are absorbed in the intestine and taken up by hepatocytes for re-excretion into bile. Measurement of bile acid concentrations is, therefore, a good indicator of hepatobiliary function, but is not specific for the type of underlying diseases.

The most important bile acids in humans are cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) (Scheme 1). All bile acids consist of two connecting units, a rigid steroid nucleus and a short aliphatic side chain [15]. The steroid nucleus of BAs has the saturated tetracyclic hydrocarbon perhydrocyclopentanophenanthrene, containing three six-member rings and a five member ring. The one or more α-oriented hydroxyl groups of BAs are put on the concave surface (α-face) of the steroid backbone and the methyl groups are positioned on the opposite convex side (β-face). Free molecules of BAs, normally cylindrical shapes of 20 Å long with a radius of about 3.5 Å, have a great surface activity and inclination to the formation of large aggregates, owing to the difference in orientation of hydrophilic and hydrophobic groups on the steroid ring systems.

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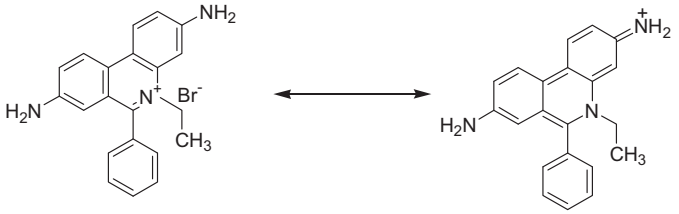


Chart 1. Structures of different resonance forms of ethidium bromide (EB).

In contrast to the spherical nature of the pseudo-particle formed due to the self aggregation of linear surfactant molecules like sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB) and/or triton-X 100 (TX-100), the BAs consist of a rigid steroid backbone giving rise to the concave side with polar hydroxyl group (α-face) and the methyl groups in the convex side (β-face). Aggregation of BAs in aqueous solution is due to hydrophobic interaction of the apolar β-faces of steroid backbones with possibility of further aggregation through hydrogen bonding in the α-faces [16]. This unique arrangement based on facial amphiphilicity renders a different aggregation pattern in BAs (Scheme 1) unlike the conventional surfactants; where, the micellar structure is mostly approximated to be spherical originated from the mutual arrangement of the head and tail groups with different hydrophobicity [17–20]. Therefore, to study the interaction of EB with aggregated BA host seems interesting and can be approximated as a biological model to mimic the DNA intercalation process. In this work we report the results of detailed steady state and time-resolved study on the fluorescence behavior of EB in several homogeneous environments consisting of neat and mixed solvent systems, and also in presence of BAs like CA, DCA and CDCA.

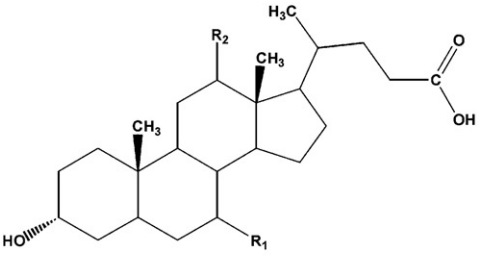
Table 1
Solvent parameters.

No.	Solvent	$\Delta f(\varepsilon,n)$[a]	$E_T(30)$[b]	α[c]	β[c]	π^*[c]
1	1,4-Dioxane	0.03	36	0	0.37	0.55
2	Ethyl acetate	0.19	38.1	0	0.45	0.55
3	Terahydrofuran	0.21	37.4	0	0.55	0.58
4	Dichloromethane	0.22	40.7	0	0.1	0.81
5	1-Butanol	0.26	49.7	0.84	0.84	0.47
6	Dimethyl sulfoxide	0.26	45.1	0	0.76	0.1
7	Dimethyl formamide	0.27	43.2	0	0.69	0.88
8	1-Propanol	0.27	50.7	0.84	0.9	0.52
9	Isopropyl alcohol	0.27	48.4	0.76	0.95	0.48
10	Accetone	0.28	42.2	0.08	0.48	0.71
11	Acetonirile	0.3	45.6	0.19	0.4	0.75
12	Methyl alcohol	0.31	55.4	0.98	0.66	0.6
13	Water	0.32	63.1	1.17	0.47	1.09

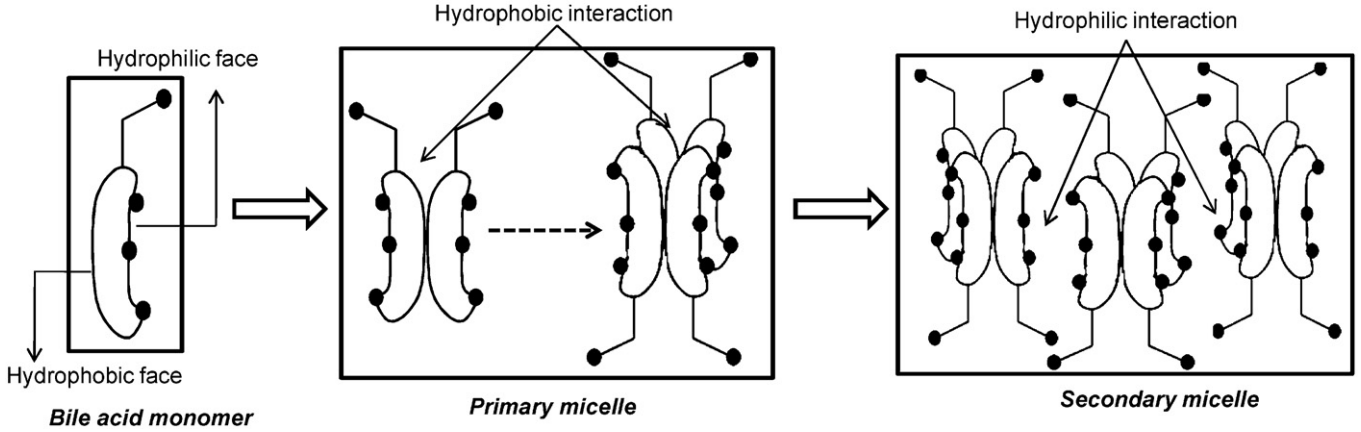
[a] Polarity parameter $\left(=\frac{\varepsilon-1}{2\varepsilon+1}-\frac{n^2-1}{2n^2+1}\right)$ where, solvent dielectric constant and refractive indices are represented by ε and n, respectively.
[b] Reichardt solvent parameter.
[c] Kamlet–Taft solvent parameters.

2. Experimental

Analytical grade ethidium bromide (EB) was procured from Sisco Research Laboratories (SRL), India (product no. 054817) and the purity was checked by chromatographic techniques before use. The organic solvents used were of spectroscopic grade (>99.5%) as received from Alfa Aesar and, in some cases, from Aldrich Chemical Company. The solvents and the corresponding solvatochromic parameters are listed in Table 1. The bile acids viz. CA, DCA and CDCA were all obtained from Sigma–Aldrich Chemical Pvt. Ltd. (product no. C1129, D2510 and C9377, respectively) and used as received in freshly prepared aqueous buffer solution of pH = 9.2 by dissolving one buffer tablet obtained from Qualigens fine chemicals (a division of GlaxoSmithkline Pharmaceuticals Ltd.), India in



Bile acid (BA)	R_1	R_2
Cholic Acid (CA)	α-OH	α-OH
Deoxycholic acid (DCA)	H	α-OH
Chenodeoxycholic acid (CDCA)	α-OH	H



Scheme 1. Structures of different bile acid (BA) used in this study. The two step aggregation pattern of BA monomers adapted from reference [33] has also been demonstrated in the lower panel.