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Full Paper

The Methylene Blue Self-aggregation in Water/Organic Solvent Mixtures: Relationship Between Solvatochromic Properties and Singlet Oxygen Production

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Abstract: In this work is presented a spectrophotometric investigation focused on the solvatochromic effects upon Methylene Blue (MB). Measurements were carried out in four different water/organic solvent mixtures: low polarity protic solvent (ethanol), polar non-protic solvent (acetonitrile), highly polar protic solvent (glycerol), and non-polar solvent (dioxane). The results showed that the photophysical behavior of MB is highly affected by self-aggregates formation at 80% of water/organic solvent blends. Besides polarity, the protic character and the coordinating properties of the solvent molecules are the key parameters for its photophysical behavior, since the sulfur atom of the cationic structure can act as a coordination center due to its Lewis acid character. In fact, water and acetonitrile coordinating properties have proved to be important to determine MB interactions intensity and its spectroscopic properties as singlet oxygen emission. It was observed that an increase of the amount of the organic solvent resulted in an enhancement of the singlet oxygen emission intensity. The presence of the water increases the dielectric constant of the medium and favors the self-aggregation process. Besides, the water molecules can act as a quencher and it decreases the quantum yield of the fluorescence of MB.

Keywords: fluorescence; methylene blue; self-aggregation; solvatochromic properties; solvent effect

1. INTRODUCTION

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Phenothiazine compounds as Methylene Blue (MB), Figure 1, and dyes from the same class, such as Azure A, Azure B and Thionine, have been extensively used for more than a century for various applications, especially in health [1]. MB is cytotoxic in the dark, being recognized by its antimicrobial action, which increases in the presence of oxygen and by the incidence of radiation with wavelength capable to promote its electronic absorption [2, 3].

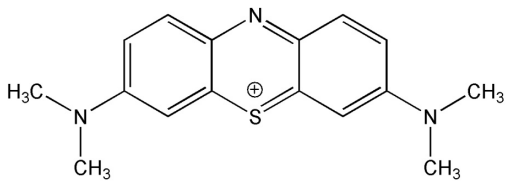

Figure 1. Structure of Methylene Blue (MB).

Besides its antimicrobial activity, MB has a variety of applications being used in dyeing of cotton, wool and paper, as temporary hair dyes, etc. Due to its strong adsorption capability on solid supports MB often serves as a model compound for the removal of dyes and organic contaminants from aqueous solutions [3].

MB presents a quantum yield of singlet oxygen ($^1O_2$) formation around 0.5 [4], with a low reduction potential, intense light absorption within the phototherapeutic window for Photodynamic Therapy [5]. Also, it displays a high photodynamic efficiency causing apoptosis of cancer cells. Currently, the use of MB has been authorized in Europe for disinfection of blood plasma, due to its efficiency in photodynamic inactivation of microorganisms and viruses [6-9]. However, its use as photodynamic agent in Photodynamic Therapy (PDT) is not very widespread since MB is reduced by NADH and FADH2 cells to the leuco form (LMB), photochemically inactive, although its efficacy in the treatment of certain tumor types has been demonstrated.

Since MB presents characteristics desirable for phototherapeutic agents, for example a good capability to sensitize the production of singlet oxygen and, despite its limitations of use, has proven to have photodynamic activity in certain physiological systems. In order to get more insight about this dye, in this study we investigate the role of the solvent (solvent mixtures of different molar fractions involving water and ethanol, acetonitrile, glycerol and dioxane) on the spectroscopic properties of MB. For this, experiments involving measurements of fluorescence, UV-Vis absorption spectroscopy and of the quantum yield of singlet oxygen ($^1O_2$) production were performed. The association of different solvents can be good alternative in order to optimize the physical-chemical behavior of phototherapeutic agents. Although MB possesses a positive charge, it has a tendency to form dimers, trimers or H-type aggregates [7-10] where self-quenching processes take place [8-12].

Often, treatment protocols require unusual preparation methods or conditions that may have many distinct characteristics of the preferred conditions. For example, aqueous solutions of MB in concentration around 2×10^{-5} mol L^{-1} is in monomeric form, but its use in topical treatments requires concentrations higher than 6×10^{-2} mol L^{-1}, in which self-aggregation is significant [10].

The photophysical properties of a dye in solution depend on the nature of its environment. The term solvatochromism is used to describe changes in UV visible absorption band following a change in the polarity of the medium. When absorption spectra are measured in solvents of different polarity it is found that not only the position but also the intensity and shape of the absorption band can vary, depending on the nature of the solvent [9-11]. In this way, MB usually show strong polarity dependent Stokes shifts, large changes in dipole moments on excitation, and very high fluorescence quantum yields. Thus, the study of the interaction of MB with different solvents having different physical-chemical properties forms an important subject that plays a significant role in the photophysics of the excited states. Therefore, it is important to investigate the processes of MB self-aggregation present in solvent mixtures and/or interaction with biomolecules [8].

2. MATERIAL AND METHODS

MB (Aldrich, 85 %) was previously recrystalized in a mixture of acetone and methanol. The solutions were prepared by using water/solvent binary mixtures (solvent = acetonitrile, ethanol, dioxane and glycerol) with different molar fractions. MB concentration was kept constant and equal to 3.13×10^{-4} mol L^{-1}. All measurements were performed at 298 ± 2 K. The organic solvents used were of spectroscopic grade. The water used was of high purity obtained by using a Millipore filtrating system.

Fluorescence spectra were recorded by using a

Jobin-Yvon Spex FluoroMax-2 and a TECAN Infinite M200 spectrofluorimeter, using the absorption at 640 nm as excitation wavelength. A 10 mm optical length quartz cuvette for fluorimetry was used in these measurements, which were done in a front face configuration mode. The electronic absorption spectra were recorded by using a Perkin Elmer Lambda 25 UV-VIS spectrophotometer.

The measurements of quantum yield of singlet oxygen ($^1O_2$) production were performed using spectrometer (Edinburg Analytical Instruments). The samples were excited with a Laser Line continuous laser with emission at 665 nm. The emission of $^1O_2$ was detected using a Hamamatsu photomultiplier model R5509 cooled at 193K. The data were analyzed by using the LP900 software from Edinburg Analytical Instruments.

3. RESULTS AND DISCUSSION

Solvent effect on the monomeric peak of Methylene Blue.

Changes in absorption intensity as a function of the solvent mixtures can be observed in the absorption spectra. Similar behavior occurs with fluorescence spectroscopy data [12]. The spectra exhibit an intense absorption band at 664 nm (π-π^*) and a shoulder at 610 nm corresponding to the 0-1 vibronic transition [13,14]. The solvatochromism observed was evaluated through solvent parameters shown in the Table 1, which in order of increasing polarity. A bathochromic shift ($\Delta\lambda_M$) (Table 1) can be seen comparing the wavelength of maximum absorption obtained in the solvents studied with the one reported for MB in the gas phase (λ= 582 nm) [15]. In the Table SI (Support Information) is presented the solvatochromic effect on MB as a function of the solvent polarity. These bathochromic shifts could be related to the solvation, since the solvated MB is positively charged, possessing high dipole moment and several groups with pairs of non-ligand electrons, including nitrogen atoms [11]. Generally, the solvent effect on the position of the absorption bands of chromophores could be associated to the polarity of the solvent [16]. However, a careful analysis of Table 1 shows that, in general, the shifts ($\Delta\lambda_M$) do not follow strictly the order of the dielectric constants, since the effect in dioxane is almost the same as it is observed in pure water. Thus, the observed changes cannot be only attributed to the dielectric constant but also to solvent polarizability, since π^* orbitals are much more polarizable than π orbitals, and to the specific solvent effects [17].

Table 1. Solvatochromic effect on MB.

Solvent	Wavelength / nm	$\Delta\lambda_M$ / nm	ε	α
Water	664	82	80.2	0.206
Glycerol	667	85	46.5	0.282
Acetonitrile	658	76	36.4	0.212
Ethanol	656	74	25.3	0.222
Dioxane	661	79	2.2	0.254

$\Delta\lambda_M = \lambda_{solvent} - \lambda_{gas\ fase}$; ε = dielectric constants and α = polarizability [15].

In general, increase in the dielectric constant of the solvent can result in the stabilization of the two orbitals involved in the electronic transition (HOMO and LUMO). Therefore, the red shift observed to the monomeric form of MB is due to the higher stabilization of the LUMO (π^*) when compared with the HOMO (π) [9]. This profile is observed increasing the polarity of the solvent from ethanol to water.

Among the solvents studied, those that have caused higher changes in the $\Delta\lambda_M$ were water and glycerol. It is known that both solvents have the highest dielectric constant and the presence of polar hydroxyl groups in their structures. Furthermore, they are protic solvents, which favor the presence of hydrogen bonds with significant intensity. These hydrogen bounds involve solvent-solvent or solute-solvent interactions, which present hydrogen bounds with an intensity that is strongly dependent on the solute structure. As a consequence of the great polarity of the water and glycerol solvents, depending on the physical-chemical properties of the solute (such as polarity, availability of non-ligand pairs of electrons (Lewis basicity) and protic or non-protic character), it can be observed very pronounced solvatochromic effects, such as the above mentioned absorption band red-shift of the electronic transitions π-π^* [18]. In this context, it is interesting to perceive that even possessing higher dielectric constant, water presents less efficiency on the stabilization of the transitions involved in MB than the glycerol. The molecular structure of glycerol, which includes three OH groups favors a more efficient interaction with π^* orbitals of the MB dye, resulting in a higher red-shift to the monomeric form of MB than the observed in aqueous solvent, in spite of the higher dielectric constant of the water.

Indeed, the stereochemical influence seems to be more important, considering the blue-shift of approximately 3 nm observed when glycerol is exchanged by water. This result could be generated by multiple hydrogen bounds between the MB molecule and the aqueous solvent. In this way, stereochemical factors (symmetrical/spatial factors) associated to a higher size of the glycerol molecule in relation to the water molecule must favor a more effective interaction of its hydroxyl groups with the π^* orbitals of MB. This implies in a higher red-shift to the glycerol as a function of the occurrence of several types of interactions in the MB-glycerol system. Furthermore, in solvents as dioxane and acetonitrile, which are non-protic solvents, it is not observed the spectroscopic effects of this kind of interaction with the MB molecules. This type of solvation must occur only for dipole-dipole and/or ion-dipole interactions.

Water/organic solvent mixtures

The addition of low contents of water to the organic solvents also resulted in a solvatochromic effect, inducing a bathochromic shift (red shift) that is related to the peak of MB monomer in comparison with the spectroscopic signals observed in the respective pure organic solvents. The shifts were of approximately 3, 6 and 8 nm to dioxane, acetonitrile and ethanol, respectively. As mentioned the presence of water increases the dielectric constant of the media, which stabilizes the LUMO (more efficiently) than the pure organic solvent.

Concomitant with the red-shift of the monomeric peak of MB, the addition of large content of water in the organic solvent also resulted in a prominent occurrence of a dimeric peak of MB, which is blue-shifted in relation to the monomeric peak, denoting the formation of H-aggregates [19]. In fact, upon aggregation many organic dyes show a remarkable change in the absorption spectrum, which is considered an important fingerprint to spectral characterization. It is the case of the H-band, which is polarized perpendicularly to the rod long-axis [20]. The aggregation of ionic dyes cannot be assigned to a specific type of interaction. There is a significant contribution of several physical-chemical influences due to van der Waals interactions, intermolecular hydrogen bonds, π-electron interactions, among others. Therefore, it is not trivial to evaluate the individual contribution of these different interactions [21].

In spite of the positive charge, MB is a molecule that contains a hydrophobic group, which is the methyl groups, i.e., the four methyl groups bonded to the two nitrogen atoms that are located out of π conjugated plane (Figure 1) [21]. The stabilization as monomer depends on the solvation associated to the repulsion between its positive charges. However, it must take into account the strong dipole-dipole interactions between the monomeric units and the strong electronic delocalization that acts distributing the charge concentration and increasing the conjugation of the π electronic density. These factors favor the π–π interactions toward to self-aggregate.

It was observed that the water addition in all organic solvents studied in the present work (acetonitrile, ethanol, glycerol and dioxane) resulted in the formation of MB dimers (D), which presented a blue-shift band in comparison to monomer (M). It is probable due to the fact the water molecules act as strong sigma bases (σ-donors) to MB. In this context, the sulfur atom, in spite of the electronic delocalization that is inherent to the π conjugated system, must be the more affected atom by the cationic charge due to its lower electronegativity, which is lower than that of the nitrogen atom. Therefore, the donor action of the non-ligand electronic pairs of the water molecule must decrease the electronic deficiency of MB, decreasing the repulsion between the monomers, which favors the dimerization process. In fact, the positive charge density of each monomeric unit of MB must decrease the electrostatic repulsion, mainly when the relative distance between the monomers is high, allowing a significant interaction of the MB units. This process occurs in such way that the van der Waals interactions can be minimally representative, favoring the dimerization process. In addition, there are the solvation effects, which are due to the hydrophobic nature of the MB molecules. Therefore, the aqueous solvent cannot effectively maintain these molecules as monomers.

In Figure 2, it is shown that the increase of water molar fraction in the organic solvent is associated to the decrease of the absorbance A_M/A_D ratio, which can be inferred through the ratio (A_M/A_D). This effect corroborates to the previous discussion, i.e., the ability of electronic donation by organic solvents is significantly lower than that of water. Therefore, the positive charge of MB is sustained by the higher percentage of organic solvents in the mixture. Thus, the charge density remains high as well as the electrostatic repulsion between the

monomers, making the dimerization process difficult.

In fact, among the organic solvents, the more efficient Lewis base would be the acetonitrile, but it is important to notice that this molecule is well known as a significant π-receptor ligand [22]. Actually, relevant works involving spectroscopic studies of several coordination compounds already demonstrated the significant π-acceptor character of acetonitrile as ligand [19, 20, 23], which has motivated studies using metallic complexes that present acetonitrile as ligand as precursor compounds of surface modifications [23, 24]. This property should decrease the interaction with the sulfur atom, at least in the first coordination sphere, considering the effective interaction that occur in orbitals of σ symmetry as well as π-orbitals. On the other hand, in terms of total electronic density, the donor influence of acetonitrile tends to be less efficient than that observed in a less electrophilic ligand, like, for instance, a water molecule, since this last ligand is considered an excellent σ-donor and also a light π-donor through its p_π orbitals. It is important to point out that the sulfur atom as coordination center can make stable interactions with ligands with high charge density, i.e., π-donor ligands, like, for example, SF_6 e SCl_6. This occurs due to the relatively low electronic density of σ- and π-orbitals of the sulfur atom. These structural characteristics must be considered in solvatochromic studies of MB because these properties are strongly related to the physical-chemical characteristics significantly different when compared with some photosensitizer (PS) agents of wide clinical application, such as porphyrins, phthalocyanines and chlorines dyes [19, 20].

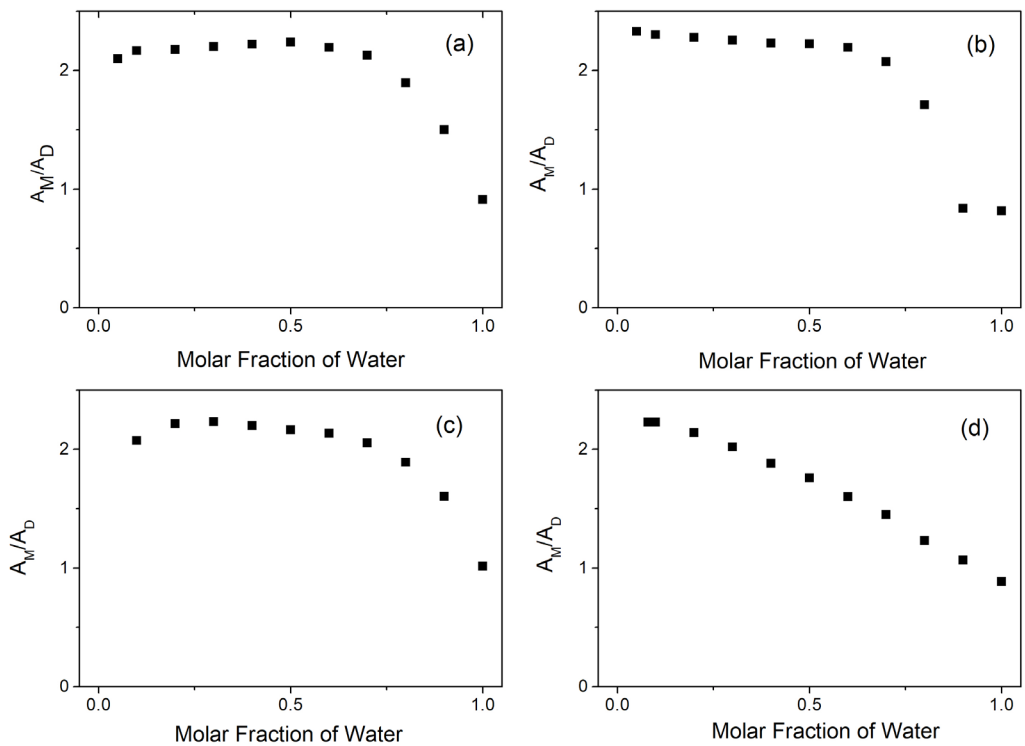

Figure 2. Changes of A_M/A_D ratio (A_M= monomer absorbance and A_D=dimer absorbance) in different binary solvent mixtures: (a) water/acetonitrile; (b) water/ethanol; (c) water/dioxane and (d) water/glycerol. [MB]= 3.13 × 10^{-4} mol L^{-1}.

It was observed an absorption maximum around 610 nm for MB dimers (H-Band) in pure water while a displacement up to +5 nm occurred with other solvents (Table 2) (in the water fractions in which the dimerization begins as show in Figure 2). It is important to note that the absorbance ratio A_M / A_D remains constant until approximately 70 % of water in organic solvent (see Figure 2), except for water/glycerol mixture, which is justified by the high polarity (high dielectric constant) of the glycerol. In fact, the solvents with higher capability of proton-donation (water and glycerol) promoted a blue-shift of the fingerprint band of the dimer.

Table 2. Maximum absorbance in the UV-Vis spectra for monomers and dimers of MB in different binary solvent mixtures.

Solvent	$\lambda_{max.}$ Dimer/nm*	$\lambda_{max.}$ Monomer/nm
Water	610	664
Ethanol/water	614	656
Acetonitrile/water	614	658
Dioxane/water	615	661
Glycerol/water	612	667

* to the water fraction that the dimer formation begins

It is well known that MB dimer does not present fluorescence, due to the deactivation of the excited state by self-quenching process. Thus, the MB fluorescence decreases with the addition of water in organic solvent, in agreement with data presented in Figure 3. These results corroborated the data presented for the A_M/A_D ratio, Figure 2. Furthermore, it is observed higher fluorescence intensity with MB in glycerol, which is related to the higher solvation of the monomer by this solvent. The decrease of the fluorescence indicates that the quantum yields of the solvents are higher than in water. The fluorescence can be influenced mainly by the stabilization of the MB molecule and it seems to be more intense when generated by monomers, except in the case of dioxane (Figure 3), in which MB is weakly solubilized. This fact explains the low fluorescence intensity when the mixture is formed with more than 90 % of dioxane.

It is also observed a shift in the maximum wavelength of emission Figure 4, with a higher dependence of the values of emission maximum of MB with the amount of water in the acetonitrile/water mixtures, in which a spectral variation of around 12 nm is observed between the solution containing pure acetonitrile and the solution that contains only water.

The distinct interactions between the water and acetonitrile molecules with MB should be quite different, since the H_2O is considered a good σ-donor and weak π-donor ligand (therefore, a ligand that acts in the same way of halides). Acetonitrile, on the other hand, is σ-donor and π-aceptor ligand [22]. These different properties influenced in the splitting of the sulfur orbitals as well as that from the π conjugated system of MB, which occurs by influence of the solvent system that would be interacting with this dye, mainly regarding polar and highly coordinating solvents, such as acetonitrile and water. Indeed, sulfur is a small electronegative atom, implying that, in spite of the cationic charge delocalization, this center must be a site with significant electronic deficiency. In this way, this atom must increase the Lewis acid character and its potential to act as coordinating center, when it is in the presence of coordinating solvents, which act as efficient sigma bases, such as water and acetonitrile. Besides, it is relevant emphasizes that the sulfur is considered a softer base, in agreement with the acid-base concept of Pearson, which means that its charge/radium ratio is low (high polarizibility).

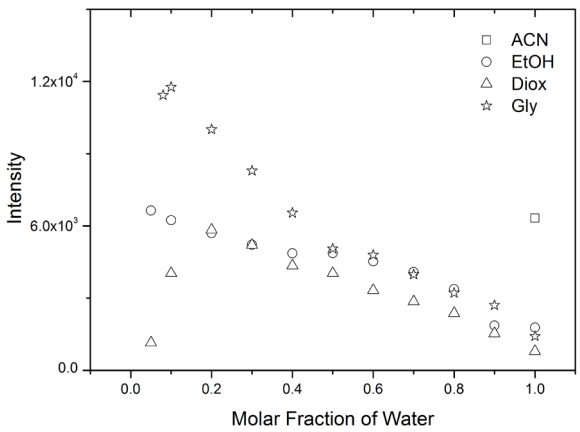

Figure 3. Changes of the fluorescence intensity measured at the maximum of emission for the binary solvent mixtures studied in this work. The excitation wavelength was adjusted at 640 nm. □) acetonitrile; ○) ethanol; Δ) dioxane; *) glycerol.

In this context, all solvents used in this work, except the acetonitrile, presents an oxygen atom as coordinating site (localization of the electronic pair in the Lewis base), which is considered an important "hard" base (high charge/radium ratio, i.e., low polarizibility). The acetonitrile coordinate through the pair of free electrons, which is located predominantly on the nitrogen atom, and it is a "softer" base than oxygen. Consequently, the sigma interaction between sulfur and nitrogen (soft-soft interaction) should be favored due to the greater orbital superposition that occurs in this linear combination than that observed in the interaction between sulfur and oxygen (soft-hard interaction) [19]. This fact emphasizes the peculiar character of the interaction between the solvent and the acetonitrile with MB, denoting the potentially high sigma interaction between the polar coordinating solvent acetonitrile and the MB dye. Considering the ethanol/water mixture, this variation is approximately 8 nm, because even for the pure ethanol solutions, the solvation model of MB already involves hydrogen bonds participation.

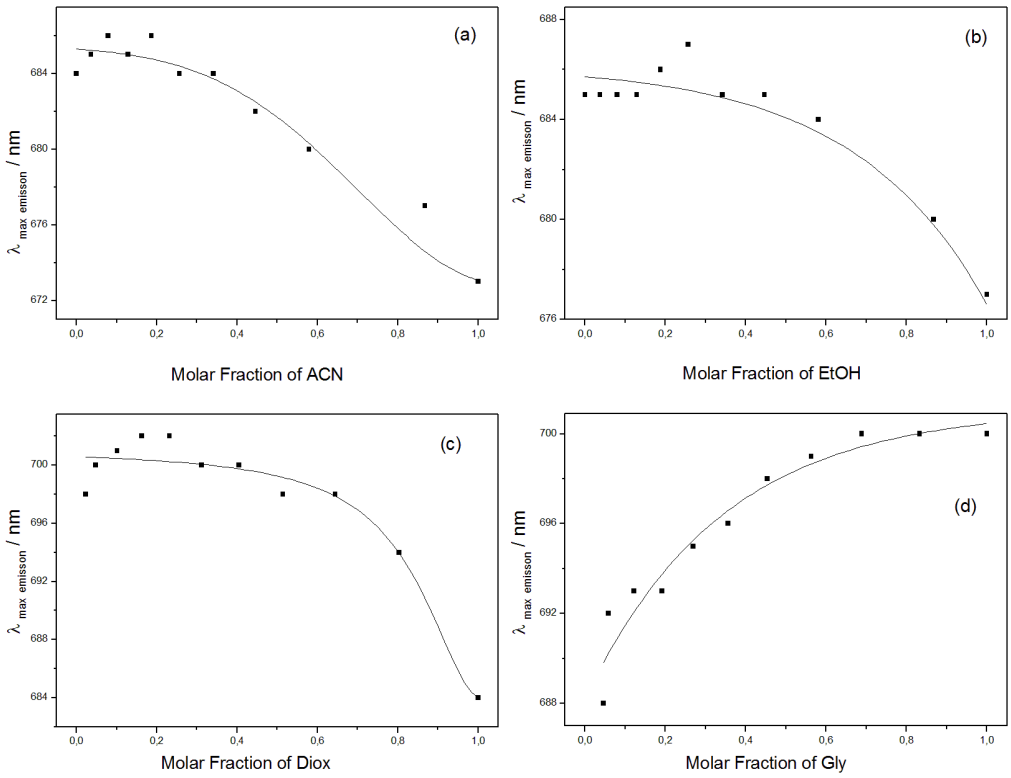

Figure 4. Shift of the emission maximum of MB as a function of the molar fraction of the solvent mixture: a) water/acetonitrile; b) water/ethanol; c) water/dioxane and d) water/glycerol. The excitation wavelength was fitted at 640 nm.

The shift of the maximum of emission suggests a significant solvation of the monomer in comparison with the monomer-monomer interactions at the concentration of 3.13×10^{-4} mol L^{-1} of MB, which is observed in the solvent mixtures studied in the present work. The excitation of MB in the mixtures dioxane/water at 640 nm in frontal face mode generated spectra with maximum emission varying from 684 to 702 nm; i.e., the shift in the maximum of emission is also relatively small when it is compared with the other solvents in mixture with water. It indicates that the energy of the singlet excited state is not suffering significant change when compared with the change that is observed to the water. Interactions between MB (positively charged) with the dipole of water, in addition to the interactions with hydrogen, are stronger than the dipole interactions of dioxane, which is a non-protic solvent with a low polar character.

Singlet oxygen emission in the binary mixtures of MB

In the same way as the intensity of fluorescence, the singlet oxygen emission is greater in each of the mixtures in which the amount of water is lower, as it can be seen on Figure 5. To elucidate this behavior, it was used the solvent polarity parameter (*Δf*). This can be calculated as defined by Lippert and Mataga [25,26]:

$$\Delta f(\varepsilon,n)=\frac{\varepsilon-1}{2\varepsilon+1}-\frac{n^2-1}{2n^2+1} \qquad \text{Eq. 1}$$

where the dielectric permittivity (ε) and refractive index (n) of the pure solvents and mixed solvents. The *Δf* has been reported to correlate nicely with a variety of physical-chemical properties of many probe molecules in different solvents and solvent mixtures. The Figure 6 shows the intensity changes of singlet oxygen emission as a function of the dielectric constant (ε) and the polarity parameter *Δf(ε,n)* obtained for each system. The values of *ε and Δf(ε,n)* were obtained from the literature [27-29]. The solvation model in these solvent mixtures must involve changes in the dielectric constant, polarizibility and dipolar moment [27]. Figure 5 show that the increase of the amount organic solvent results in an enhancement of the singlet oxygen emission intensity. These factors associated to the different ways of hydrogen bond formation and polarity of the medium would generate the solvatochromic effect

observed in the measurements involving MB [16]. In theses mixtures when *ε and Δf(ε,n)* decrease the organic solvent portion is increased, i.e., in solvents with low polarity higher values of singlet oxygen were obtained. This indicates greater changes between interactions with more polar solvents relative to those less polar [30]. In fact, the addition of water increases the dielectric constant of the medium and promotes the monomer destabilization, resulting in the self-aggregation process. The aggregation mechanism together with the possibility of dynamic quenching of excited states by water molecules (that act as quencher) decreases the quantum yield of fluorescence in the mixtures with higher amount of water [19]. For the four organic solvents evaluated in this work, it is possible to identify a greater intensity of singlet oxygen emission when the presence of aqueous solvent is lower. Certainly, the presence of a higher quantity of water molecules might favor a more efficient dynamic quenching of the respective excited states, decreasing the lifetime of the excited state of the dye, as well as of the molecular oxygen, which the excited state corresponds to the singlet oxygen ($^1O_2$). In this way, a very intense quencher effect could increase both the processes of relaxing of the molecular oxygen and the photosensitizer agent (PS), promoting a synergic relaxation, which in association with the increase of the singlet oxygen relation increases the return to the fundamental state of the dye, precluding a more intense generation of the own singlet oxygen that is already relaxed. The decrease of the fluorescence and singlet oxygen formation is related directly to the increase of the dimerization and, in the second case, is correlated to the increase in the water concentration, where the time of life is very low. Besides, the higher interaction between water and the MB by hydrogen bounds and ion-dipole interactions result in vibronic deactivations and decrease in the time of life of fluorescence as well as the quantum yield, favoring the non-radioactive decays.

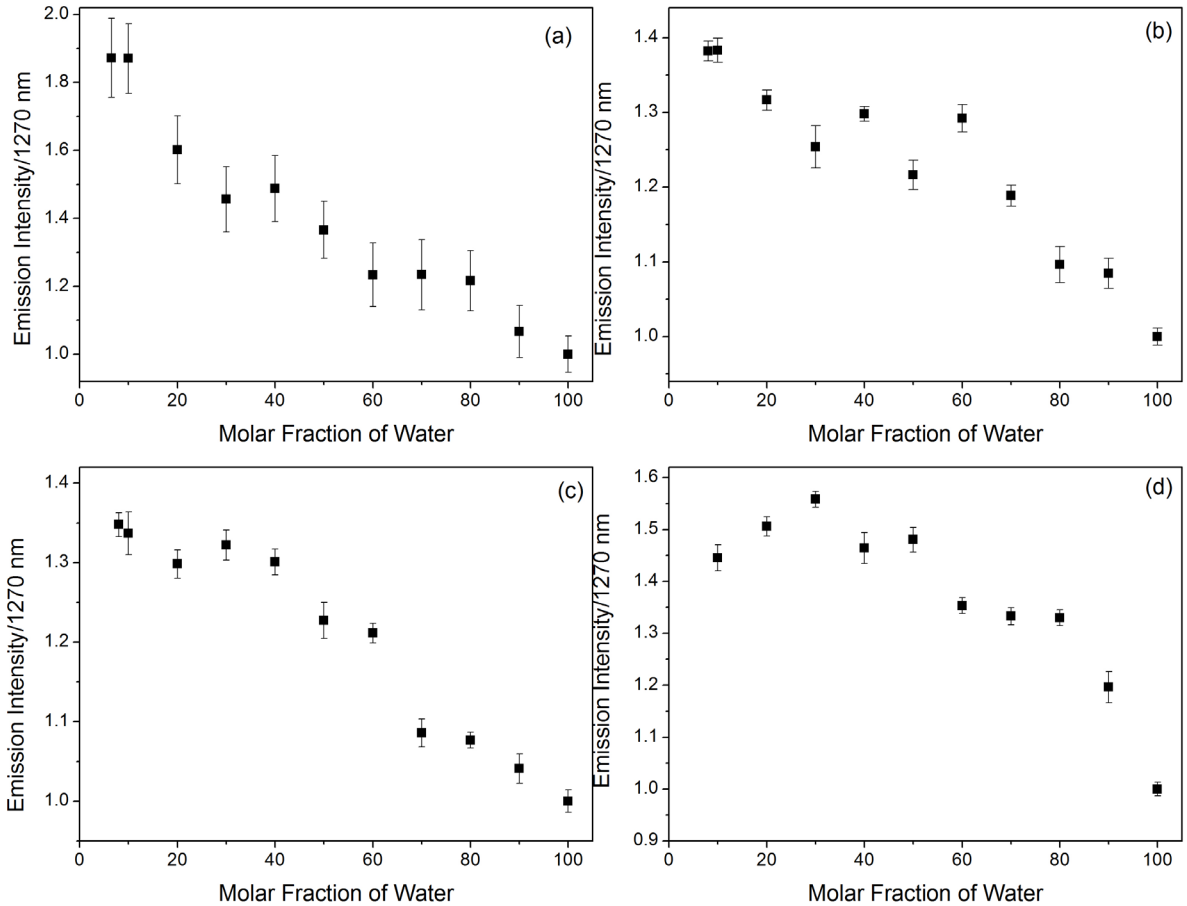

Figure 5. Changes in singlet oxygen emission intensities in (a) acetonitrile, (b) ethanol, (c) glycerol and (d) dioxane as a function of the water content. MB concentration of 3.13×10^{-4} mol L^{-1}. The excitation wavelength was fitted at 665 nm.

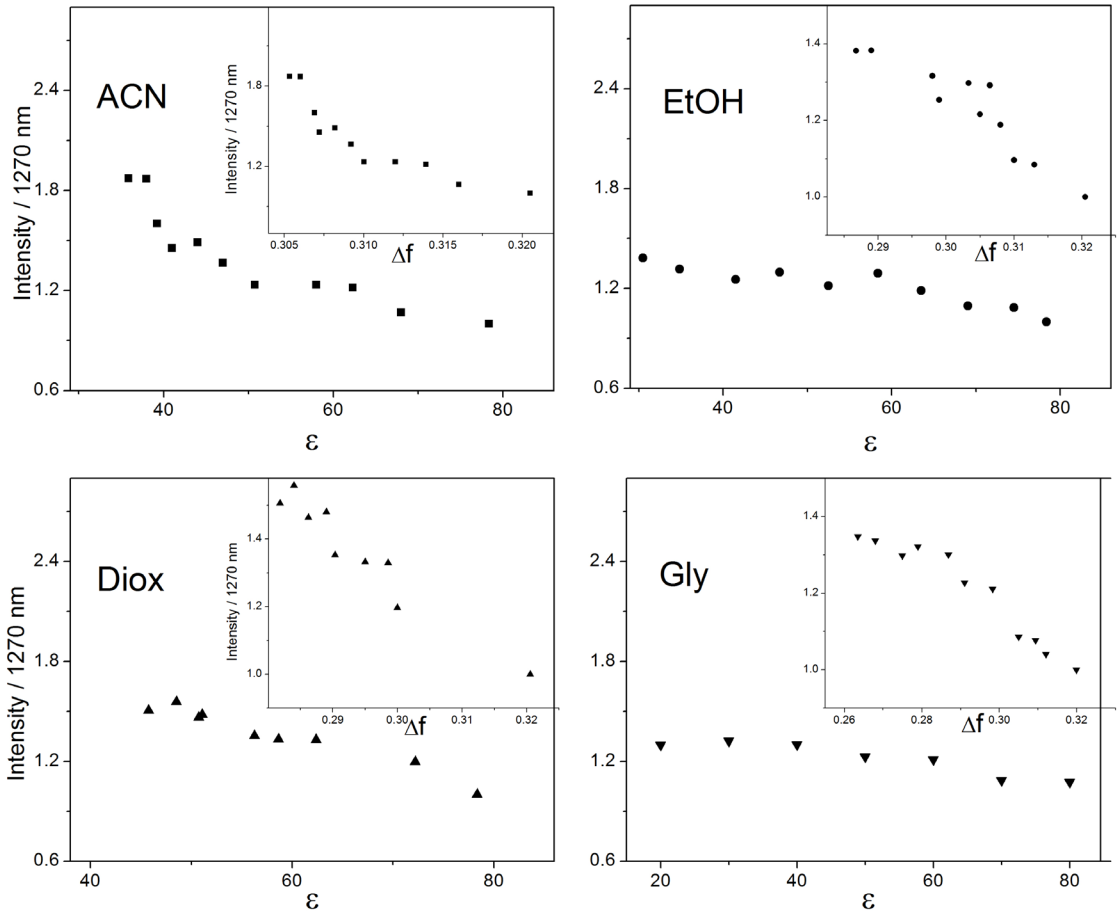

Figure 6. Intensity change of singlet oxygen emission in ACN (acetonitrile), EtOH (ethanol), Gly (glycerol) and Diox (dioxane) as a function of the dielectric constant and solvent polarity parameter, *Δf* (inset). MB concentration of 3.13 × 10^{-4} mol L^{-1}. The excitation wavelength was fitted at 665 nm.

It is interesting to notice that even in environments with high water concentration, the fluorescence is not quite quenched; indicating that, in the respective conditions, the aggregation processes involving the MB molecules is not completed. The solvent mixtures with more than 30% of water (molar fraction = 0.563), there is a substantial decrease in the fluorescence intensities, which is caused by the self-aggregation process of MB in aqueous medium. The solvent polarity and the specific interactions cause significant shifts in the respective wavelengths of maximum absorption and emission as well as affected the potential singlet oxygen production. Singlet oxygen emission is greater in each of the mixtures in which the amount of water is lower; in solvents with low polarity, higher values of singlet oxygen were obtained. The model of solvation in these solvent mixtures must involve changes in the dielectric constant, polarizibility and dipolar moment. In this context, it is important to note that the coordinating or non-coordinating characters of the solvent system play an important role on the photophysical properties presented by MB as function of the presence of sulphur atom in the molecular structure, since this atom can act as coordination center. This tendency is significantly accentuated in a dye as methylene blue, which is due to its cationic character that increases the electronic affinity of the sulphur atom, favoring its action as Lewis acid and as a center of coordination.

It was also observed a drastic interruption in the change of the fluorescence maximum with 50% of acetonitrile in water (Figure 4b). A similar effect is observed in the change of the fluorescence intensity (Figure 4). These results corroborated to the fact that the solvation effect only affect the excited singlet state of the acetonitrile/water system (considering all solvents studied in the present work), which is supported by previous results. Probably, the coordinating properties of acetonitrile and water is an

important factor for the determination of this solvatochromic behavior. Indeed, the presence of a potential center of coordination accentuates the factors related to the interaction of coordinating solvents and/or non-coordinating solvents with a cationic dye, such as methylene blue, changing the physical-chemical properties of this compound [11]. Another interesting result was observed in the glycerol/water system in relation to the proportion between dimer and monomer as a function of the quantity of water, which was also only observed in this solvent binary mixture.

4. CONCLUSION

It could be observed that the presence of water in organic solvents affects directly the MB aggregation process and, as consequence, changes the photophysical properties in the binary mixtures of solvent. In fact, different organic solvent-water mixtures were studied, which were acetonitrile-water, ethanol-water, dioxane-water and glycerol-water systems. Furthermore, it is observed that the monomeric form of methylene blue (MB) is favored in several solvent mixture conditions, which was showed by the higher fluorescence intensities when the amount of organic solvent is higher. It is an extremely auspicious fact when considering the potential applications of MB, since the monomeric form of this dye presents quantum yield significantly higher when compared with the respective dimeric and aggregated forms (H-aggregates). In any case, some interesting aspects of the dye-solvent interaction were observed in this study, as the influence of the coordinating properties of the solvent molecules employed in each solvent mixture. Indeed, the obtained results showed that the presented a strong interaction with the MB molecule, mainly with respect to the action of the sulphur atom. This work demonstrated that the mixture of different solvents can be an efficient alternative in order to optimize the physical-chemical properties of dyes aiming its employment in several biological applications, for example as a photosensitizer agent in PDT.

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