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Study between solvatochromism and steady-state and time-resolved fluorescence measurements of the Methylene blue in binary mixtures

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ABSTRACT

In this work, the study on the influence of binary mixtures of solvents (water-acetonitrile, water-ethanol and water-glycerol) upon the spectroscopic properties of methylene blue (MB) was done. In addition, the photophysical characterization of the MB in different concentrations in the solvent mixtures was done. In the mixtures, the increase in the quantity of water has decreased the fluorescence quantum yield together with other photophysical alterations. The studies of time-resolved fluorescence have demonstrated a first-order decay, with lifetimes between 328 and 550 ps. These values increase as the organic solvent proportion is increased. The results have shown a direct relationship between the viscosity and the rotational lifetime, correlating with the interference in the processes of deactivation of the excited state, which are slower in media with higher viscosity. The conformation of the clusters in the binary mixtures was also identified as a key factor to determine the results obtained in this work.

1. Introduction

Recently, the interest in the phenothiazinium compounds has increased significantly due to their application in several areas, such as Photodynamic Therapy (PDT), biosensors, photo initiators of vinylic polymerization, biological markers and DNA-labels [1–3].

The mechanism of the photophysical processes is based on the light absorption, in which the photosensitizer molecule (PS) is excited to the singlet electronic state ($S_0 \rightarrow S_1$) and, by intersystem conversion to the triplet electronic state ($S_1 \rightarrow T_1$). The PS in the triplet state can transfer energy to the molecular oxygen ($^3O_2$) and generate the singlet oxygen ($^1O_2$), (Type II mechanism) [4,5].

The methylene blue (MB) has been used in the treatment of methemoglobinemia [6], malaria [7], carbon monoxide or cyanide poisoning [8], against Gram-positive as well as Gram-negative bacteria [9,10] and in PDT [11]. The MB is a phenothiazinium compound that presents a planar heterocyclic aromatic structure (Fig. 1), being characterized spectroscopically by an intense electronic absorption band in the red spectral region (~664 nm) [4].

The MB is very soluble in ethanol and water. It has quantum yield of singlet oxygen formation in the order of $\Phi\ ^1O_2 = 0.5$ [12], with quantum yield of fluorescence emission of 0.04 in water [4]. The MB has low reduction potential and a moderated toxicity. In addition, it presents high photodynamic efficiency in the death of some types of cancer cells [4,13–16]. Studies showed that the MB molecules can generate self-aggregates in the presence of several agents, such as cell organelles, organic solvents and water, which affect the photophysical properties of the chemical system. The perturbations can decrease the fluorescence and the quantum yield of the singlet oxygen, affecting the photodynamic action of the MB [17].

The MB is employed frequently as optical sensor in biophysical systems due to its high molar absorptivity coefficient that allows

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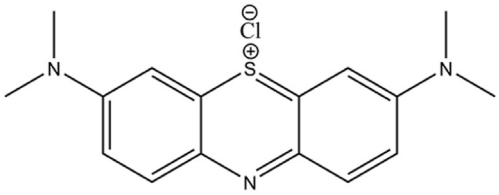

Fig. 1. Molecular structure of the phenothiazinium compound Methylene Blue (MB).

this dye to be used in solutions with low concentration [2]. The MB, for example, is a suitable model compound to study interaction of sensors with DNA [3]. On the other hand, in the studies that use fluorescent sensors in homogeneous media, the effects of the solvents and the solvent mixtures upon the various physico-chemical phenomena that affect the chemical action of a sensor can be elucidated through the analysis of the influence of a series of different parameters. These parameters are, for example, acidic-basic behavior, polarizability, density, viscosity, dielectric constant, numbers of acceptor-donor of electrons, solvent polarity, surface tension, refraction index, among others [18,19]. In the case of the binary aqueous solutions, distinct interactions can occur between water and organic solvents, depending on the intrinsic properties of these solvents.

Considering several applications of the MB, it is important to investigate the changes in its photophysical properties in different binary solvent mixtures, including water and organic solvents such as ethanol (polar-protic), acetonitrile (polar-aprotic) and glycerol (polar-protic). These information are important to the understanding of the photophysical-spectroscopic properties of the MB in these chemical environments.

In particular, the characterization of the excited state is very important to explain the chemical reactivity in the energy transfer and/or electronic transfer reactions, whose processes are typically associated to PDT. In the present work, the studies focused on the influence of the solvent in the electronic absorption of the dye. The fluorescence quantum yield and the fluorescence lifetime of the MB were carried out in different solvent mixtures.

2. Materials and methods

The MB and the solvents were obtained from Sigma–Aldrich and J.T. Baker, respectively. The MB was analytical grade and was used without further purification. The solvents of analytical degree used in this work were ethanol (EtOH), acetonitrile (ACN), glycerol (Gly) and water (Milli-Q, Millipore system).

At a first step, it was prepared a MB stock solution (2.5×10^{-3} mol L^{-1}) in high purity water. Subsequently, the MB solutions were prepared through successive dilutions of the stock solution (v/v), in different solvent mixtures (water-acetonitrile, water-ethanol and water-glycerol). The resulting MB concentrations were 1.0×10^{-6}, 5.0×10^{-6} and 3.5×10^{-5} mol L^{-1}.

The electronic absorption spectra were obtained by using a UV–Vis spectrophotometer (Perkin Elmer Lambda 25 UV-VIS spectrometer). The fluorescence measurements were carried out by using a fluorescence spectrophotometer (Perkin Elmer LS 55 fluorescence spectrometer). The excitation wavelength (λ_{exc}) used was 612 nm. Each spectrum was recorded at least thrice, at 140 nm min^{-1} and with slit width of 5 and 4 nm.

The fluorescence quantum yield (Φ_F) was determined to the different solvents using cresyl violet dissolved in methanol as a reference solution ($\Phi_F = 0.54$) [20], according to the following equation:

$$\Phi_{FS} = \Phi_{FS}[(S_{FS}S_{AS})/(S_{FR}S_{AR})]\left[n_S^2/n_R^2\right] \quad (1)$$

where Φ_F is the quantum yield, S_A is the integrated absorbance band area and S_F is the integrated fluorescence emission band area, n is the solvent refractive index. Subscript S and R refer to the sample and the reference (standard), respectively [21]. All fluorescence measurements were conducted for dilute solutions with an absorbance near 0.1.

The fluorescence anisotropy was measured in the Hitachi spectrofluorometer model F-7000, in which a xenon lamp acts as a source of excitation light. This equipment has a thermostatized sample holder by a circulating water bath. The polarizing films were added in the beams of excitation and emission for performing anisotropy measurements of steady-state ($\lambda_{exc} = 290$ nm and $\lambda_F = 680$ nm).

The fluorescence anisotropy was evaluated in the conventional way from Ref. [22,23].

$$r = \frac{i_{\parallel} - i_{\perp}}{i_{\parallel} + 2i_{\perp}} \quad (2)$$

where $i_{\parallel}$ and $i_{\perp}$ are the parallel and perpendicular fluorescence intensities with respect to the polarization direction of the incident radiation.

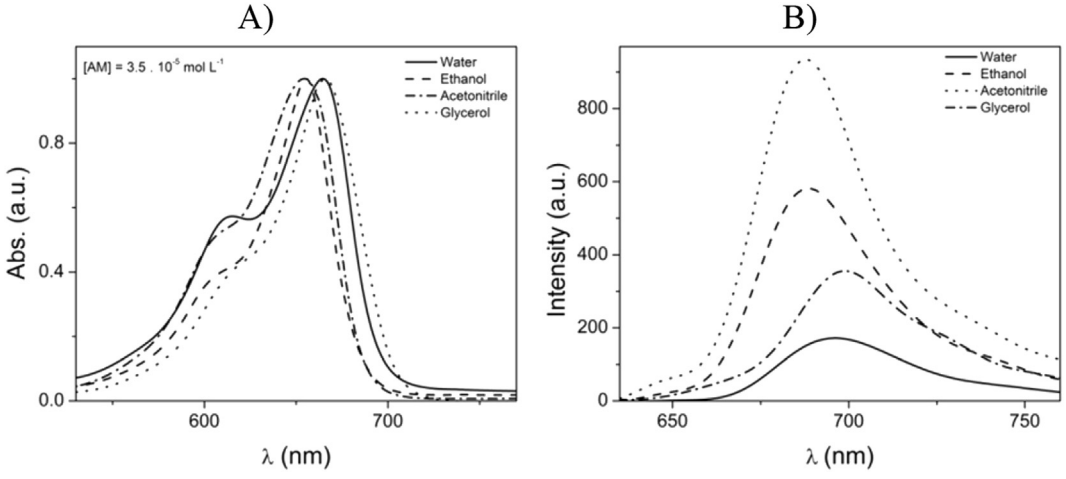

Fig. 2. Normalized electronic absorption spectra (A) and fluorescence emission spectra (B) of the MB (3.5×10^{-5} mol L^{-1}) in different solvents.