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Sensing different micellar microenvironments with solvatochromic dyes of variable lipophilicity

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ABSTRACT

The UV/Vis spectral behavior of three solvatochromic *N*-alkyl α-pyridones of varying lipophilicity was investigated in aqueous solutions of a block copolymer at variable temperatures. The solvatochromic responses of the three analogues to the distinct microenvironments were compared and interpreted in terms of their different location in the microheterogeneous system. The interpretation was supported by ^{1}H NMR measurements and by molecular dynamics simulations that mimicked the interactions of the dyes with the polymeric micelle in water.

1. Introduction

Solvatochromic betaine dyes have been employed for some time as polarity sensors in homogeneous and microheterogeneous media [1–4]. The position of their longest-wavelength band in a series of solvents has been used to characterize their polarity. Ideally, the same betaine dye should be employed in all media, so that its spectral responses in different solvents can be compared. However, because of solubility problems, this is not always possible, and betaine dyes with different solubilities are sometimes employed as sensors for different solvents. In such cases, the solvatochromic sensors should differ in their solubility, as a consequence of their different lipophilicity, but not in their spectral response to the medium. This assumption had traditionally been taken for granted, until it was shown that, even for analogous probes in homogeneous media, their lipophilicity is a factor to be considered in characterizing their microenvironment. Analogous compounds with a common donor–acceptor pair but different lipophilicities have shown different spectral responses in pure solvents and in binary solvent mixtures [5–7]. In consequence, in the characterization of micellar microenvironments with the aid of solvatochromic dyes, we are faced with an ambiguous situation even if a pair of analogous sensors with different lipophilicities is employed. A different spectral response by the pair may either be caused (a) by their differences in lipophilicity, which affect their intrinsic response to the same environment, or (b) by the fact that they actually sense different microenvironments.

In a previous work in which we compared the spectra of the two analogous α-pyridone betaine dyes **1** and **3** in pure solvents and in a binary solvent mixture, we drew attention to the fact that they behaved differently even in homogeneous media [7].

Their different behavior in the presence of a micelle was cautiously interpreted by us as arising from their sensing different microenvironments, a conclusion that was based on the observation that their differences were larger in that medium than in pure solvents. Our caution was motivated by the complex nature of micellar solutions. Conclusions on the polarity of these systems from the position of the solvatochromic band of a dye in their presence may be meaningless. A solvatochromic probe in the presence of a micelle may be partitioned between different microenvironments, yielding a complex response that depends on factors such as relative concentrations and temperature.

We have recently examined this situation in a study of the thermochromism of the $E_T(30)$ betaine dye **4** in micellar solutions of block polymers ("poloxamers") [8] (Scheme 2).

These copolymers are well known for their tendency to form micelles in water by aggregation of their individual chains ("unimers") in a temperature range that depends on the polymer composition and its concentration [9–11].

We showed that, as the temperature was raised, the solvatochromic dye was partitioned between two microenvironments, the aqueous unimeric solution and the polymeric micelle. Thus, these

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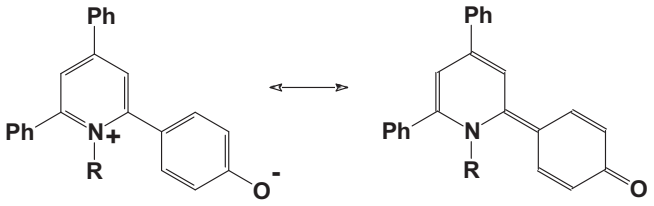


1, R = CH_3
2, R = n-C_4H_9
3, R = n-C_8H_{17}

Scheme 1.

systems offered the possibility of clearly distinguishing different microenvironments for a particular probe in a microheterogeneous medium. Furthermore these systems should also allow us to identify, for a series of analogous probes with different lipophilicities, the factors that govern their spectral response in these media.

With these goals in mind, we decided to compare the behavior of three analogous α-pyridone betaine dyes in aqueous solutions of a block copolymer. Our analysis was supported by molecular dynamics simulations that mimicked their interactions with the micellar environment (Scheme 1).

2. Experimental

2.1. Materials and experimental methods

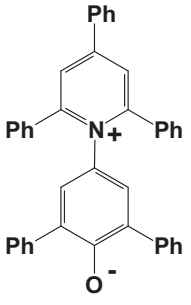
UV–Vis spectra were recorded with a SCINCO S-4100 spectrometer equipped with 10-mm-pathlength thermostatted cells. ^{1}H NMR spectra were obtained with a Bruker Avance 400 MHz equipment.

Polymer poly(ethyleneglycol)-block-poly(propylene glycol)-block-(polyethylene glycol) [$(PEG)_m$-$(PPG)_n$-$(PEG)_m$], of average mass M_n 1900 and PEG 50 wt%, was purchased from Aldrich.

Betaine dyes **1**, **2** and **3** were prepared from the corresponding pyridinium fluoroborates [12], as described previously [7]. Basic micellar solutions of the betaine dyes were prepared from aqueous 0.5 M NaOH solutions and a polymer concentration of 0.06 M. This concentration was chosen so as to ensure a convenient temperature range for the observation of the equilibrium between unimers and micelle.

2.2. Theoretical calculations

For the dynamics simulation studies, we employed as a model an A-B-A copolymer composed of two blocks of eleven ethyleneglycol units and one block of sixteen propyleneglycol units, $(PEG)_{11}$-$(PPG)_{16}$-$(PEG)_{11}$. This model, with a PEG content of *ca* 60 wt %, was small enough for our calculations and at the same time reasonably similar to the structure of the employed polymer.



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Scheme 2.

The molecular structure of our model polymer was optimised with the AM1 method. The molecular structures of dyes **1** and **3** were optimised with the Gaussian03 package [13], at the HF/6-31G* level. Partial charges for both molecules were then calculated with the CHELPG option, employing the hybrid DFT B3LYP/6-31G* method.

The micellar structure was built from a spherical aggregate of *ca*. 135 Å diameter, composed of 50 U-shaped polymeric chains that were assembled with Packmol [14]. Movement restraint for all atoms could be varied from 0 (complete freedom) to 1 (complete restraint). In order to maintain the original spherical structure, a restraint of 0.2 was applied to all carbon atoms of the chains. This aggregate was then inserted into a solvent box of 155 × 155 × 155 Å^3 containing 96,160 water molecules. Each of the water molecules was treated as a rigid three-point charge model, with an OH distance of 0.9572 Å and HOH angle of 104.5° (TIP3 water model). A dynamics simulation was then performed with the Nanoscale Molecular Dynamics (NAMD) code [15], employing periodic boundary conditions and an isothermal-isobaric (NPT) ensemble, attaining a final temperature of 310 K. The simulation protocol consisted of an initial minimization of the system in 10,000 steps. It was then heated to 310 K in 3000 steps, and then equilibrated in 1,000,000 steps, until its root-mean-square deviation (RMSD) varied by less than 10%. Finally, an acquisition period of 1.0 ns was adopted, with frames stored in intervals of 1.0 fs.

The dye molecule **1** or **3** was then positioned in the interface between the micelle and the aqueous bulk solution and the same dynamics protocol was followed to obtain the final position of the dye inside the micelle.

The radial distribution function (RDF) of the water molecules around the phenolate oxygen of the dye was obtained with the RDF.tcl script of the Visual Molecular Dynamics (VMD) program [16], which was also employed for 3-D visualization of all systems.

3. Results and discussion

3.1. UV–Vis spectroscopic results

In order to compare the effect of their different lipophilicities on their solvatochromic behavior in a 0.5 M NaOH aqueous solution, the spectra of dyes **1**–**3** were first recorded in water at 25 °C. The obtained λ_{max} values at 390 ± 2 nm were practically the same for the three dyes.

The thermochromic behavior of the dyes in water was next examined, by recording their spectra in the range of temperatures 27–47 °C. In all cases, very small (<5 nm) bathochromic shifts of the longest-wavelength band were observed with the increasing temperature. This small effect, illustrated for dye **1** in Fig. 1, was nearly the same for the three analogs.

We could thus conclude that the intrinsic responses of the three analogs in water, at constant or variable temperatures, did not differ appreciably.

When the block copolymer (0.06 M) was added to the basic solution at 27 °C, the solvatochromic band of the dyes, recorded previously in the homogeneous basic medium, shifted bathochromically by 20–40 nm. These shifts were larger than any previously observed shift in a homogeneous medium and varied with the dye lipophilicity, increasing with the size of the *N*-alkyl chain in the order *N*-methyl (21 nm) < *N*-butyl (30 nm) < *N*-octyl (41 nm). Fig. 2 compares the spectra of the three dyes at this temperature.

As the temperature was raised from 27 °C to 47 °C, the maxima observed at a lower temperature gradually disappeared, giving rise to new maxima at higher temperatures. The whole process is

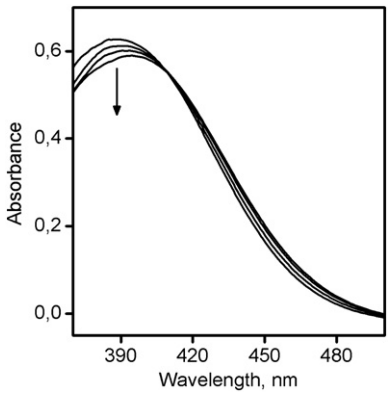


Fig. 1. Variation with temperature of the spectrum of betaine dye **1** (*ca.* 1.3 mM) in an aqueous 0.5 M NaOH solution. Spectra were recorded in the range 27–47 °C.

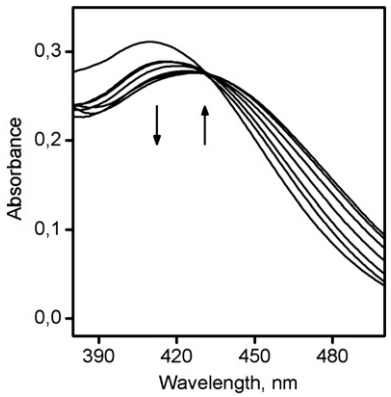


Fig. 3. – Variation with temperature, in the range 27–47 °C, of the spectrum of dye **1** (*ca.* 0.6 mM) in an aqueous 0.5 M NaOH solution, in the presence of the block polymer (0.06 M).

illustrated in Figs. 3–5 for each of the three dyes. Again, the λ_{max} values shifted differently for the three dyes, with the rise in temperature. Bathochromic shifts increased in the order of the increasing *N*-alkyl length: *N*-methyl (18 nm) < *N*-butyl (27 nm) < *N*-octyl (30 nm). The existence of isosbestic points for **1**–**3** in the three graphs was an indication that in each case an equilibrium was established between the dye in two different microenvironments, as observed previously for the $E_T(30)$ dye in aqueous solutions of different block copolymers [8]. At a lower temperature the block copolymer exists in the form of disperse unimers in solution, in what is described as a pre-micellar stage (Fig. 2). As the temperature is raised, the unimer chains start to aggregate forming micelles.

Table 1 compares the λ_{max} values for the three betaine dyes in the three different microenvironments (basic aqueous solution, pre-micellar and micellar stages). For the sake of comparison, λ_{max} values for solutions of the three dyes in pure solvents or solvent mixtures are also given, with values close to those recorded in the presence of the surfactant [$(PEG)_m$-$(PPG)_n$-$(PEG)_m$] employed in this study. An inspection of Table 1 eliminates "intrinsic" lipophilic contributions to account for the distinct behavior of the dyes in the micro-heterogeneous system. In water their λ_{max} values were practically the same, and were not affected greatly by a rise in temperature. However, in the presence of the block polymer, different affinities for the hydrophobic domains generated by the added surfactant are reflected in different λ_{max} values for the three dyes, both in the pre-micellar and the micellar stage. If we compare these values with those of the same dyes in homogeneous media, it must be concluded that the "polarity" of the polymer solution, both in the pre-micellar and in the micellar stages, is differently assessed by the three dyes. For example, dye **1** characterized the polarity of the polymer solution in the pre-micellar stage as equivalent to that of a 1:2 methanol-water mixture (λ_{max} = 409 nm), dye **2** as a 1:1.3 methanol-water mixture (λ_{max} = 419 nm) and dye **3** as a 5:1 methanol-water mixture (λ_{max} = 430 nm). For the micellar stage, the solution polarity was found equivalent to that of a 5:1 methanol-water mixture (λ_{max} = 428 nm), according to dye **1**; to that of a 1:2 2-propanol-water mixture (λ_{max} = 448 nm), according to dye **2**; and to a 5:1 2-propanol-water mixture (λ_{max} = 462 nm), according to dye **3**.

In the pre-micellar stage, in spite of their differences, all betaine dyes sensed a less hydrophilic environment than that of pure water, as a result of their proximity to isolated polymer chains or unimers. At this stage, dynamics simulation suggests that while the more hydrophilic PEG chains of the unimers are freely solvated by bulk water, the PPG block coils up to provide a more hydrophobic domain where the lipophilic dye is lodged [8]. Affinity for this hydrophobic domain increases with the dye lipophilicity.

In the micellar stage, more hydrophobic than the pre-micellar stage, some degree of insertion of the dye into the polymeric micelle is postulated, to account for the observed bathochromic shifts. Again, the degree of insertion into the micelle depends on the lipophilic *N*-alkyl chain, being largest for the *N*-octyl derivative **3**.

It is clear then, that the "polarity" of the polymer solution, in the pre-micellar or in the micellar stage, is differently assessed by compounds **1**, **2** and **3**, not only because they have slightly different intrinsic responses, due to their different lipophilicities, but also

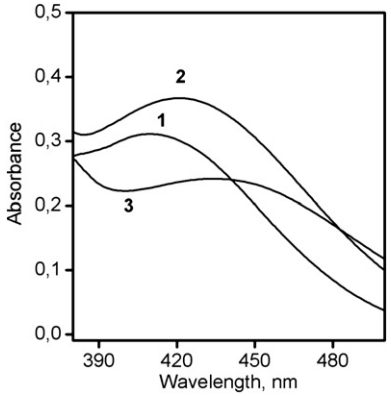


Fig. 2. Spectra of betaine dyes **1**, **2** and **3** (average concentration *ca.*1 mM) in an aqueous 0.5 M NaOH solution of the block polymer (0.06 M), at 27 °C.

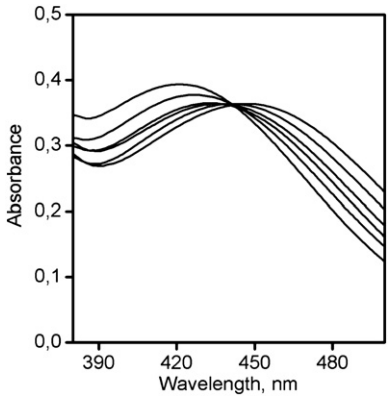


Fig. 4. Variation with temperature, in the range 27–47 °C, of the spectrum of dye **2** (*ca.* 0.8 mM) in an aqueous 0.5 M NaOH solution, in the presence of the block polymer (0.06 M).

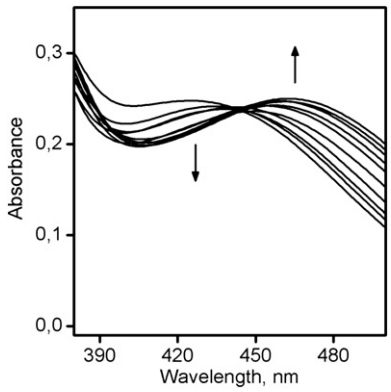


Fig. 5. Variation with temperature, in the range 27–47 °C, of the spectrum of dye **3** (*ca.* 0.5 mM) in an aqueous 0.5 M NaOH solution, in the presence of the block polymer (0.06 M).

and mainly because they are situated in different microenvironments in solution.

3.2. ^1H NMR results

A comparison of the ^1H NMR spectra of the polymer solution in D_2O at different temperatures was carried out, in the absence and presence of dyes **1** and **3**. The location of an aromatic substrate within a polymeric micelle may be investigated with this technique, which relies on the effect of the aromatic ring current on the chemical shift of neighboring polymeric chain protons [17–19]. Examples pertinent to our discussion are studies, employing this technique, of the interactions of 2,6-diisopropylphenol ("propofol") [19] and of 2-hydroxy-4-methoxyacetophenone ("paeonol") [20] with different poloxamers in water.

At 27 °C, the spectrum of the studied poloxamer in D_2O (80 mg in 10 mL, *ca.* 6 mM) exhibited a doublet at δ 1.09, corresponding to the PPG methyl group, a singlet at δ 3.63, corresponding to the more hydrophilic PEG methylenes, and a multiplet in the range of δ 3.44–3.58, that was assigned to the OCH–CH_2O protons of the hydrophobic PPG block. At 50 °C these signals became broader. The methyl doublet coalesced to a broad singlet at δ 1.36, the OCH–CH_2O protons to a broad singlet at δ 3.73 and the PEG methylenes to a broad signal at δ 3.90. Addition of dye **1** or **3** (*ca.* 1% w/w relative to the copolymer) to the poloxamer solutions at 27 °C caused practically no change to the spectrum obtained in the absence of the dye. However, at 50 °C, all poloxamer protons exhibited detectable low-frequency shifts of their signals (>0.01 ppm). Table 2 lists all the observed shifts, for the two dyes at 27 °C and 50 °C.

In the case of dye **1**, at 50 °C, all signals corresponding to the PEG and PPG methylenes, and to the PPG methyl group exhibited low-frequency shifts of 0.02, when compared with the spectrum of the pure polymer at this temperature. In the case of the more hydrophobic dye **3**, a similar trend was observed, but the low-frequency shifts were larger (0.03 ppm).

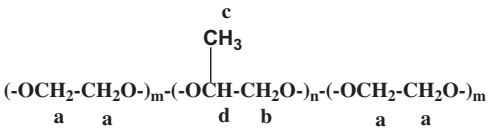
These results may be compared with a systematic study of the interactions of a hydrophobic drug ("paeonol") with poloxamer P103 in water at various temperatures [20]. Under experimental conditions that approached the relative concentrations employed by us, in the presence of the drug, the signal corresponding to the PPG methyl group of poloxamer P103 exhibited a low-frequency shift at temperatures above the polymer critical micelle concentration (CMT), that was of the same magnitude as those observed in the present work. This shift was accompanied by a signal broadening at high temperatures, also in agreement with our observations. The signal shift and broadening were taken as evidence for the replacement of water molecules by the hydrophobic drug in the vicinity of the PPG methyl groups. The low-frequency shift was an indication of the increased binding affinity of the hydrophobic compound for the polymeric micelle at higher temperatures [20]. The similarity with our observations allows a similar interpretation

Table 1
Values of the solvatochromic band of betaines **1**–**3** in different microenvironments, in aqueous basic solutions of the block copolymer.

Dye	λ_{max}/nm		
	I[a]	II[b]	III[c]
1	388	409[d,e]	427[d,f]
2	390	420[d,g]	447[d,h]
3	392	433[d,i]	463[d,j]

[a] In a 0.5 M NaOH solution.
[b] In the pre-micellar stage.
[c] In the micellar stage.
[d] Solvent mixtures of equivalent polarity.
[e] 1:2 MeOH–H_2O (λ_{max} = 409 nm).
[f] 5:1 MeOH–H_2O (λ_{max} = 428 nm).
[g] 1:1.3 MeOH–H_2O (λ_{max} = 419 nm).
[h] 1:2 2-PrOH–H_2O (λ_{max} = 448 nm).
[i] 5:1 MeOH–H_2O (λ_{max} = 430 nm).
[j] 5:1 2-PrOH–H_2O (λ_{max} = 462 nm).

Table 2
Variations of the chemical shifts of the studied poloxamer in D_2O, at 27 °C and 50 °C, in the absence and presence of the betaine dyes **1** and **3**.



Added dye[a]	t/°C	Chemical shifts/δ			
		H-a	H-b	H-c	H-d
None	27	3.63	3.44–3.54	1.09	3.56–3.58
None	50	3.90	3.73	1.36	–[b]
1	27	3.63	3.44–3.54	1.09	3.56–3.58
1	50	3.88	3.71	1.34	–[b]
3	27	3.64	3.45–3.53	1.10	3.56–3.58
3	50	3.87	3.71	1.33	–[b]

[a] 1% w/w of added dye, relative to the polymer (concentration *ca.* 6 mM).
[b] merged with the H-a peak.

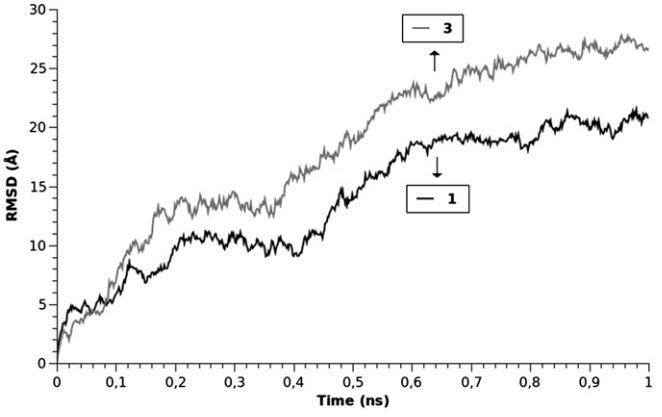


Fig. 6. Variations of the ratios of minimum-square-distances (RMSD) with the number of steps for the dynamics simulation of the insertion of dyes **1** and **3** inside the polymeric micelle.

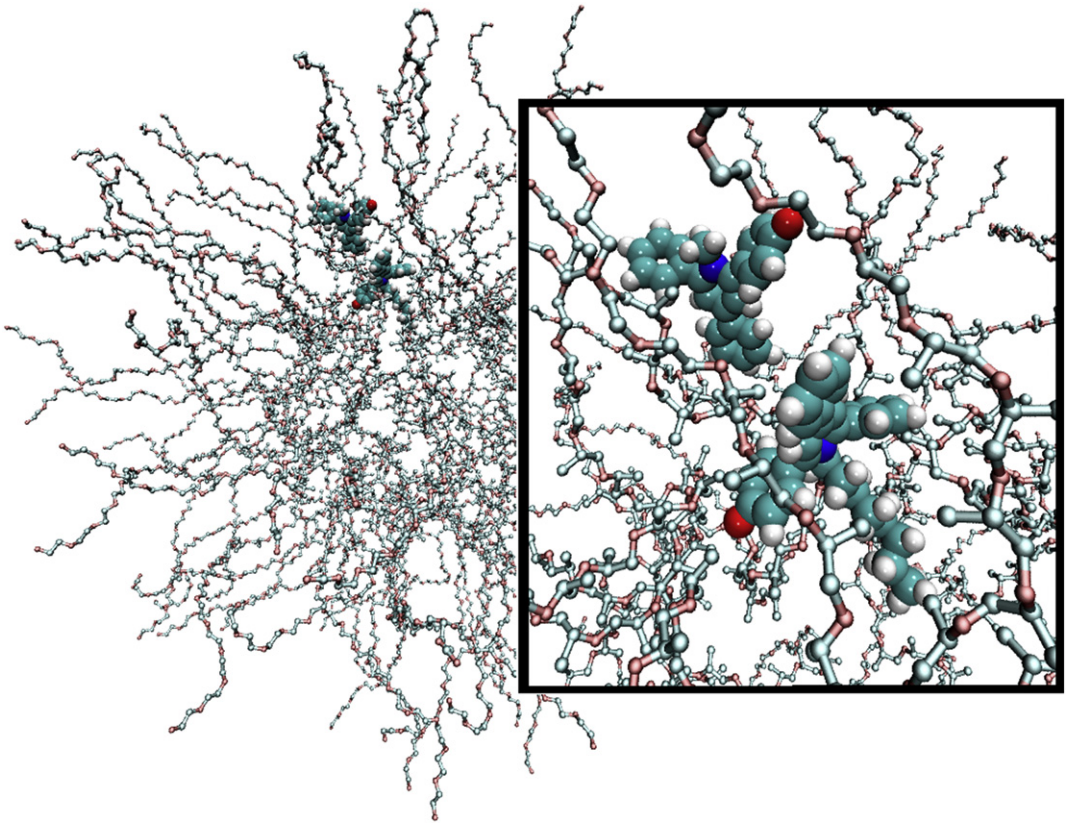


Fig. 7. Positions of betaine dyes **1** and **3** inside the micelle at the end of the dynamics simulation process.

for the qualitative behavior of dyes **1** and **3** in the presence of the block copolymer: inside the micelle, they do not discriminate between the crown PEG groups and the more hydrophobic PPG methylene or methyl groups. However, betaine dye **3** interacts more strongly with the polymer than betaine dye **1**, a conclusion that stems from the larger shielding effect of its aromatic rings on the polymeric chain protons.

3.3. Dynamics simulation results

Dynamics simulations of the studied system were then performed to seek support to the conclusions reached with the spectral analyses.

A micelle composed of 50 polymeric chains was generated, following suggestions from the literature that mentioned this as a "typical aggregation number" for aqueous poloxamer solutions [21]. A molecule of dye **1** was placed on the water-micelle borderline and a 1-ns simulation was performed. The same protocol was applied to dye **3**.

A plot of the root-mean-square-deviation (RMDS) of the system against the number of simulation steps for both dyes is shown in Fig. 6. The figure depicts the paths of both dyes, as they gradually penetrate the hydrophilic crown of the micelle. Starting from the same position as its more hydrophilic analog **1**, the more lipophilic betaine dye **3** penetrates deeper inside the micelle, ending up in a more hydrophobic environment than **1**. Their relative positions inside the micelle at the end of the simulation are reproduced in Fig. 7. Notice that both betaine dyes are localized in the interface between the crown and core regions, so that in their interactions they do not discriminate between the PEG and PPG units of the polymeric chain. This theoretical result is thus in agreement with the conclusions of the ^{1}H NMR chemical shift studies at variable temperature of Section 3.2. The stronger interactions between the polymeric micelle and the more hydrophobic betaine dye **3**, suggested by these studies, are reinforced by a more quantitative picture of their relative insertion, provided by Fig. 8, which is a plot of the distance of the oxygen atom of dyes **1** and **3** from the center of the micelle in the course of the simulation. It can be seen that both dyes reduced their distance from the center of the micelle until, after 1 ns, both systems reached an equilibrium, with dye **3** buried deeper inside the micelle (with an average distance between its O atom and the micelle center of 4 Å), than dye **1** (with a corresponding distance of 5.5 Å). Their environmental differences may be compared more clearly with a graph of the radial distribution functions (RDF) of the water molecules in the vicinity of the oxygen atom of both dyes. These graphs for dyes **1** and **3** are compared in Fig. 9. The relatively open micellar structure hosts a significant number of water molecules, so that, even inside the micelle, a solvation layer of water molecules builds around the negatively

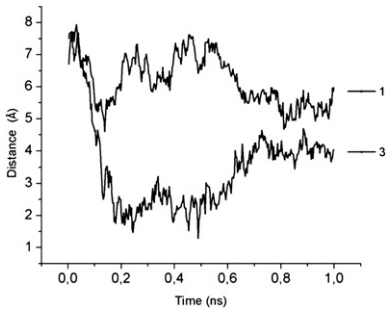


Fig. 8. Variation of the distance of the oxygen atom of dyes **1** and **3** from the micelle center in the course of the simulation process.

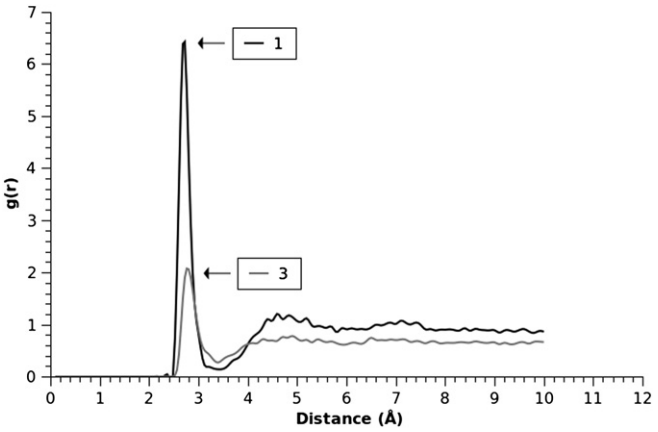


Fig. 9. Radial distribution functions of the water molecules in the vicinity of the oxygen atom of betaine dyes **1** and **3**. The area under the curves integrates to *ca.* 3 and 1 water molecules for the solvation layers of the two betaine dyes, respectively.

charged oxygen atom of these betaine dyes. Fig. 9 reveals that, although these solvation layers occur at the same distance from the oxygen for both dyes, the layer of the more hydrophilic dye **1** comprises a higher (*ca.* 3) number of water molecules than the layer of **3** (*ca.* one solvating molecule). Thus, dye **3** senses a less hydrophilic environment than dye **1**, a result that is also in agreement with the larger shielding of the polymeric chains by the *N*-octyl derivative **3** inside the micelle.

4. Conclusions

Both the experimental evidence from UV–Vis and ^{1}H NMR spectroscopic measurements and the results of molecular dynamics simulations presented in this paper shed light on the role of the lipophilicity of analogous solvatochromic dyes in assessing the polarity of microheterogeneous systems. It was shown that, although analogous dyes with different lipophilic chains may exhibit slightly different solvatochromic responses in homogeneous media, such intrinsic differences cannot account for their distinct behavior in a microheterogeneous system. In the presence of a micelle, the different responses of these dyes reflect their different degrees of insertion into the hydrophobic core of the micelle. Conclusions based on the response of a single probe about the "polarity" of a micellar system are thus shown to be too simplistic. The use of a set of analogous solvatochromic probes of variable lipophilicity for measuring the polarity of micelles opens the possibility of assessing different microenvironments within these systems and of mapping them more precisely.

Acknowledgements

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