The thermochromism of the E-T(30) betaine in a micro-heterogeneous medium: A spectral and dynamics simulation study

4 AUTHORS, INCLUDING:

Carolina Aliaga
University of Santiago, Chile
44 PUBLICATIONS **541** CITATIONS

Marcos Caroli Rezende
University of Santiago, Chile
141 PUBLICATIONS **1,181** CITATIONS

Cristian Tirapegui
University of Chile
4 PUBLICATIONS **34** CITATIONS

The thermochromism of the $E_T(30)$ betaine in a micro-heterogeneous medium: A spectral and dynamics simulation study

Carolina Aliaga [a,b], Luis Briones [c], Marcos Caroli Rezende [a,*], Cristián Tirapegui [a]

[a] Facultad de Química y Biología, Universidad de Santiago, Santiago, Chile
[b] Centro de Desarrollo de Nanociencia y Nanotecnología, CEDENNA, Universidad de Santiago, Santiago, Chile
[c] Facultad de Ingeniería, Universidad de Talca, Talca, Chile

ARTICLE INFO

ABSTRACT

A spectral investigation of the thermochromic behavior of Reichardt's $E_T(30)$ betaine in aqueous solutions of block copolymers ("poloxamers") P407, P237 and P105 was carried out as a function of temperature and concentration. The betaine microenvironment at various stages of the micellization process in these systems was mimicked with the aid of molecular dynamics simulations of model systems. These consisted of the $E_T(30)$ probe in boxes of water molecules, in the presence of an isolated block copolymer of formula $(PEO)_{11}–(PPO)_{16}–(PEO)_{11}$, and of a micelle formed of 50 of these unimers.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Reichardt's $E_T(30)$ dye **1** is perhaps the most conspicuous member of a large family of solvatochromic phenolate betaines [1] (see Scheme 1).

It is remarkably sensitive to changes in its microenvironment, being able to assess differences in medium polarity (solvatochromism), temperature (thermochromism), electrolyte concentration (halochromism), medium pressure (piezosolvatochromism) and medium chirality (chirosolvatochromism). The absorption intensity of its longest wavelength band is also dependent on the acid-base equilibrium between the protonated (colorless) and the zwitterionic dye, a property that may shed light on local acidities in a micro-heterogeneous system. This wide range of responses to different environment variables has led to the coinage of the term "perichromism" to characterize its general behavior as a probe.

The thermochromism of the $E_T(30)$ betaine has been investigated in pure solvents [2–4], and in binary solvent mixtures [5–8]. In pure solvents, two different situations have emerged from the spectral variations of the probe with the temperature. In one case, in solutions of methanol [4], the observation of an isosbestic point was interpreted as arising from two differently solvated forms of the betaine in equilibrium. A second behavior was ob-

served in solvents like ethanol [4] and acetonitrile [3], where a small, but perceptible shift of the solvatochromic band of **1** was recorded with the rising temperature.

The solvatochromic behavior of **1** has also been extensively investigated in micellar systems at room temperature [9–15].

By contrast with the abundance of papers on the thermochromism of the $E_T(30)$ betaine in pure solvents and solvent mixtures, and on its solvatochromic responses in the presence of micelles at room temperature, to our knowledge, there is no study of its thermochromism in the presence of a surfactant. This observation led us to search for a convenient system to carry out these studies. Our choice of aqueous solutions of the block copolymers commonly known as "poloxamers" or "pluronics" was motivated by their well-documented temperature-dependent behavior as micelle-forming polymers.

Poloxamers are copolymers of polyethyleneoxy (PEO) and polypropyleneoxy (PPO) blocks, of general formula $[PEO]_m–[PPO]_n–[PEO]_m$. They have attracted permanent interest in the past decades, not only because of their industrial applications, but also because of their behavior in aqueous and organic solutions. The temperature-dependent formation of polymeric micelles in water is well-documented, and has been studied with a variety of techniques [16–21]. Among them, various research groups have employed dyes and fluorescent probes for the study of the micellization process and for assessing the polarity of the heterogeneous microenvironment of these surfactant solutions [22–28].
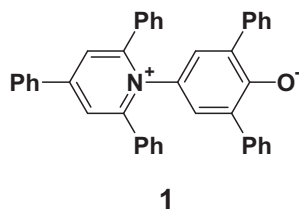
Scheme 1.

There is one report on the use of the $E_T(30)$ betaine as a polarity sensor of poloxamer solutions [29]. The reported spectroscopic results were obtained at 298 K and "apparently at the cmc" [30], making the interpretation of the obtained polarity values open to criticism. Ignoring the effect of the temperature when assessing the polarity of temperature-dependent micro-heterogeneous systems may lead to partial or ambiguous interpretations. Several questions remain unanswered, regarding the perichromic behavior of **1** in these systems: what is the meaning of a polarity value in a micro-heterogeneous system? Does it correspond to fully developed micelles, to pre-micellar unimers, or to both? Where is the probe located? How is it solvated, in comparison with its solvation in pure water?

In order to answer these questions a more detailed and systematic study is needed, that takes into consideration the effect of the temperature in these systems.

We therefore decided to investigate in detail the thermochromic behavior of the $E_T(30)$ betaine in aqueous solutions of three poloxamers (P407, P237 and P105), at variable temperature and polymer concentrations. In addition, in order to gain insight into its location and the way it is solvated in these systems, we resorted to molecular dynamics simulations. This theoretical tool had been employed before, by other groups and by us, for the description of solute–solvent interactions of the $E_T(30)$ dye [31] and of related phenolate betaines [32–34] in pure solvents. As a natural extension to a more complex system, not yet described in the literature, we applied it in the present paper to mimic the interactions of **1** with the micro-heterogeneous medium of aqueous poloxamer solutions.

2. Materials and methods

2.1. Materials

Poloxamers P407, P237 and P105 were purchased from ICI.

The $E_T(30)$ dye **1** was obtained by treatment with base of the corresponding N-hydroxyphenylpyridinium perchlorate. This compound was prepared following a published procedure [35] from the pyrylium perchlorate [36], m.p. 273 °C, lit. [37] m.p. 273–274 °C.

The N-hydroxyphenylpyridinium perchlorate thus obtained was converted into the corresponding betaine by treatment with $NaOCH_3$ in methanol, as described [37]. Spectra of the betaine prepared by us were recorded in twelve protic and non-protic solvents. The obtained maxima coincided with the tabulated values in the literature [38], in a final test of the spectral purity of our probe.

2.2. Methods

The UV–Vis spectra of **1** in aqueous poloxamer solutions were recorded with a photodiode-array UV–Vis spectrophotometer Scinco S-3100, in thermostatted cell compartments.

For the dynamics simulation studies, we employed as a model an A-B-A copolymer composed of two blocks of eleven monomeric

ethyleneoxy units and one block of 16 monomeric propyleneoxy units, $(PEO)_{11}$–$(PPO)_{16}$–$(PEO)_{11}$.

The structure of this polymer was built with InsightII [39] and optimised with the AM1 method. The structure of dye **1** was optimised with the Gaussian03 package [40], 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.

Dye **1** was docked close to the polymer molecule employing Autodock4 [41]. About half of the obtained conformations presented similar orientations of the dye, with close values of energy (*ca.* −6.5 kcal mol^{-1}). The bimolecular complex with the smallest energy was then inserted into a solvent box of $90 \times 100 \times 140$ Å^3, containing 40,330 TIP3 water molecules. A dynamics simulation was carried out with NAMD [42], employing periodic boundary conditions and an NPT ensemble, at 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, followed by its equilibration 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.

For the simulation of the dye-micelle system, a spherical aggregate of *ca.* 135 Å of diameter was built with 50 poloxamer units, starting from U-shaped polymeric chains and assembling them with Packmol [43]. In order to maintain the original spherical structure, a restraint of 0.2 was applied to all carbon atoms of the unimers. This aggregate was then inserted into a solvent box of $155 \times 155 \times 155$ Å^3 containing 96,160 TIP3 water molecules, and the above dynamics protocol followed to obtain the equilibrated micelle in water. The dye molecule **1** was then inserted into 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 within the micelle. The radial distribution function of the water molecules around the phenolate oxygen of the dye was obtained with the RDF.tcl script of VMD [44].

3. Results and discussion

3.1. Spectral observations

The three poloxamers employed in the present study constitute examples of block polymers with variable masses of the PPO and PEO blocks. They were chosen so as to compare the perichromic behavior of **1** in aqueous solutions of a large (P407), medium (P237) and small (P105) polymer, with a relatively large (P407), medium (P237) and small (P105) hydrophobic PPO core. For the sake of comparison, Table 1 illustrates their differences [45].

As can be seen in the table, the micellization temperatures (T_m) of the chosen systems cover a convenient range, with low (P407), intermediate (P237) and high (P105) T_m values for the same polymer concentration.

Spectra of **1** in aqueous solutions of poloxamers P407, P237 and P105 were recorded at variable temperatures, and with variable concentrations of the polymer.

Table 1
Compositions and temperatures of micellization of the three studied poloxamers.

Total and block masses (Da)[a]				T_m (°C)[b]	
Poloxamer	Total mass	M_{PPO}	M_{PEO}	5 mM	10 mM
P407	12,000	4000	8000	22	15
P237	7700	2250	5450	31	29
P105	1900	950	950	49	47

[a] Data from Ref. [45].
[b] Temperatures of micellization T_m for polymer concentrations of 5 and 10 mM. T_m values for other poloxamer concentrations are listed as Supplementary material.
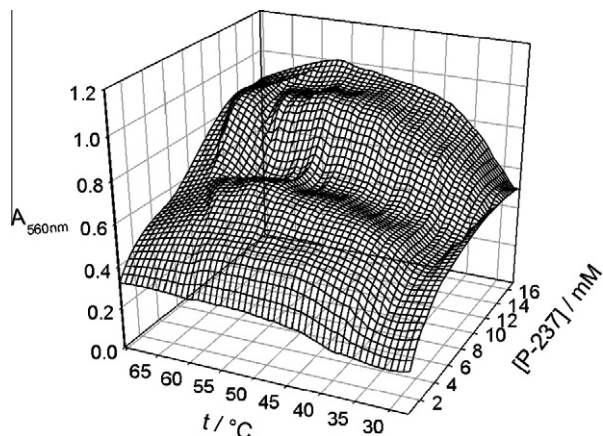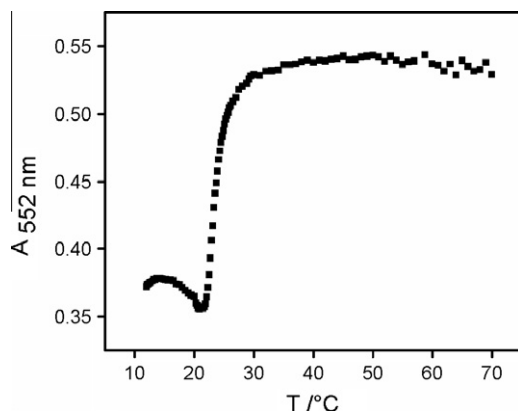
Fig. 1. Variation of the absorbance of the perichromic band of dye **1** (*ca.* 560 nm) in aqueous solutions of poloxamer P237, at variable temperature and polymer concentration.

The perichromic band of **1** was a reliable witness for the subtle changes that took place during the micellization process of aqueous poloxamer solutions, as the temperature or the polymer concentration changed. Fig. 1 illustrates this for poloxamer P237, in a three-dimensional graph of the band absorbance at 560 nm as a function of the temperature and polymer concentration.

When the polymer concentration was kept constant, a graph like the one shown in Fig. 2, for a 6-mM solution of P407, was obtained.

After a temperature range of practically constant absorbance readings, the intensity of the perichromic band at 552 nm rose steeply around 22 °C, reaching a plateau value by 30 °C. This abrupt change corresponded to the temperature where the polymeric chains ("unimers") started to aggregate to form micelles. This micellization temperature (T_m) varied inversely with the polymer concentration. Decreasing the polymer concentration led to higher T_m values. Examples of this are given in the Supplementary material, where critical micellar concentrations at various temperatures are listed for each of the three poloxamers. This and the observation that the micellization temperature also depended on the polymer structure (see Table 1) were in agreement with previous studies [16,22,24–26,45].

The steep rise in absorbance shown in Fig. 2 corresponds to an equilibrium process between molecules of probe **1** in two different environments. To illustrate this, spectra were recorded for different poloxamers at different concentrations, near the corresponding micellization temperature. As can be seen in Fig. 3, in
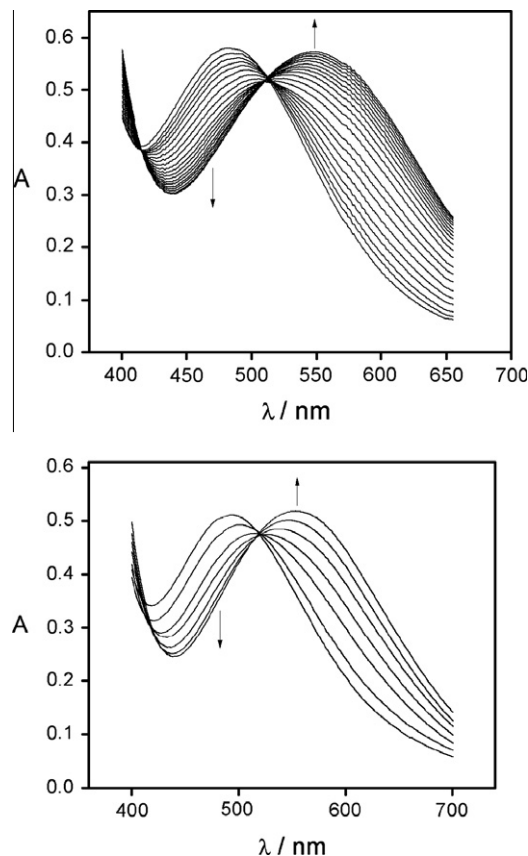
Fig. 3. Variation of the spectrum of **1** (*ca.* 40 μM) in a 10-mM solution of P407, in the temperature range of 14–20 °C (top); variation of the spectrum of **1** (*ca.* 40 μM) in a 20-mM solution of P105, in the temperature range of 42–55 °C (bottom).

spite of their structural differences, concentrations and micellization temperatures, the same behavior was observed: Fig. 3 (top), reproduces the spectra obtained for a 10-mM P407 solution, between temperatures 14 and 20 °C. A clear isosbestic point at 513 nm is an indication of an equilibrium between two species, with bands at 480 and 552 nm. Fig. 3 (bottom) shows the spectra of a 20-mM solution of P105, in the temperature range of 42–55 °C, with a very similar behavior.

The 480-nm band, corresponding to molecules of the probe in a more hydrophilic environment ($E_T(30)$ value of 59.5 kcal mol^{-1}), arises from the association between the hydrophobic sensor and isolated molecules of the polymer ("unimers"), in a pre-micellar stage. This is a less hydrophilic environment than pure water ($E_T(30)$ value of 63.1 kcal mol^{-1} [38]), but is still appreciably "open" to the aqueous bulk solution. As the temperature is raised, aggregation of these unimeric molecules to form micelles generates a second, less hydrophilic environment, characterized by a new band at 552 nm ($E_T(30)$ value of 51.8 kcal mol^{-1}), with a polarity close to that of ethanol ($E_T(30)$ of 51.9 kcal mol^{-1} [38]). As more unimers aggregate, all the betaine is transferred from the first, "open", environment to the more hydrophobic micellar core. Interestingly, the existence of an isosbestic point and the essential constancy of the λ_{max} values of both species in equilibrium, as the temperature is raised, are indications that the two environments remain essentially constant during the micellization process. A comparison of the two graphs of Fig. 3 also shows that the environments sensed by the dye at the pre-micellar and micellar stages are essentially the same for different poloxamers, in spite of different temperatures of micelle formation and polymer concentrations.

Fig. 2. Variation of the absorbance of the perichromic band of **1** at 552 nm with the temperature in an aqueous solution of poloxamer P407 (*c* = 6 mM).

Thus, the thermochromic behavior of the $E_T(30)$ betaine in these systems during the micellization process is similar to its behavior in methanol at variable temperature [4].

The existence of well-defined isosbestic points is an indication in both cases of two differently solvated forms of the betaine in equilibrium.

A different behavior had been observed in solvents like ethanol and acetonitrile [3,4], where a small, but perceptible shift of the solvatochromic band of 1 was recorded with the rising temperature. This situation was also observed in our systems, after the polymeric micelle had been formed. Further rise of the temperature led to a small but discernible bathochromic shift of the solvatochromic band. Fig. 4 depicts the spectral variations of 1 in an aqueous solution of P407 (6 mM) with the increased temperature (>40 °C), after complete formation of the polymeric micelle around 30 °C. The absence of an isosbestic point and the small bathochromic shift of the solvatochromic band are indications that, as the temperature is raised, the dye senses different, increasingly more hydrophobic environments.

Thus, depending on the temperature and polymer concentration, the spectral responses of dye 1 suggest three stages in the micellization process. In the first stage, at lower temperatures, unimers associate with the dye in an "open", more hydrophilic environment. As the temperature is raised, they start to form micelles, and the dye is gradually transferred to their more hydrophobic core. In this second stage, micelles increase in number, at the expenses of their constituting unimers, but are not altered in form or size by the increasing temperature. Dye 1, accordingly, senses the same microenvironment, the hydrophobic core of a presumably spherical micelle. With the further rise of the temperature these micelles coalesce to larger aggregates, with different size and form, lodging betaine 1 in increasingly more hydrophobic microenvironments. Limiting temperatures for the occurrence of these transformations vary with the nature of the poloxamer and its concentration.

The above interpretation rules out the existence of some contribution from the bulk aqueous medium to the observed thermochromic shifts. This may be questioned, and the matter would only be settled through a parallel study on the thermochromism of 1 in pure water. Unfortunately, the scarce solubility of betaine 1 in water [46] made this study not feasible. This poor solubility, however, is a strong indication that direct interactions between 1 and the bulk water should be much less important than between 1 and the polymer. The observed maxima of Figs. 3 and 4 point, in all cases, to the existence of microenvironments that are significantly less hydrophilic than pure water.
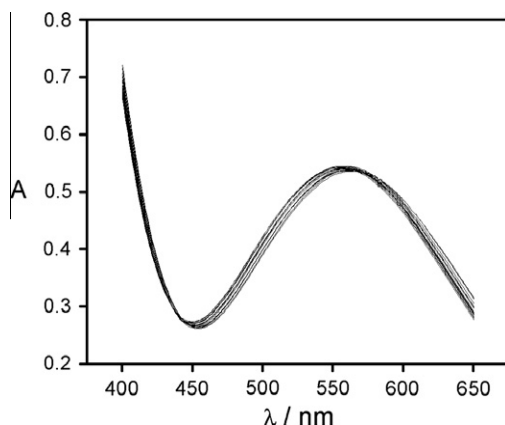
3.2. Dynamics simulation studies

Further support to our interpretations was next sought in molecular dynamics simulations of a model system.

We generated a block copolymer of the type A-B-A, where the polyethyleneoxy fragment A was made up of 11 monomeric units, and the polypropyleneoxy block of 16 monomeric units. This model polymer was conveniently small for our calculations, being at the same time reasonably similar to P105, with a total mass of ca. 2100 Da. We next docked one molecule of betaine 1 close to the obtained unimer, and soaked the resulting system into a box containing ca. 40,000 water molecules. After thermal equilibration and relaxation, a dynamics simulation of 1.0 ns resulted in partial coiling of the polymeric blocks, this coiling being more significant for the less hydrophilic PPO fragment. The betaine was situated close to the more hydrophobic PPO fragment, as shown in Fig. 5. This pose is an adequate picture of the pre-micellar unimer-dye complex, where the betaine is partially shielded from the bulk aqueous medium by the proximity to the hydrophobic coiled PPO block.

In order to investigate the interaction of the dye with a micelle of the simulated poloxamer, a spherical aggregate composed of 50 unimers was built and inserted into a cubic box of water molecules. The system was then submitted to the same protocol described above. One betaine molecule was then positioned in the interface between the micelle and the aqueous bulk solution. After the usual relaxation and equilibration, a 1.0-ns dynamics simulation was carried out. The betaine migrated to the interior of the spherical micelle, locating itself inside the crown region, very close to the its hydrophobic core (Fig. 6).

At this stage, the environment sensed by the betaine is more hydrophobic than the "open", aqueous environment of the pre-micellar phase. Nevertheless, it is still reasonably hydrophilic, because of the water molecules that permeate the crown region of the spherical micelle. This is confirmed by the radial distribution function of the water molecules around the phenolate oxygen of 1, shown in Fig. 7.

Though the concentration of water molecules in this microenvironment is smaller than in the bulk solution, they build a solvation layer centered around 2.8 Å. Integration of the corresponding peak yields the average number of water molecules that comprise this layer. Inside the micelle, it amounts on the average to nearly one molecule. This number is to be compared with the value of 2.0–2.2 molecules calculated by Mente and Maroncelli [31] for an
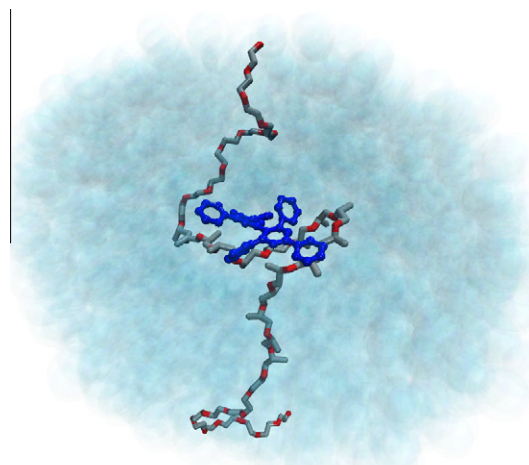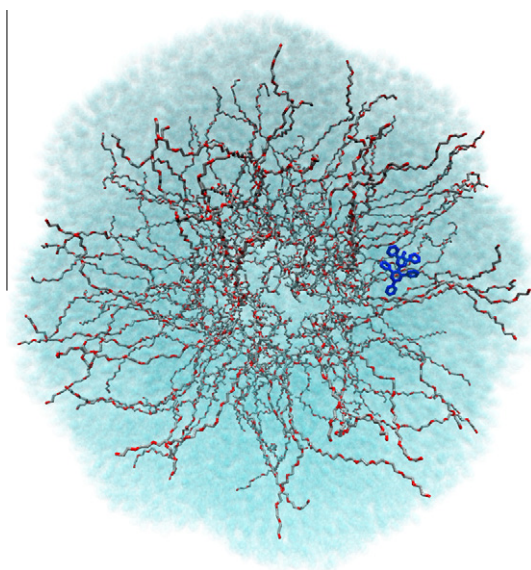

Fig. 4. Variation of the spectrum of 1 (ca. 40 μM) in a 6-mM solution of P407, in the temperature range of 40–55 °C.

**Fig. 5.** Dynamics simulation of a unimer-dye complex in a water pool, in an aqueous poloxamer solution built with a $(PEO)_{11}–(PPO)_{16}–(PEO)_{11}$ poloxamer. For the sake of clarity, hydrogen atoms are omitted.

Fig. 6. Dynamics simulation of betaine **1** inside a spherical micelle comprising 50 units of a block polymer $(PEO)_{11}$–$(PPO)_{16}$–$(PEO)_{11}$ in water. For the sake of clarity, hydrogen atoms are omitted.
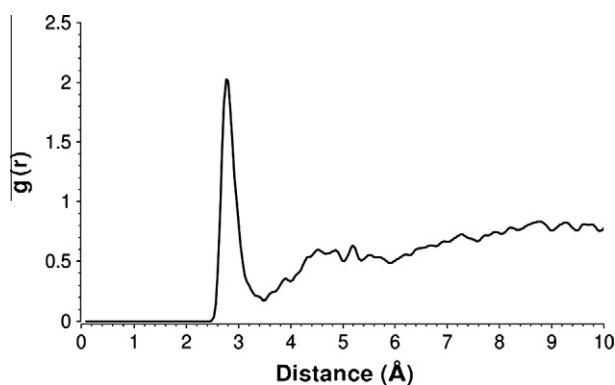
Fig. 7. Radial distribution of the water molecules around the phenolate oxygen of **1**, when the dye is buried inside the crown region of the spherical micelle of Fig. 6.

$E_T(30)$ solution in pure water, and with values of 2.3–2.8 molecules, calculated by us for other less sterically hindered phenolate betaines in water [34]. The smaller number of water molecules present in the solvation layer of the phenolate group of **1** inside the micelle is in agreement with the spectral observations of a less polar microenvironment, with a polarity value close to that of ethanol.

The above results from dynamics simulations provide us with a clear picture of the microenvironments sensed by betaine **1** in poloxamer solutions, as the temperature is raised. Interestingly, our results confirm what other authors had cautiously suggested, nearly 10 years ago, for the behavior of **1** in aqueous solutions of another polymer (PEG-2000) [30]: "the dye appears to have "partitioned" to a discreet and observable region of the solution and, thus, the solution is apparently not homogeneous with respect to the solute that it contains."

4. Summary

The present work was an investigation of the nature of the microenvironments sensed by the $E_T(30)$ dye in aqueous solutions of three different poloxamers, with varying temperature and polymer concentrations. The $E_T(30)$ betaine proved faithful to its description [47], as a true princess of Christian Andersen's fairytale "The Princess and the Pea", capable of feeling a pea through layers of several mattresses, and of sensing subtle environmental differences during the micellization process of these polymers.

Spectra of the dye in aqueous solutions of poloxamers P407, P237 or P105, recorded at increasing temperatures, revealed the existence of an equilibrium between two differently solvated dye molecules, one in a more hydrophilic environment, identified as a dye-unimer complex, in a relatively "open" aqueous medium, and the other, more hydrophobic, attributed to the dye inside the fully formed polymeric micelle. A subtle environmental change was also detected within the micelle of a P407 solution, as the solution temperature was raised. This was attributed to geometry modifications and further aggregation of the spherical micelle with temperature, leading to more dehydrated, hydrophobic microenvironments.

Simulations of a model block copolymer in a water box yielded more detailed pictures of the different stages of the micellization process, and of the interactions of the $E_T(30)$ dye with the premicellar unimer and with the polymeric micelle. The resulting pictures lent support to our interpretation of the solvatochromic shifts of **1** in the various systems. The polarity value of the micellar microenvironment, which was very close to that of pure ethanol, was also rationalized by the simulation results, by consideration of the radial distribution of water molecules that solvate the phenolate oxygen of **1**. The number of water molecules in this solvation layer was significantly smaller than the value calculated by us [34] and other groups [31] for phenolate betaine dyes in pure water. This result was thus an indication of a moderately polar microenvironment sensed by the dye in the micelle, permeated by quite a few water molecules.

In conclusion, our results describe and interpret the thermochromism of the $E_T(30)$ betaine in the presence of a surfactant, rationalizing the spectral observations by means of molecular dynamics simulations.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2010.05.088.

References

[1] C. Reichardt, Pure Appl. Chem. 80 (2008) 1415.
[2] W. Linert, R.F. Jameson, J. Chem. Soc. Perkin Trans. 2 (1993) 1415.
[3] X. Zhao, J.A. Burt, F.J. Knorr, J.L. McHale, J. Phys. Chem. A 105 (2001) 11110.
[4] X. Zhao, F.J. Knorr, J.L. McHale, Chem. Phys. Lett. 356 (2002) 214.
[5] E. Bosch, M. Rosés, K. Herodes, I. Koppel, I. Leito, I. Koppel, V. Taal, J. Phys. Org. Chem. 9 (1996) 403.
[6] E.B. Tada, P.L. Silva, Phys. Org. Chem. 16 (2003) 691.
[7] P.L. Silva, E.L. Bastos, O.A. El Seoud, J. Phys. Chem. B 111 (2007) 6173.
[8] C.T. Martins, M.S. Lima, E.L. Bastos, O.A. El Seoud, Eur. J. Org. Chem. 7 (2008) 1165.
[9] M.A. Kessler, O.S. Wolfbeis, Chem. Phys. Lipids 50 (1989) 51.
[10] J. Kriwanek, R. Miller, Colloids Surf., A 105 (1995) 233.
[11] N.A. Vodolazkaya, N.O. Mchedlov-Petrossyan, G. Heckenkemper, C. Reichardt, Liquids 107 (1–3) (2003) 221.
[12] R.V. Pereira, M.H. Gehlen, Spectrochim. Acta A 61 (2005) 2926.
[13] S. Basu, D.R. Vutukuri, S. Thayumanavan, J. Am. Chem. Soc. 127 (2005) 16794.
[14] N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, A.A. Kornienko, E.L. Karyakina, C. Reichardt, Langmuir 21 (2005) 7090.
[15] N.O. Mchedlov-Petrossyan, Pure Appl. Chem. 80 (2008) 1459.
[16] P. Alexandridis, J.F. Holzwarth, T.A. Hatton, Macromolecules 27 (1994) 2414.
[17] B. Chu, Langmuir 11 (1995) 414.
[18] K. Lee, S.C. Shin, I. Oh, Arch. Pharmacal Res. 26 (2003) 653.

[19] J. Ma, Ch. Guo, Y. Tang, J. Xiang, Sh. Chen, J. Wang, H. Liu, J. Colloid Interface Sci. 312 (2007) 390.
[20] X. Liang, Ch. Guo, J. Ma, J. Wang, Sh. Chen, H. Liu, J. Phys. Chem. B 111 (2007) 13217.
[21] H. Walderhaug, O. Söderman, Curr. Opin. Colloid Interface Sci. 14 (2009) 171.
[22] K. Nakashima, T. Anzai, Y. Fujimoto, Langmuir 10 (1994) 658.
[23] T. Nivaggioli, P. Alexandridis, T.A. Hatton, A. Yekta, M.A. Winnik, Langmuir 11 (1995) 730.
[24] R. Wang, H. Knoll, F. Rittig, J. Kärger, Langmuir 17 (2001) 7464.
[25] C.D. Grant, M.R. DeRitter, K.E. Steege, T.A. Fadeeva, E.W. Castner Jr., Langmuir 21 (2005) 1745.
[26] R. Bandula, M. Vasilescu, H. Lemmetyinen, J. Colloid Interface Sci. 287 (2005) 671.
[27] P. Sen, S. Ghosh, K. Sahu, S.K. Mondal, D. Roy, K. Bhattacharyya, J. Chem. Phys. 124 (2006) 204905.
[28] N.A. Begum, N. Roy, S. Mandal, S. Basu, D. Mandal, J. Lumin. 129 (2009) 158.
[29] J.R. Lopes, W. Loh, Langmuir 14 (1998) 750.
[30] J.G. Huddleston, H.D. Willauer, S.T. Griffin, R.D. Rogers, Ind. Eng. Chem. Res. 38 (1999) 2523.
[31] S.R. Mente, M. Maroncelli, J. Phys. Chem. B 103 (1999) 7704.
[32] M.Z. Hernandes, R. Longo, K. Coutinho, S. Canuto, Phys. Chem. Chem. Phys. 6 (2004) 2088.
[33] T.L. Fonseca, K. Coutinho, S. Canuto, Chem. Phys. 349 (2008) 109.
[34] C. Mascayano, M.C. Rezende, C. Mendez, G. Núñez, V. Chiang, J. Solution Chem. 38 (2009) 363.
[35] M.C. Rezende, C.M. Radetski, Quim. Nova 11 (1988) 353.
[36] K. Dimroth, Angew. Chem. 72 (1960) 331.
[37] K. Dimroth, C. Reichardt, T. Siepmann, F. Bohlmann, Liebigs Ann. Chem. 661 (1963) 1.
[38] C. Reichardt, Solvents and Solvent Effects in Organic Chemistry, third ed., VCH-Wiley, Weinheim, 2003.
[39] I.I. Insight, Accelrys, San Diego, USA, 1998.
[40] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford CT, 2004.
[41] G.M. Morris, D.S. Goodsell, R. Huey, W. Lindstrom, W.E. Hart, S. Kurowski, S. Halliday, R. Belew, A.J. Olson, Autodock 4.0: A free Docking Software <http://autodock.scripps.edu>, 2007.
[42] J.C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kalé, K. Schulten, J. Comput. Chem. 26 (2005) 1781.
[43] J.M. Martínez, L. Martínez, J. Comput. Chem. 24 (2003) 819.
[44] W. Humphrey, A. Dalke, K. Schulten, J. Mol. Graphics 14 (1996) 33.
[45] A.E. Beezer, W. Loh, J.C. Mitchell, P.G. Royall, D.O. Smith, M.S. Tute, J.K. Armstrong, B.Z. Chowdhry, S.A. Leharne, D. Eagland, N.J. Crowther, Langmuir 10 (1994) 4001.
[46] C. Reichardt, D. Che, G. Heckenkemper, G. Schäfer, Eur. J. Org. Chem. (2001) 2343.
[47] C. Reichardt, Chem. Soc. Rev. 21 (1992) 147.