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MONITORIZING METHYLENE BLUE INCLUSION IN REVERSE MICELLAR NANOSTRUCTURES

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Methylene blue (MB) inclusion in nanostructured systems formed by sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelles, water and organic solvents (*i.e.*, *iso*-octane, *n*-hexane) is investigated by UV-Vis, static fluorescence and dynamic light scattering. The spectroscopic signals of methylene blue are blue shifted in reverse micelles compared to those in water. The probe senses a more nonpolar microenvironment in reverse micelles formed in *n*-hexane compared to that in *iso*-octane. The results are discussed in terms of optimization the methylene blue solubilization in nanoconfined media.

INTRODUCTION

Reverse micelles are aggregates formed by surfactants in nonpolar solvents in presence of very small amounts of water. In such structures, the hydrophilic head groups of the surfactants are oriented towards water, the hydrocarbon tails are embedded into the nonpolar solvent, and the systems are isotropic and thermodynamically stable.[1,2] The micellar core is characterized by the hydration degree, w_0, which is the water to surfactant molar ratio. The nanoscopic water drops are a model of those in the live systems and this explains the popularity of reverse micelles as model of biomembrane/water interface. The inner core of reverse micelles, the "water pool", is able to solubilize hydrophilic biomolecules and reverse micelles are unique medium for enzymatic catalysis,[3-6] protein extraction,[7-9] and protein refolding.[10-12]

Dye molecules are often employed to probe the structure of live systems. MB is exploited to sentinel lymph node mapping,[13] to treat oncological, cardiovascular and ophthalmic diseases,[14] or to delay the onset of Parkinson's and Alzheimer's syndromes.[15-17] Inspired by the medical applications of methylene blue, our attention was directed towards the use of MB in the spectroscopic investigation of confined water in reverse micelles. The spectroscopic data were correlated with those obtained from dynamic light scattering measurements. This work aims to shed more light on the oil/water interface following the location of MB in the reverse micelles obtained with sodium bis(2-ethylhexyl) sulfosuccinate (AOT) in different solvents, and at various hydration degrees.

EXPERIMENTAL

Reagent grade *iso*-octane, *n*-hexane, AOT from Aldrich, and methylene blue from Fluka were used as received. The molecular structures of the MB and AOT are shown in Fig. 1.

The water was Milli-Q, and the reverse micellar solutions are prepared by the injection method.[18] The concentrations of surfactant and dye in reverse micelles are of 0.1 M and 1.3 x 10^{-5} M, respectively.

Optical absorption spectra are recorded on a Varian Cary 100 Bio spectrophotometer. Fluorescence measurements are

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carried out on an Edinburgh Instruments FLS 900 spectrofluorimeter with excitation set at 514 nm. Dynamic light scattering data are collected on a ZetaSizer, Nano ZS, Malvern Instruments. The analysis was performed at a laser wavelength of 633 nm and an angle of 173°.

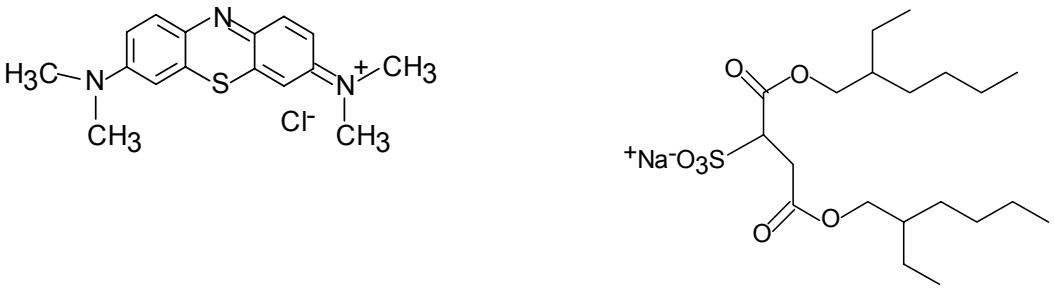

Fig. 1 – The molecular structures of methylene blue and sodium bis(2-ethylhexyl) sulfosuccinate.

RESULTS AND DISCUSSION

Fig. 2 presents our dynamic light scattering data in terms of the hydrodynamic diameter of reverse AOT micelles in *iso*-octane and *n*-hexane. They show a linear dependence of the hydrodynamic diameter on w_0. The results are in agreement with the data obtained by Sechler *et al.*[19] who state for AOT reverse micelle in *iso*-octane a linear increase in water droplet size with the hydration degree. In the present study we use an organic solvent with a linear alkyl chain, *n*-hexane, that shows a similar size/hydration degree linear behavior and a marked decrease of nanodroplet size as compared to that in micellar systems formed with branched alkyl chain hydrocarbons like *iso*-octane. The progressive raising of water concentration in the AOT/organic solvent system allows the formation of reverse micelles whose dimensions depend on the water to surfactant molar ratio.

Fig. 3A shows the absorption behavior of MB in bulk water and AOT/water/*iso*-octane reverse micelle at different hydration degrees. The spectra reveal the presence of two species of MB (monomer and dimer) that have been previously identified in water.[20] In aqueous solution, the MB monomer has the absorption peak at 664 nm and the MB dimer appears as a shoulder around 600 nm. The absorption spectra in reverse micelles resemble those in water. Moreover, our results show the increase of absorbance of the MB monomer with the hydration degree and the blue-shift of the absorption peak by 6 nm as compared to water.

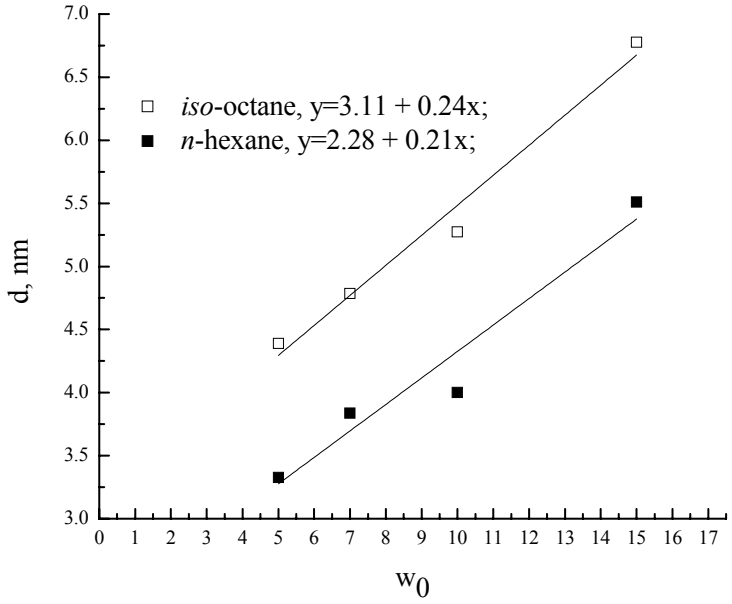

Fig. 2 – The hydrodynamic diameters of AOT reverse micelles *vs*. the hydration degree. The solid lines are the least-squares fits, and the squared correlation coefficients from bottom to top are r^2=0.9173 and r^2=0.9708.

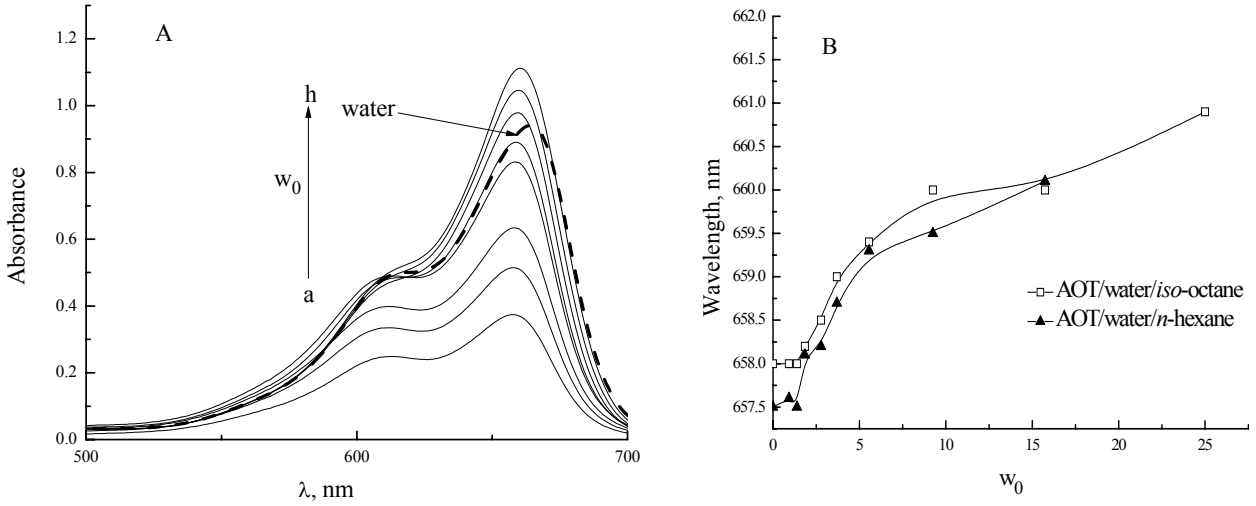

Fig. 3 – Absorption spectra of 1.3 x 10^{-5} M methylene blue in bulk water (dashed line) and at different hydration degrees (w_0) of reverse micelles (solid line; From a→h, w_0=0; 0.93; 1.4; 1.85; 3.7; 5.56; 9.3; 15.74) (A). The variation of MB absorption maximum wavelength in different AOT/water/organic solvent systems, *vs*. hydration degrees (B).

The variation of MB monomer absorption maximum in the AOT reverse micelles obtained with two solvents, *iso*-octane and *n*-hexane, at different hydration degrees appears in Fig. 3B. The shape of curves is similar in both solvents. At low hydration degrees up to w_0 = 1.4, the absorption maximum doesn't change. By raising the hydration degree a steep red shifting is recorded, followed by a slight increase of the absorption maximum wavelength. The results are along with those previously reported in the reverse micellar system of Triton X-100, *n*-hexanol and cyclohexane investigated using methyl orange.[21] Other results pointing out a blue shift of the maximum absorption of MB as compared to water were interpreted by a smaller polarity sensed by the probe.[5,22] Our data show that the micelles prepared in *n*-hexane have the smallest polarity. This organic solvent has a short linear alkyl chain able to accommodate easier into the surfactant hydrophobic tail than the more bulky *iso*-octane diminishing the water amount from the micellar host. Yuan *et al*., studied the structure of water in AOT/water/*iso*-octane system by dissipative particle dynamics simulation method and the data reveal the presence of several types of water.[23] There is water located in the hydrophobic palisade of the micelle (captured water), at the periphery of micelles around the polar beads of surfactant molecules (bound water) and in the micellar core (free water). For low hydration degrees, one may consider that, in the interfacial region of our systems, there are those water molecules captured and not bonded to the surfactant polar groups. This assumption relies on the disordered structure of the micelles at molecular level that allows to some water molecules to stay around the hydrocarbon tails of AOT in micelles producing water of very low density. These water molecules behave like monomers or dimers and are capable to penetrate the interfacial layer.[24] In such regions, even if the density of water is very low and compactness of micelle is high, the probe is captured and solubilized. For w_0 in between 1.4 and 9.3, the ionic layer of AOT/water/*iso*-octane reverse micelle contains water molecules bound to the sodium ions of the sulfonate groups. Due to its cationic character, MB tends to be linked to negatively charged groups of the surfactant. By increasing the hydration degree, the interactions between the water and counterions enhance to the detriment of those between the cationic MB and negatively charged polar groups, leading to a further weakening of the electrostatic interaction between SO_3^- and Na^+. The plateau begining at w_0=9.3 can be safely ascribed to the free water from the micellar core by analogy with previous data by Hou *et al.*[25] In the Triton X-100/1-butanol/*n*-octane/water systems they observed bulklike water at w_0=5.3 a much lower value than in our systems.

In the next approach we did aging test of the reverse micelles. Fig. 4 shows the change of absorbance maximum of the dye monomer *vs*. the

hydration degree at a time span from two hours to three weeks. The recorded absorbancies did not change denoting stable systems in time. The results show a good relationship between the MB inclusion in reverse micelles and the stability in time of micellar matrix structure. At the same time, the obtained data reveal that at $w_0<3.7$ there is a hyperchromic shift of the absorption peak of MB with increasing of w_0 denoting a further inclusion of dye in the micellar host. For $w_0>3.7$, the absorption remain constant suggesting the saturation of micellar host with dye. At this hydration degree, the absorbance peak of MB monomer is a maximum (Fig. 3A), being higher than the dye's signal in *n*-hexane and bulk water. This demonstrates that in the water-pool of micelles the methylene blue reaches a higher local concentration than in bulk water. Such a phenomenon is similar to that observed in micellar catalysis, when the rate constant increases because the reactants are concentrated inside the micelles.[26,27]

The effect of water content of the reverse micelles upon the MB solubilization was investigated by static fluorescence. Fig. 5 shows the variation of emission maxima of the MB monomer solubilized in reverse micelles formed in *iso*-octane and *n*-hexane as a function of w_0. The curves have an ascending part, eventually followed by a plateau and a descending zone. The fluorescence data have a similar trend to those of UV-VIS, and show a raise of emission to $w_0=3.7$ (in the system with *iso*-octane). This means that the probe is gradually solubilized within the water pool of micelles. Above a hydration degree of 3.7, the emission is constant but gradually decreases when $w_0=9.3$. Fig. 5 also shows that MB is less concentrated into the micellar nanocage, since its emission signal diminishes ($w_0>9.3$). Comparing the shift in the MB emission in AOT/water/*n*-hexane with that in AOT/water/*iso*-octane it is observed that the probe presents a weaker fluorescence signal for the same value of w_0. This indicates that the probe senses the difference in size of the water droplet in the systems prepared with *n*-hexane and *iso*-octane.

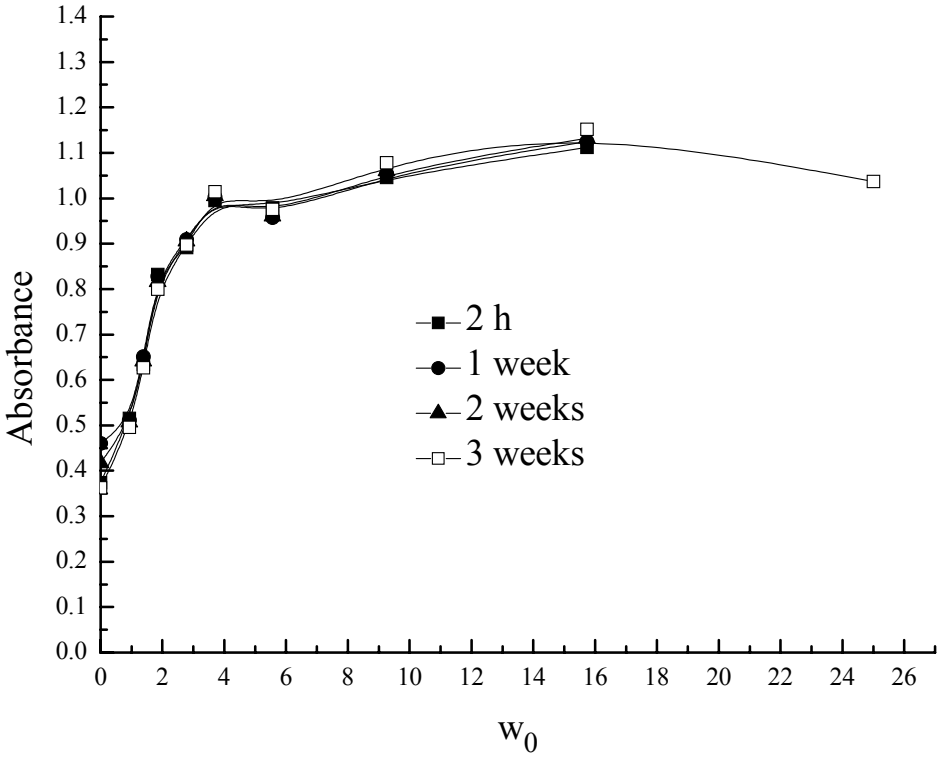

Fig. 4 – Maximum of absorbance values
at different times *vs*. hydration degrees of AOT/water/*iso*-octane micelles.

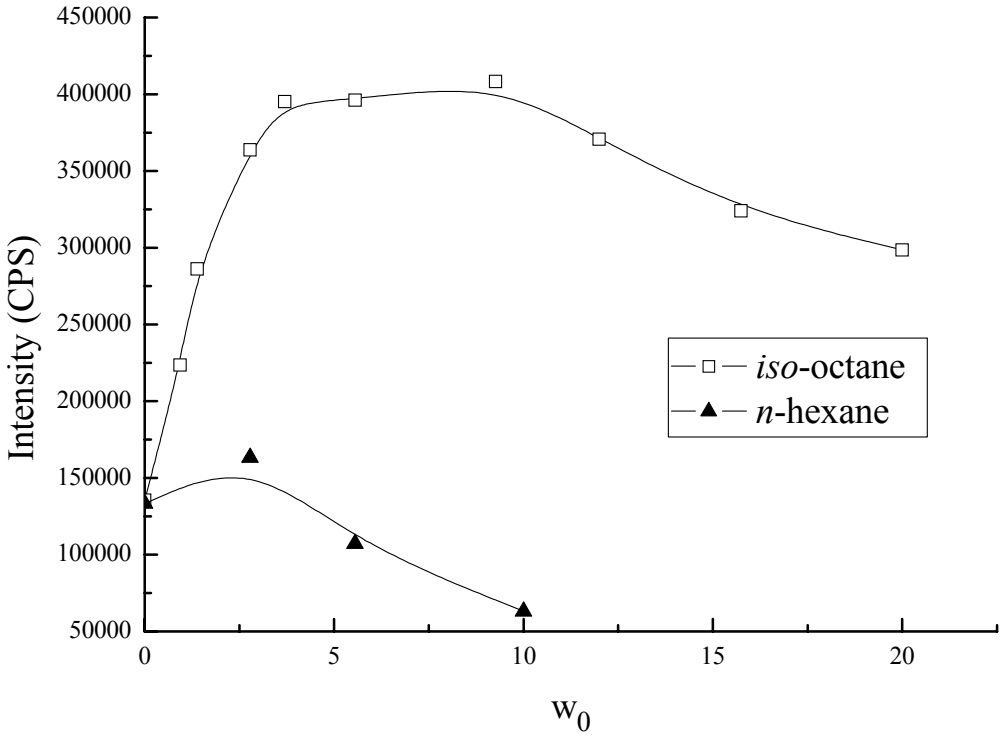

Fig. 5 – The variation of MB emission maxima in AOT reverse micelles at different w_0.

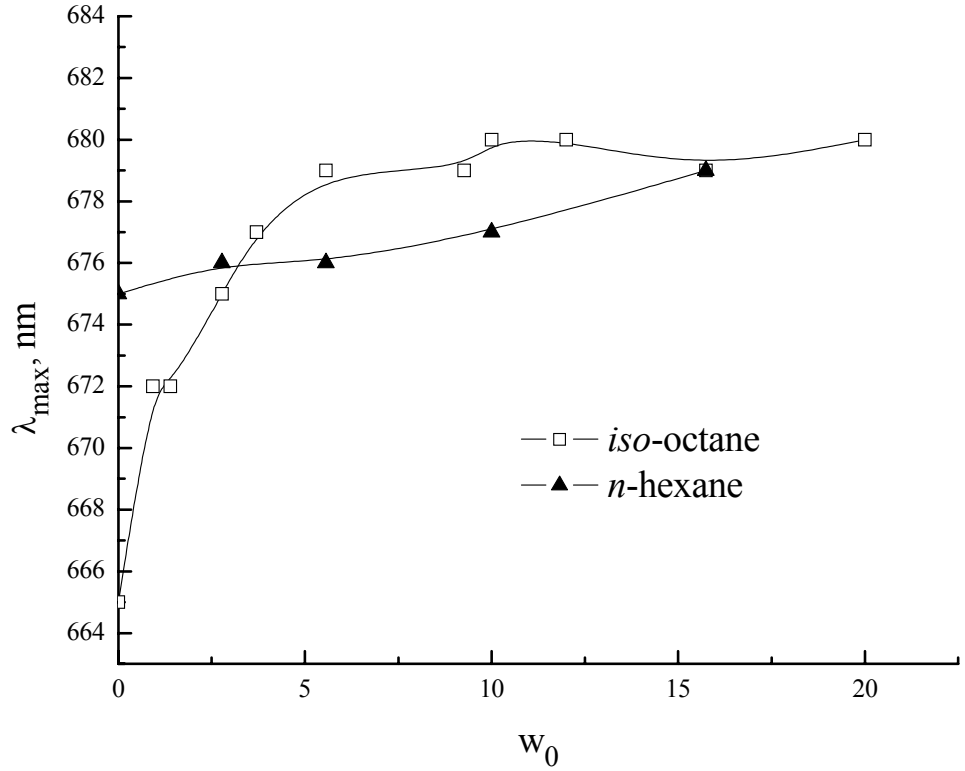

Fig. 6 – The variation of MB emission maximum wavelength (λ_{max}) as a function of hydration degree in reverse micellar systems.

Fig. 6 illustrates the change of the maximum wavelength (λ_{max}) of emission in reverse micelles prepared with *iso*-octane or *n*-hexane. A blue shift of the fluorescence maximum compared to that in water ($\lambda_{max,water}$=686 nm) was observed. By analogy with the electronic spectra discussed above, a blue shift denotes that the microenvironment is less polar than bulk water.

The shift induced by the organic solvent is of 21 nm for *iso*-octane and of 11 nm for *n*-hexane. At the same time, one may observe that λ_{max} of MB in *iso*-octane displays a progressive red shift of 12 nm (from 665 to 677 nm) with the increase of w_0. This change became smaller and reaches a plateau when the hydration degree is higher than 3.7, meaning that MB is completely dissolved into the water pool of reverse micelles. The phenomenon can be explained admitting that MB senses, at low w_0, the interfacial region of reverse micelle. As w_0 increases, the probe moves towards the bulk water. A different situation appeared in case of AOT/water/*n*-hexane when a small increase of λ_{max} with w_0 is recorded. A weak dependence of λ_{max} on w_0 for this system indicates that the probe is located in a region of the reverse micelles that is hardly penetrated by water.

The fluorescence measurements confirm that linear hydrocarbons penetrate better the surfactant layer, raising the spontaneous curvature of the reverse micelle and decreasing the amount of incorporated water. As a consequence, in the reverse micelles formed in *n*-hexane, the quantity of solubilized MB decreases as proved by our absorbance and fluorescence data.

CONCLUSIONS

Spectroscopic absorption and fluorescence properties of methylene blue are different in the water pool of the AOT reverse micelles as compared to those in bulk water. MB experiences a less polar microenvironment in the reverse micelles formed in *n*-hexane than in *iso*-octane and the bulk water. The dye has a higher local concentration in the micellar host than in bulk water. This depends on the hydration degree and the organic solvent.

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