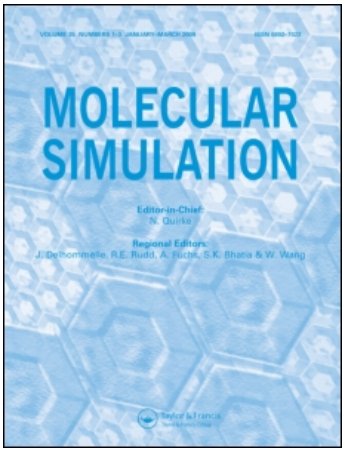

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Amphiphilic Block Copolymer Micelles with Hydrophobically Modified Shells

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The conformational behavior of hydrophobically modified shell-forming poly(methacrylic acid) (PMA) blocks in 1,4-dioxane–water solutions of polystyrene-*block*-poly(methacrylic acid) (PS-PMA) micelles was studied by a combination of several experimental methods (static and quasielastic light scattering, fluorescence techniques and atomic force microscopy). The nonradiative energy transfer (NRET) studies with fluorescently tagged samples show that the hydrophobic anthracene groups attached to the ends of PMA blocks try to avoid the aqueous medium and return closely to the PS core, forcing PMA chains to loop back. Computer modeling (Monte Carlo and mean-field calculations) was performed to get better insight into the conformational behavior. Computer studies reproduce the behavior on the semi quantitative level.

Keywords: Micelles; Conformation; NRET; Copolymers

INTRODUCTION

Diblock and triblock copolymers AB and ABA form multimolecular spherical micelles with compact insoluble cores B and protective shells A, when dissolved in a selective organic solvent/precipitant (solvent for block A and a precipitant for B) [1]. Micellization usually occurs spontaneously upon dissolution of copolymer samples. Due to the compactness of the core, micelles are fairly small for their molar mass, which is usually in the range 10^6–10^7 g/mol. The core radius is *ca.* 10–14 nm, while the hydrodynamic radius of the micelle ranges from 25 to 40 nm. The micellization obeys the scheme of closed association, which means that fairly monodisperse micelles coexist in equilibrium with a low fraction of nonmicellized chains (unimers).

Amphiphilic copolymers, e.g. hydrophobic (nonpolar)-hydrophilic (polyelectrolyte) diblocks, so called block polyelectrolytes, also form multimolecular micelles in aqueous media. Since water is a very strong precipitant for nonpolar blocks, the high molar mass samples are insoluble in water and micelles have to be prepared indirectly. Several years ago we developed a dialysation technique which yields well defined and fairly monodisperse block polyelectrolyte micelles with high reproducibility [2]. The sample, e.g. polystyrene-*block*-poly(methacrylic acid) (PS-PMA), is dissolved in a mixture of 1,4-dioxane (80 vol%) and water. This mixture is a mild selective precipitant for PS and micelles with swollen PS cores form spontaneously. Then the solution of micelles is dialyzed against mixtures with increasing water content and finally against aqueous buffers. During dialysis the thermodynamic quality of the solvent deteriorates for PS. The association number increases and the core shrinks. At certain water content that depends on the lengths of blocks, the mixture becomes very strong precipitant for PS and the micellization equilibration freezes. The unimer concentration drops to zero and cores become so compact that any exchange of chains between micelles stops. With the further increase in the water content, the association number does not change. It is important to realize that the association number of frozen

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micelles is not controlled by the thermodynamic quality of the solvent in which the micelles are studied, but by the quality of that aqueous solvent in which the equilibrium freezes. However the behavior of the shell depends on the final solvent.

Water-soluble polymeric micelles and kinetically frozen polymeric nanoparticles have been studied by a number of research groups in the last decade because they offer interesting potential applications in various fields, e.g. in drug and gene delivery [3,4]. The number of experimental studies is so vast that it is futile to give all relevant references. Therefore we refer only to a book by Hamley, which reviews recent studies in the field [5]. Some authors have been considering the attachment of recognition groups at the ends of water-soluble blocks of amphiphilic block copolymers [6,7]. If the recognition groups have some degree of hydrophobicity, they do not have to be localized at the periphery of micelles. Therefore the spatial distribution of modified ends of the shell-forming blocks has to be investigated in detail. Because of the complexity of biocompatible nanoparticles, experimental studies on simple, stable and well-defined model systems together with Monte Carlo and mean-field calculations are needed.

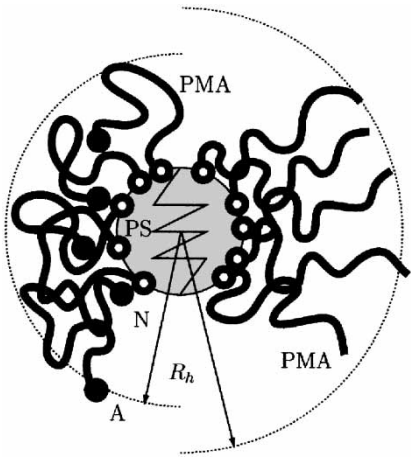
We have been studying micellization of amphiphilic block copolymers (mostly block polyelectrolytes) both experimentally and theoretically for more than ten years. Recently we investigated micellar systems with modified ends of the water-soluble blocks [8,9]. In this paper, we study the shell structure and the distribution of hydrophobic tags in the shell by Monte Carlo and mean-field calculations. According to referee's suggestions, the results of experimental studies will be reported elsewhere. In the next part, we summarize only the most important experimental findings necessary for understanding the simulations, their strategy and aims.

DESCRIPTION OF THE SYSTEM

Two specifically tagged PS-PMA samples, single tagged by one pendant naphthalene (N) group between both blocks (PS-N-PMA) and hydrophobically modified, i.e. double tagged by one pendant naphthalene group between both blocks and by one anthracene (A) group attached at the end of poly(methacrylic acid) (PMA) block (PS-N-PMA-A) were used in the experimental study. Both samples have similar weight-average molar masses M_w (*ca.* 58 kg mol^{-1}) and weight fractions of polystyrene close to one half. The lengths of blocks were similar in both samples, which allows for direct comparison of experimental results and determination of effects caused by the hydrophobic modification of PMA blocks. The double tagging by naphthalene (energy donor) and anthracene (energy trap) allows for fluorometric study of the nonradiative excitation energy transfer aimed at the estimate of distribution of traps in the shell [8–12].

The micelles studied were prepared as follows: The solutions of micelles (5×10^{-3} g/l) in a 1,4-dioxane–water (20 vol%) mixture were prepared by direct dissolution of samples in the solvent under a vigorous overnight stirring. All other solutions with higher content of water were prepared by dialysis. The characterization and study of the behavior of micelles in mixed and aqueous media were performed by standard experimental techniques using the up-to-date apparatuses described either in our recent studies or by other authors by static and quasielastic light scattering (SLS, QELS) [13], steady-state and time-resolved fluorometry [13], fluorescence correlation spectroscopy (FCS) [14] and by atomic force microscopy (AFM) [15].

When PS-PMA micelles are formed in mixed polar or aqueous media (1,4-dioxane–water), PS blocks form the compact micellar cores and PMA chains form the shells. Density of the core is spatially constant while that of the shell decreases towards the micellar periphery. The PMA chains are preferentially stretched in the radial direction (see the R.H.S. in Scheme 1). In hydrophobically modified PS-N-PMA-A micellar systems, the hydrophobic anthracene tags at the ends of PMA blocks try to avoid the polar solvent and return back into a less polar shell close to the nonpolar core. They force the PMA chains to loop back. The distribution of anthracene tags in the shell (see the L.H.S. in Scheme 1), which is a result of the enthalpy-to-entropy interplay, may be studied by monitoring the nonradiative energy



SCHEME 1 The structure of the shell in hydrophobically modified PS-N-PMA-A micelles (L.H.S.) and in regular (non-modified) PS-N-PMA micelles (R.H.S.).

transfer (NRET) from energy donors (naphthalene embedded in the core/shell interface) to acceptors (terminal anthracene tags) and analyzing the fluorescence decays [9,12].

Results of our experimental studies will be published separately, nevertheless the most important experimental characteristics have been included in this paper since they are needed for developing a suitable model and for the comparison of the results. Molar mass and size of micelles in different solvents were studied by a combination of several techniques (SLS, QELS, FCS and AFM). Hydrodynamic radius, R_H, of micelles measured by QELS during dialysis together with the NRET transfer efficiency, χ^{tr} (the fraction of the total excitation energy transferred from the excited donor to the ground state acceptors) as functions of the solvent composition are depicted in Fig. 1. A representative AFM scan of micelles deposited on mica surface is shown in Fig. 2.

The included experimental results clearly demonstrate the formation of multimolecular micelles in the mixed solvents (containing more than 20 vol% of water) and the return of hydrophobically modified PMA ends towards the PS core in solvents with a considerable amount of water. For the theoretical study, we have chosen the micellar system in a 1,4-dioxane (70 vol%)–water mixture. This mixture is a very strong selective precipitant for PS, which means that we simulate the behavior of kinetically frozen micelles, but the dissociation of PMA chains is negligible and does not have to be taken into account. The micellar core is compact and kinetically frozen, there is no chain exchange and thus the micelles can be considered as inert PS spheres covered and protected by a PMA brush.

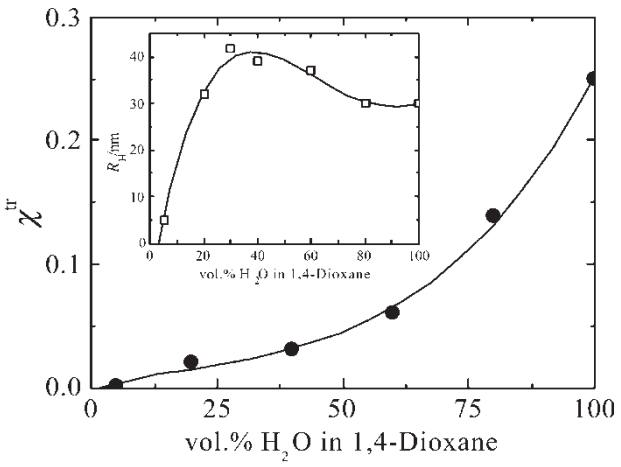


FIGURE 1 Efficiency χ^{tr} of NRET from naphthalene to anthracene in PS-N-PMA-A micelles as a function of 1,4-dioxane–water solvent composition. *Inset*. Hydrodynamic radius R_H of PS-N-PMA-A micelles as a function of the solvent composition.

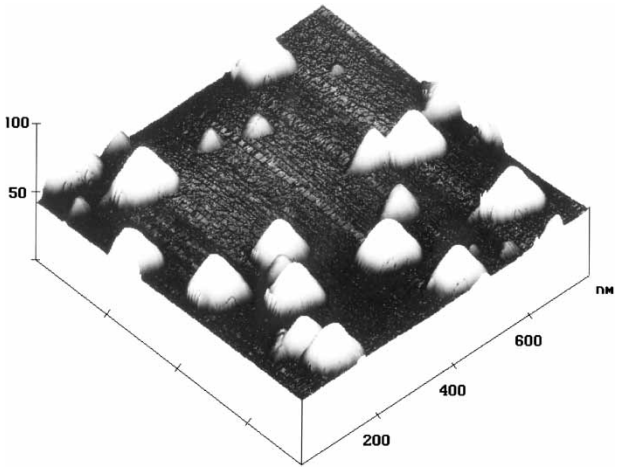


FIGURE 2 A tilted $800 \times 800\,nm^2$ AFM scan of modified PS-N-PMA-A micelles on mica surface.

COMPUTER SIMULATIOS

For the system that we studied experimentally and that is briefly described and specified above, we attempted to study the structure of its micellar shell by means of both lattice Monte Carlo (MC) and self consistent field (SCF) methods. The basic underlying model in both cases is essentially the same.

The experimentally studied micellar solution was dilute and this allows us to simulate one micelle only. The polydispersity of samples was not taken into account in computer studies. The core is modeled as an impenetrable sphere. The experimental association number and the core radius were used in the simulations. The unknown parameters of the model that remain to be set-up are the lattice constant, effective number of segments, interaction energies (ϵ_{ij} in MC) and interaction parameters (χ_{ij} in SCF). The proper number of segments in a simulation is not necessarily the same as the experimental number of segments due to the different flexibility of real and simulated chains. The unknown lattice constant can be eliminated by requiring that the experimental contour length is reproduced. There are five distinct components in our system, the core (PS), segments (PMA), donors (N), traps (A) and mixed solvent that we consider to be effectively a single-component medium. The core, donors and acceptors are chemically very similar and can be treated as one type only. In order to evaluate the interaction energy effectively, we set the MC interaction energies of all components with solvent to zero. This can be viewed as an effective shifting of the energy scale. The SCF interaction parameters between molecules of the same component χ_{ii} are zero by definition. The effective number of segments and interaction energies, respectively interaction parameters (except the trap interaction), were obtained by simulating the system without traps and varying the unknown

parameters until the best agreement with available experimental values of R_g [16] for several different polymer lengths and PS contents was obtained. The remaining interaction energy, respectively interaction parameter, was obtained by fitting the values of R_g and χ^{tr} available for the studied tagged system. The fluorescence behavior was calculated within the common direct dipole–dipole (Förster) exchange mechanism. It was found experimentally that the donors are immobilized in a fairly viscous core-shell interface. Their distances and orientations were found to be unfavorable for both the NRET and the energy migration between them. This allows us to simulate the NRET from a single excited donor to traps within one micelle only. The probability ρ that a donor is still excited at time t is given by [17]

$$\frac{d\rho}{dt} = -\left[\frac{1}{\tau_D} + \sum_{i=1}^{N} k(r_i)\right]\rho, \tag{1}$$

where τ_D is the effective fluorescence lifetime of the donor (unaffected by NRET to traps), $k(r_i)$ are the transfer rate constants depending on the donor-to-trap distances r_i and N is the number of traps. The rate constants are given by $k(r) = (1/\tau_D)(R_0/r)^6$, where R_0 is the Förster radius with experimentally determined value in nonpolar media of 2.1 nm [18]. The low polarity in our case is justified by the presence of the hydrophobic PS core. The fluorescence intensity $I(t)$ for a single micelle is given by

$$I(t) = I_0 \exp\left\{-\frac{t}{\tau_D}\right\} \prod_{i=1}^{N} \int_0^{\infty} \exp\left\{-\left[\frac{R_0}{r_i}\right]^6 \left[\frac{t}{\tau_D}\right]\right\} w_{DT}(r_i)\, dr_i, \tag{2}$$

where I_0 is the initial fluorescence intensity and w_{DT} is the probability density of the donor-to-trap distances. The NRET efficiency χ^{tr} can be calculated using the formula

$$\chi^{tr} = \frac{R_0^6 \int_0^{\infty} \frac{w_{DT}(r)}{r^6}\, dr}{R_0^6 \int_0^{\infty} \frac{w_{DT}(r)}{r^6} dr + \frac{1}{N}}. \tag{3}$$

In the MC simulations, we used a dynamic configuration-bias method on a simple cubic lattice. The chains are modeled as SAWs with interactions. Only the contact interactions between the nearest neighbors are taken into account. The chains are tethered in a narrow core-shell interface layer. The basic MC move consists of dissolving and rebuilding a randomly chosen chain. The new chain is accepted or rejected according to the modified Metropolis algorithm. Each MC run was 2^{28} steps long, first half of which was devoted to pre-equilibration and second half to data accumulation. The statistical errors of calculated quantities were estimated by the blocking method [19], calculated density functions were estimated by the kernel density estimation (KDE) method with the triweight kernel [20]. Further details can be found in Ref. [10].

In the SCF simulations, we used the method developed by Scheutjens and Fleer [21], where the chains are modeled as the first order Markov chains. The position of a given segment depends only on the position of the previous one. The steps are allowed in all possible directions including the immediate reversal step. The interaction potential is controlled by the concentration of components and interaction parameters. We model the micellar shell by chains on a spherical lattice that are tethered to the surface of a sphere. The chain segments can be located only outside this sphere. After making the first estimate of the concentration profiles of all components, the numerical iterative procedure is run until the self-consistence is achieved. As a result from SCF calculations, we obtain the radial distribution functions of shell segments and traps from which we calculate the NRET fluorescence behavior by an auxiliary static Monte Carlo simulation. Further details can be found in Ref. [11].

RESULTS AND DISCUSSION

In this paper, we focus on how the simulations compare with and explain the experiments and what they predict. The two types of simulations will be mutually compared only briefly, leaving the full discussion for the future publication [22].

The optimized MC interaction energies obtained by the above described parameterization are $\epsilon(\text{PMA}-\text{PMA}) = 0.8\,kT$, $\epsilon(\text{PMA}-\text{nonpolar}) = 0.6\,kT$ and $\epsilon(\text{nonpolar}-\text{nonpolar}) = -3.0\,kT$, while the optimal SCF interaction parameters are $\chi(\text{PMA} - \text{solvent}) = 0.5$, $\chi(\text{PMA} - \text{nonpolar}) = 9.0$ and $\chi(\text{nonpolar} - \text{solvent}) = 9.0$. The lattice constants were found to be $d = 1.0$ and 1.1 nm, for MC and SCF, respectively. We have found that the SCF model is more flexible and is able to reproduce the experimental values more closely, while the MC model is stiffer. We estimated the experimental errors about ± 5 nm in the case of gyration radii and about ± 0.02 in the case of NRET transfer efficiencies. The SCF method could reproduce the values with even smaller uncertainties while the MC method predicts the value of R_g to be larger than the experimental by about the experimental error. The two sets of interaction energies and parameters can be directly compared using equation [23]

$$\chi_{AB} = \frac{Z}{2kT}(2\varepsilon_{AB} - \varepsilon_{AA} - \varepsilon_{BB}), \tag{4}$$

where Z is the lattice coordination number ($Z = 6$ in our case). The values of χ (PMA–nonpolar) and χ (nonpolar–solvent) are essentially the same in both methods, the value of χ (PMA–solvent) is smaller in

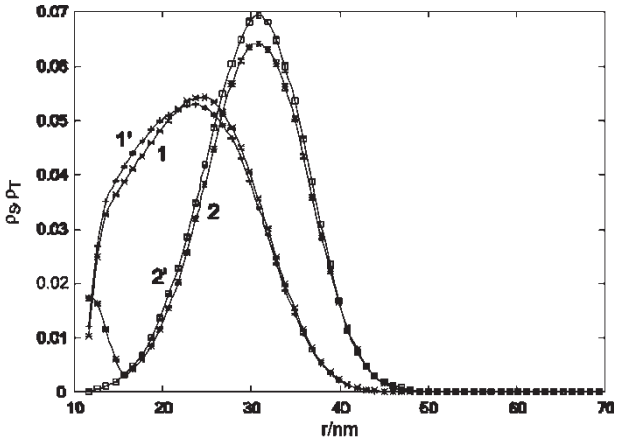


FIGURE 3 The probability density of the shell-forming segments (including donors and traps) $\rho_S(r)$ (curve 1), the probability density of end-segment (trap) distances $\rho_T(r)$ (curve 2) from the core center for the hydrophobically modified system as obtained in the MC simulations. The results for the unmodified systems are also shown (the curves are labeled as 1′ and 2′, respectively).

the MC case. This corresponds to somewhat larger values of R_g in the latter case.

The results of simulations will be presented as distribution functions of distances of segments or traps from the center of the micelle. Figure 3 shows the probability density of the shell-forming segments (including donors and traps) $\rho_S(r)$ (curve 1) and the probability density of end-segment (trap) distances $\rho_T(r)$ (curve 2) from the core center for the hydrophobically modified system as obtained in the MC simulations. The results for the unmodified systems are also shown (the curves are labeled as 1′ and 2′, respectively). Figure 4 shows the corresponding probability distribution functions from the SCF calculations. The results of both methods agree qualitatively. The segment profiles

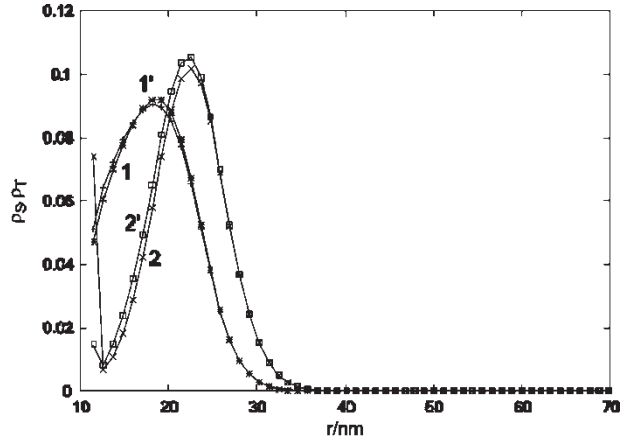


FIGURE 4 The probability density of the shell-forming segments (including donors and traps) $\rho_S(r)$ (curve 1), the probability density of end-segment (trap) distances $\rho_T(r)$ (curve 2) from the core center for the hydrophobically modified system as obtained in the SCF simulations. The results for the unmodified systems are also shown (the curves are labeled as 1′ and 2′, respectively).

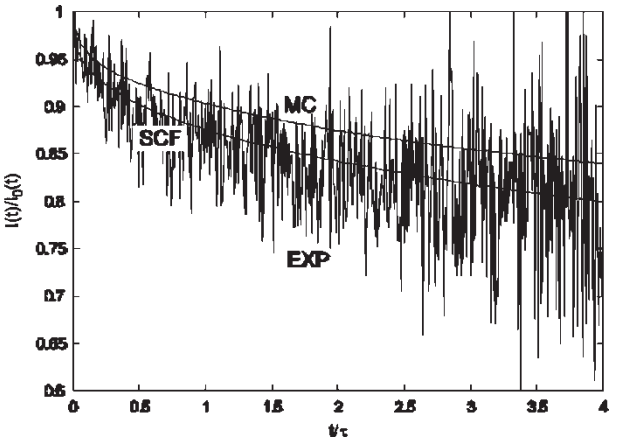


FIGURE 5 Relative fluorescence decays $I(t)/I_0(t)$, where $I_0(t)$ is the decay unaffected by traps for PS-N-PMA-A micelles in a mixed 1,4-dioxane–water (70 vol% water) solvent.

for the hydrophobically modified and unmodified systems are similar, but the maximum density for the modified system is shifted slightly closer to the core/shell interface. The segment density near the core is in both cases reduced due to the entropy penalty imposed by the impenetrable core. The density of end-segments for the reference system shows a single peak, while the density for the modified system has two peaks. One is in approximately the same distance as in the case of reference system, the other one is in the vicinity of the core. The latter peak is due to favorable interactions of nonpolar tags with the core. The relatively high values of the NRET efficiency seen in experiments reproduced reasonably by simulations are due to a non-negligible fraction of traps quite close to the core.

The comparison of experimental and simulated decay curves $I(t)/I_0(t)$, where $I_0(t)$ is the fluorescence intensity unaffected by the NRET to traps, is shown in Fig. 5. The agreement with the experiment is good, although a more ramified model is be needed to achieve a good quantitative agreement between the experimental and simulated behavior of this relatively complex system. However, the most decisive features, including the double peak distribution of donor-to-trap distances, that may explain peculiar experimental shapes of time resolved decays, were obtained. In the future work, we plan to study charged micellar shells in water which will require incorporating long-range electrostatic interactions to our model.

CONCLUSIONS

(1) The hydrophobically modified PS-N-PMA-A micelles, prepared and investigated in our experimental papers, were studied by computer-based simulations.

(2) Since the time-resolved fluorescence studies suggest that the hydrophobic anthracene tags at the ends of PMA blocks try to avoid the aqueous solvent and return in the shell close to the PS core, forcing the PMA blocks to loop back, the computer-based study was aimed at the distribution of traps in the shell and the analysis of the time-resolved fluorescence decays (influenced by the NRET).
(3) The simulations suggest that the distribution of traps is bimodal. Some traps return closely to the core while others remain distributed in the shell.
(4) The computer simulations confirm the most decisive trends in the conformational behavior of the modified PMA that may be drawn from experimental studies. The comparison of experimental and simulated data is good at the semi-quantitative level. As concerns a quantitative comparison, there is still room for improvement.

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