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Environmental Effect on the Laser-Excited Fluorescence Spectra of Methylene Blue and Methylene Green Dyes

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Abstract

A laser-induced fluorescence investigation of the phenothiazine dyes Methylene Blue (MB^+) and Methylene Green (MG^+) is reported. To study the effect of environment, fluorescence spectra of these molecules in a variety of solvents with different polarity and various concentrations of an anionic surfactant were measured. The polarization and anisotropy analysis showed that there is no abrupt behavior except at the surfactant concentrations corresponding to the critical micellar concentration.

Introduction

The fluorescence probe is a useful tool for the investigation of micro-environments of model systems as the excited states are sufficiently long lived to interact with their immediate environment prior to their decay. Surfactant molecules which contain a polar head group and hydrophobic chain are capable of producing supramolecular assemblies that possess properties distinct from those of the individual monomeric molecules prior to aggregation [1]. Upon dispersion in water hydrocarbon segments in a surfactant tend to minimize water exposure and thus prefer to self aggregate and organize closely. The force that drives this aggregation is entropic in origin and facilitates the release of structured water molecules [2].

Methylene Blue (MB^+) is used as a stain for elastic fibres and connective tissues and a counterstain after basic fuchsin for staining tubercle and leprabacilli in mammalian tissues [3]. It is also used as a component of tetrachrome stain for differentiation of blood corpuscles. The potential use of Methylene Blue (MB^+) and Methylene Green (MG^+) dyes in light energy conversion systems warrants detailed investigation on the elementary process induced by light excitation. Inspired by the technological applications of these dyes, a laser-induced fluorescence investigation was carried out in various environments such as solvents and surfactants to study the dye molecules location and the nature of the interactions with such systems.

Experimental

The purest commercially available MB^+ and MG^+ dyes from Sigma and the anionic surfactant sodium dodecyl sulfate (SDS) from BDH were used as received. The solvents acetone, isopropanol, methanol, ethanol, dimethylformamide (DMF) and acetonitrile were spectroscopic grade. Double distilled water was used in the preparation of aqueous solutions.

The requisite volume of aqueous stock solution of MB^+ (0.12 mM) and MG^+ (0.5 mM) were prepared and mixed with various solvents in the volume ratio of 1:4. About 10 mL of the mixture of dye solution and SDS were prepared by varying the SDS concentrations from 2 to 30 mM. The mixtures were thoroughly mixed before sampling for fluorescence measurements.

The experimental set up used for the laser-induced fluorescence measurements is described in detail in [4]. The 514.5 nm green line from a Spectra Physics (2020-04) argon-ion laser was used as the excitation wavelength and the laser power at the sample was always maintained at 70 mW. All the above measurements were carried out at room temperature.

Results and discussion

The dyes (MB^+ and MG^+) used in these studies are cationic and their molecular structures are shown in Figure 1a and b respectively. Sodium dodecyl sulphate (SDS) is an anionic surfactant ($C_{12}SO_4$) and possesses 12-carbon alkyl chains and oppositely charged head groups. It is well known that the surfactant forms nearly spherical micellar aggregates at its critical micellar concentration (cmc) and such aggregates contain 100–150 surfactant molecules each and have a radius of the order of around 30 Å [5].

The incorporation of probe molecules into aqueous micelles effectively reveals such parameters as the critical micellar concentration, the roughness of the micelle surface

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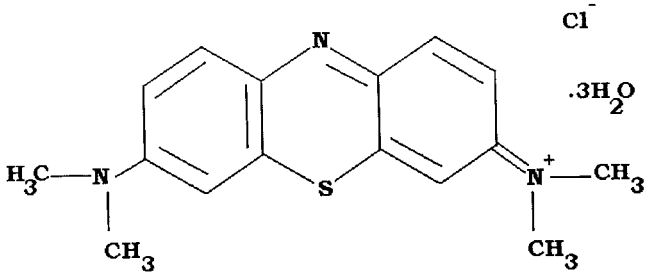

Figure 1a. Structure of Methylene Blue MB^+.

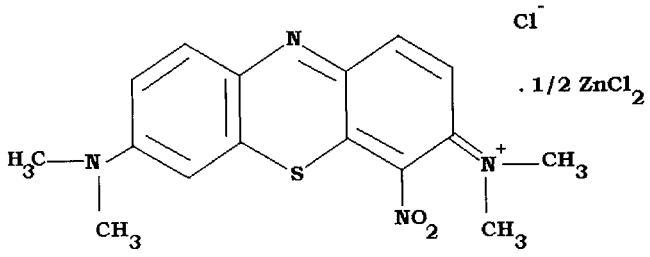

Figure 1b. Structure of Methylene Green MG^+.

and the degree of water penetration into these surfactant aggregations. The excitation wavelength of the laser was fixed at 514.5 nm and emission spectra in the region 630–750 nm were studied.

It was noticed that both dye molecules exhibit appreciable fluorescence intensities in various solvents (Figures 2 and 3). There was no appreciable shift in the emission maxima with the variation of the polarity of the solvents and also with the SDS concentration. However, there may be changes in the fluorescence intensities and they have been used in the determination of the cmc of surfactants [6–8]. Such features are expected for MB^+ and MG^+ dyes in the ~8 mM region of SDS concentration and they are related to the formation of surfactant micelles in which the dye molecules are incorporated. As there is no shift in the emission maxima, the dye molecules are not moved from the polar aqueous phase to the less polar micellar region. These dye molecules are most likely to reside at the water-surfactant interface, i.e., on the surface of a micelle on a polar disposition.

These dye molecules at the interface of SDS micelles exhibit emission at a longer wavelength consistent with the polar microenvironment. The enhanced polarity of the surroundings can be attributed to greater water penetration of the micelle interface. The variable accessibility of water to dye molecules at the interface can be further supported by an estimate of the surface area per head group. The larger aggregation number of SDS micelles suggests a smaller effective surface area per head group. Hence the amount of void space around the SDS head group is higher thereby providing a higher degree of water permeability. This causes a more polar environment for these dye molecules near the SDS head groups

The measurement of fluorescence polarisation or anisotropy is a good measure of the motional dynamics of the molecules. It also provides information on processes such as energy transfer between various molecules in the sample. The environment surrounding the fluorophore can affect the

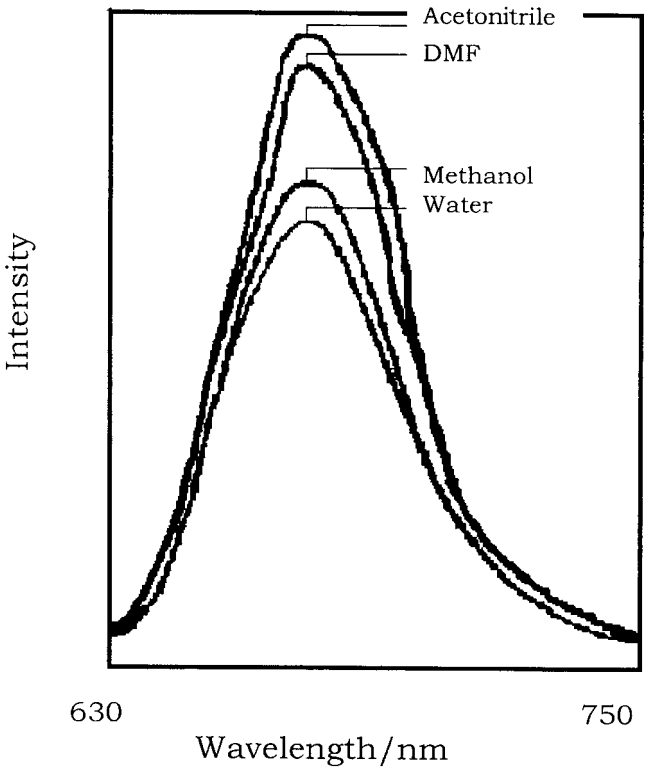

Figure 2. Emission spectra of Methylene Blue.

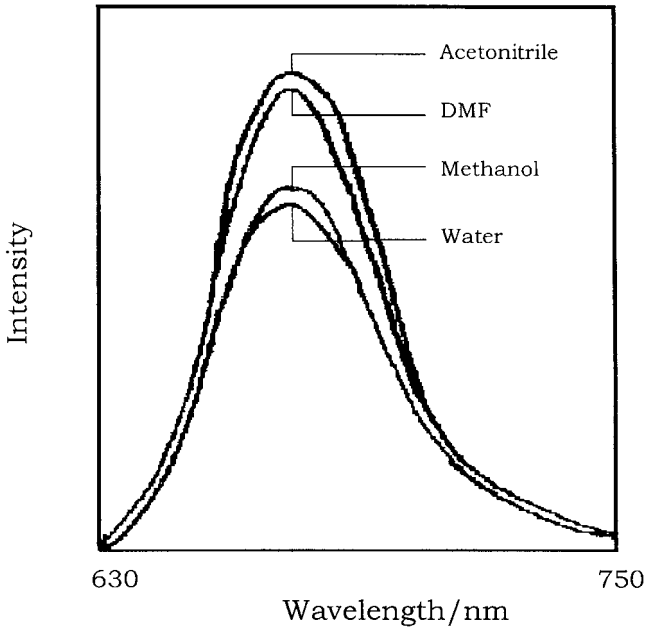

Figure 3. Emission spectra of Methylene Green.

intrinsic polarization P_0 and the anisotropy values r_0. So the fluorescence intensities of the emitted light polarized parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the polarisation of the exciting light were recorded for various SDS concentration in solvents of different polarity.

The fluorescence polarization values of the MB^+ dye in various SDS concentrations are given in Figures 4 and 5 for the MG^+ dye. From the figures it is observed that there is a sharp variation in the polarization values at the cmc of SDS (8 mM). The anisotropy values of the MB^+ and MG^+

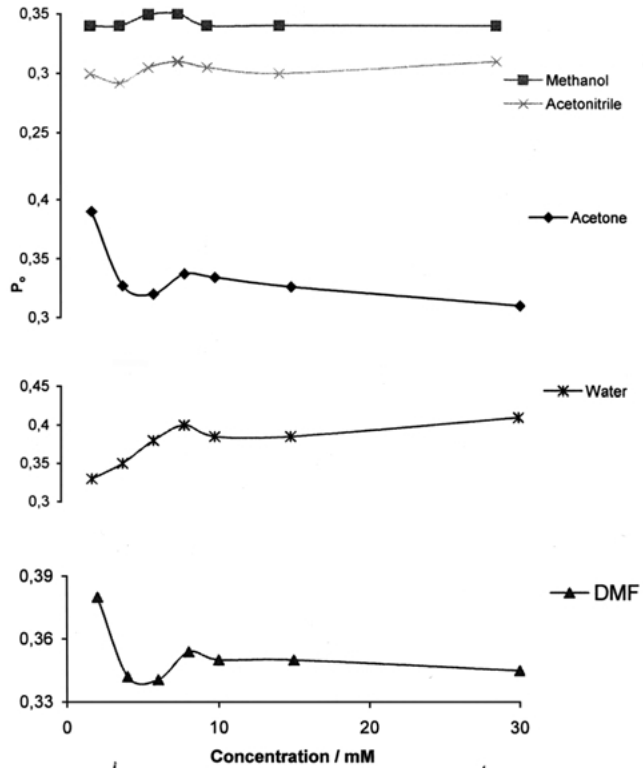

Figure 4. Behavior of the fluorescence polarization P_0 of Methylene Blue in different solvents for various SDS concentrations.

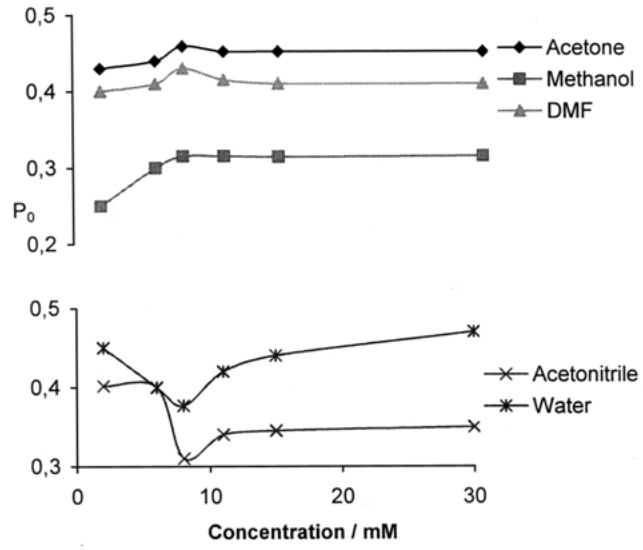

Figure 5. Behavior of the fluorescence polarization P_0 of Methylene Green in different solvents for various SDS concentrations.

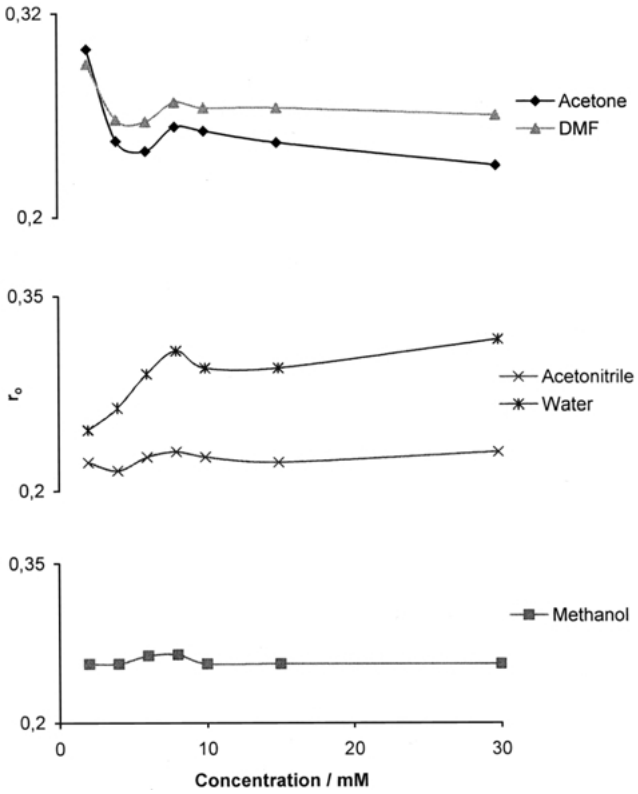

Figure 6. Behavior of the polarization anisotropy r_0 of Methylene Blue in different solvents for various SDS concentrations.

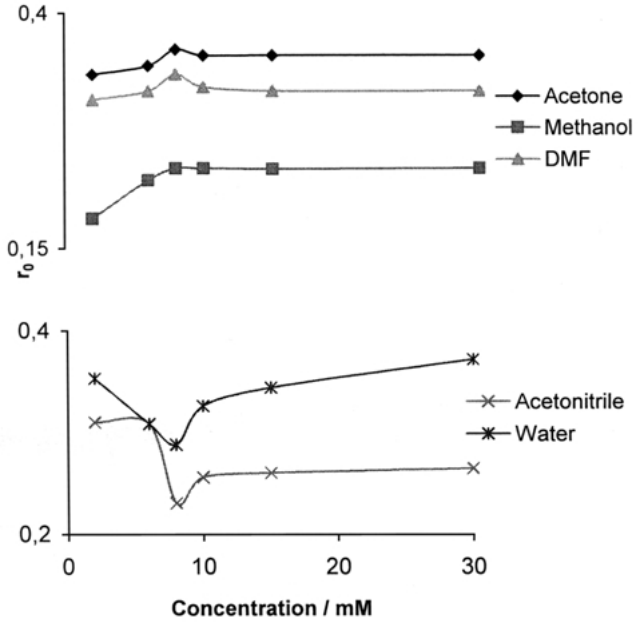

Figure 7. Behavior of the polarization anisotropyr_0 of Methylene Green in different solvents for various SDS concentrations.

dyes measured in various SDS concentrations and are given in Figures 6 and 7 respectively. Further, it is observed that there are no significant variations for the anisotropy values on increasing the SDS concentrations above the cmc value.

At the cmc the dye molecules are incorporated into the surfactant micelles and the dye molecules are placed in the more polar aqueous phase. Since they experience a high polar environment, there is an abrupt change in the polarization value.

Conclusion

Both the dyes MB^+ and MG^+ exhibit appreciable fluorescence intensities in all the solvents. The variation of SDS concentrations has a significant effect on the fluorescence polarisation and anisotropy values in both cases. However, there is no appreciable shift in the emission maxima on varying the polarity of the solvents and the SDS concentra-

tion. This indicates that the dye molecules are most likely to reside at the water-surfactant interface.

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