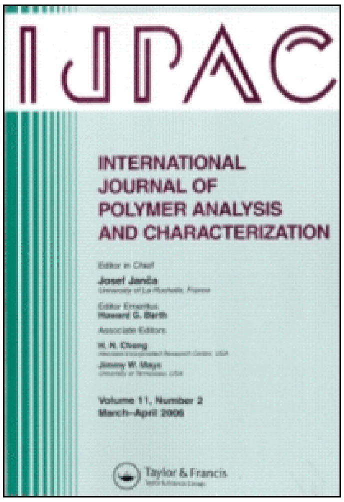

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Experimental Study of the Electrophoretic Mobility and Effective Electric Charge of Polystyrene-Block-Poly(Methacrylic Acid) Micelles in Aqueous Media

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Abstract: Kinetically frozen polystyrene-block-poly(methacrylic acid) (PS-PMA) micelles were prepared by dialysis of reversible micelles from 1,4-dioxane-rich aqueous solutions in solvents with increasing content of water and finally in pure aqueous media. Dynamic light scattering (DLS) and capillary zone electrophoresis (CZE) were used to determine the hydrodynamic radius and electrophoretic mobility of micelles as functions of pH. The electrophoretic curves show broad and relatively fast moving micellar peaks, indicating fairly high electrophoretic mobility and a non-negligible net electric charge of micelles. The experiments show that CZE is a suitable technique for studying polyelectrolyte micelles.

Keywords: Capillary zone electrophoresis; Polystyrene-block-poly(methacrylic acid) micelles; Static and dynamic light scattering

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INTRODUCTION

The self-assembled nanoparticles formed by block copolymers in aqueous media offer a number of important applications in modern bio- and medicinally oriented nanotechnologies, e.g., as carriers of hydrophobic drugs in targeted drug delivery.[1] This is why new block copolymers have been synthesized and their self-assembly has been studied by a number of research groups for almost two decades.[2] Recent studies have been aimed either at directly applicable drug transport systems or at the investigation of general principles of self-assembly, the behavior of formed particles, and processes of controlled uptake and release.

High molar-mass diblock copolymers containing a long hydrophobic block, such as polystyrene (PS) and a water-soluble polyelectrolyte block, such as poly(methacrylic acid) (PMA), are usually insoluble in aqueous media. Nevertheless, water-soluble nanoparticles can be prepared indirectly, e.g., by dialysis of associates formed in 1,4-dioxane-rich against water-rich mixtures and finally against aqueous buffers. During dialysis, the thermodynamic quality of the solvent for the core-forming PS blocks deteriorates and the association number increases. At a certain water content, depending on the copolymer composition, the mixed solvent becomes very poor for PS and the micellization equilibrium kinetically freezes. The unimer concentration drops virtually to zero, and the unimer exchange between micelles stops. The core-shell nanoparticles in aqueous media may be regarded as inert PS spheres decorated with a fairly monodisperse PMA brush tethered to their surface. They represent stable stimuli-responsive nanoparticles that respond to pH, ionic strength, and temperature changes, but do not dissociate upon dilution because they contain kinetically frozen cores.

When discussing the behavior of core-shell polyelectrolyte nanoparticles, it is necessary to differentiate between systems with shells formed by quenched and annealed polyelectrolytes. The former behave like typical spherical polyelectrolyte brushes. The density of charged groups attached to the shell-forming polymer chains is high, and a considerable electrostatic force prevents the entropy-driven escape of small counterions in the bulk solvent. The micelles are effectively neutral and do not interact over long distances. Depending on the concentration of the added salt, the shell obeys either the osmotic or salted brush regime. The quenched systems have been amply studied by both experimentalists and theoreticians, and their properties are now sufficiently understood.[3–5]

The behavior of micelles with shells formed by annealed (weak) polyelectrolytes is fairly complex. We have been studying the behavior of PS-PMA micelles by different experimental[6,7] and computer-based techniques[8] for a long time. Recently, we have shown that a considerable fraction of counterions escapes to the bulk solvent under certain conditions, and the micelles act as fairly charged particles.[7] However, the question to what extent it affects the behavior has not yet been unambiguously answered.

In this article, we address the above problem by using capillary zone electrophoresis (CZE) in combination with static (SLS) and dynamic light scattering (DLS). CZE has been widely used for analytical purposes, mainly for studying mixtures of small ions.[9,10] It has also been applied for systems containing surfactant micelles and moderately large molecules.[11] However, to our best knowledge, it has rarely been used for studying charged polymer micelles.[12,13] The aim of the article is twofold: we would like (i) to demonstrate the importance of the electric double layer around micelles and its effect on the behavior of micellar systems and (ii) to show that a combination of recognized "benchmark" techniques with those not often used in polymer science considerably increases the research potential.

EXPERIMENTAL SECTION

Polymers, Buffers, and Preparation of Micellar Solutions

The diblock copolymer PS-PMA was prepared at the University of Texas at Austin. The molar mass is 41.6×10^3 g/mol (68 wt.% of polystyrene). Details of the preparation and characterization are given in Ramireddy et al.[14] The micelles were prepared indirectly using the following procedure: the sample was dissolved in a 80%-1,4-dioxane/20%-water solution, which is a mild selective solvent for PS. The solution was stepwise dialyzed in aqueous solutions with decreasing 1,4-dioxane content (60%, 40%, 20%). The final dialysis was done in buffers with a constant ionic strength $I = 20\,\mathrm{mM}$ in the pH range 5.6 to 8.2. TAPS (N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid), MOPS (3-(N-morpholino)-propane-sulfonic acid), and MES (2-(N-morpholino) ethanesulfonic acid) buffers with a constant ionic strength were used for both DLS and CZE. The components for their preparation were purchased from Aldrich (purity > 99.5%) and were used without purification. The pH values were measured using a Radiometer PHM 93. The solutions of micelles and the buffers were filtered through a 0.45 µm Acrodisk filter (Sigma-Aldrich, St. Louis, Mo., USA). The micelles were characterized by SLS and DLS techniques and pertinent data are given Table I.

Capillary Zone Electrophoresis

The electrophoretic measurements were carried out using a Hewlett-Packard 3D CE apparatus (Agilent Technologies, Waldbronn, Germany), equipped with an inline variable wavelength detector. The

Table I. Characteristics of PS-PMA micelles measured by DLS, SLS, and CZE in buffers with a constant ionic strength $I = 20\,\text{mM}$ and different pH

pH	$R_H{}^a$ (nm)	$R_G{}^b$ (nm)	$M_W{}^b$ (g/mol)	$\mu_M{}^c$ (m^2/Vs)	$q_M{}^d$ (e)	ζ^e (mV)
5.57	44	43	25.7×10^6	2.62×10^{-8}	136	– 37.0
6.69	56	48	27.3×10^6	3.00×10^{-8}	198	– 42.4
7.52	59	48	28.9×10^6	3.06×10^{-8}	213	– 43.2
8.17	59	49	25.0×10^6	3.05×10^{-8}	212	– 43.1

[a] Hydrodynamic radius measured by DLS.
[b] Apparent radius of gyration and apparent weight-average molar mass measured by static light scattering.
[c] Electrophoretic mobility.
[d] Calculated negative charge using the Stokes equation, $e = -1.6 \times 10^{-19}$ C.
[e] Calculated zeta potential.

data were collected at three different wavelengths (200 nm, 214 nm, and 254 nm). An untreated silica capillary (50 µm internal diameter; total length, l_C 47.5 cm; distance to the detector, l_{D1} 39 cm; TWC-S50, Caco, Slovakia) was used. The applied voltage was usually 20 kV and the experiments were carried out at 25°C. The capillary was conditioned sequentially by 0.1 M sodium hydroxide (30 min), water (3 × 2 min), and finally by the buffer to be used for the subsequent measurement (2 × 2 min). The micelles dissolved in TAPS buffers (ionic strength $I = 20\,\text{mM}$, pH 8.17) were injected hydrodynamically at a pressure of 20 mbar for 6 s. Thiourea (1 mM) was added as marker for the electroosmotic flow (EOF).

The mobility μ_M of micelles, defined as $\mu_M = \frac{v_M}{E}$ (where v_M is the velocity of the micelles and E is the applied electric field strength), assumes negative values for the negatively charged micelles (providing the inlet is negatively charged). In the standard inlet injection method, the velocity of EOF, v_E, and the apparent velocity of the motion of micelles, v_A, can be evaluated on the basis of times of their detection, t_E and t_M, respectively, $v_E = \frac{l_{D1}}{t_E}$ $v_A = \frac{l_{D1}}{t_M}$. Since the micelles and EOF move in opposite directions, the electrophoretic mobility of micelles can be calculated as

$$\mu_M = \left(\frac{1}{t_E} - \frac{1}{t_M}\right) l_{D1} l_C / U \tag{1}$$

In our study, a modification of the CZE technique was necessary because the apparent electrophoretic motion of micelles changes direction in a series of buffers used due to a strong dependence of EOF velocity on pH. In the case that $v_E < v_M$, a "double injection method" at both sides of the capillary was used. A buffer/thiourea mixture was injected into the outlet at a pressure of 20 mbar for 12 s, and subsequently the sample was injected into the inlet (20 mbar, 6 s). The electrophoretic mobility can be calculated as

$$\mu_{\mathrm{M}} = \left(\frac{l_{\mathrm{D1}}}{t_{\mathrm{M}}} + \frac{l_{\mathrm{D3}}}{t_{\mathrm{E}}}\right) \cdot l_{\mathrm{C}}/U \tag{2}$$

Static and Dynamic Light Scattering

The light scattering setup (ALV, Langen, Germany) consists of a He-Ne laser (wavelength 633 nm), an ALV CGS/8F goniometer, an ALV QE APD, detector and an ALV 5000 multibit, multitau autocorrelator. The measurements were carried out for solutions of different concentrations (approximately 1.4–0.3 mg/mL) with angles in the range from 60 to 150° (10° steps) at a temperature of 20°C.

Static light scattering measurements were evaluated using the Standard Zimm technique. Dynamic light scattering measurements (DLS) were evaluated by fitting of the electric field autocorrelation function, $g_{(1)}(t)$, related to the measured intensity autocorrelation function, $g_{(2)}(t)$, by the Siegert relation, $g_{(2)}(t) = 1 + \beta|g_{(1)}(t)|^2$, where β is the coherence factor accounting for deviation from the ideal correlation. To obtain the averaged hydrodynamic radius of the particles, R_{H} (z-average for ${R_{\mathrm{H}}}^{-1}$), the $g_{(1)}(t)$ functions measured for various copolymer concentrations and scattering angles were fitted to the function

$$g_{(1)}(t) = \exp(-D_{\mathrm{app}}q^2t + \mu_2t^2/2) \tag{3}$$

where D_{app} is the apparent diffusion coefficient and μ_2 is the second moment of the distribution function of relaxation times. The D_{app} values obtained from the fits were further extrapolated to zero q and c to yield the z-averaged diffusion coefficient of the particles, $\langle D\rangle_{\mathrm{z}}$.

The Einstein-Smoluchowski and the Stokes-Einstein formulas

$$q_{\mathrm{M}} = k_{\mathrm{B}}T\mu_{\mathrm{M}}/\langle D\rangle_{\mathrm{z}}, \qquad R_{\mathrm{H}} = \frac{k_{\mathrm{B}}T}{6\pi\eta\langle D\rangle_{\mathrm{z}}} \tag{4}$$

enable direct calculations of the electric charge q_{M} and the hydrodynamic radius of micelles, respectively. Here k_{B} is the Boltzmann constant, T is the temperature, and η the viscosity of the solvent.

RESULTS AND DISCUSSION

The kinetically frozen PS-PMA micelles prepared as described in the Experimental section were characterized by light scattering. Their molar masses and sizes were measured in a series of medium ionic strength buffers ($I = 20\,\text{mM}$, pH 5.6–8.2,), i.e., under conditions where the long-range electrostatic interactions are screened enough and do not influence or invalidate the results of light scattering measurements. The refractive index increment, $\mathrm{d}n/\mathrm{d}c = 0.204$, was calculated as the weight-average of values for PMA and PS (based on latex particles) in aqueous media.[15] The light scattering results are given in Table I. Since the micelles are kinetically frozen in aqueous media, their molar mass should be constant. Slight variations are caused by the fact that we used the refractive index increment in pure water. A typical (fairly regular) Zimm plot of micelles in a neutral buffer (pH 7.52) is shown in Figure 1.

Diffusion coefficients of PS-PMA micelles were measured by DLS and recalculated in hydrodynamic radii using the Stokes-Einstein formula. The radii are plotted in Figure 2 as a function of pH. The R_H versus pH dependence compares well with our earlier data on similar micellar systems.[16]

The main focus of this article consists in the study of the electrophoretic mobility, μ, and the net charge of micelles as functions of pH and ionic strength. Two typical electrophoregrams are shown in (Figure 3(a) and (b). The micellar peaks are fairly broad (for the detection time of approximately 15 min, the half width is approximately 2 min). At present, we believe that the broadening is mainly due to the polydispersity of micelles, and it

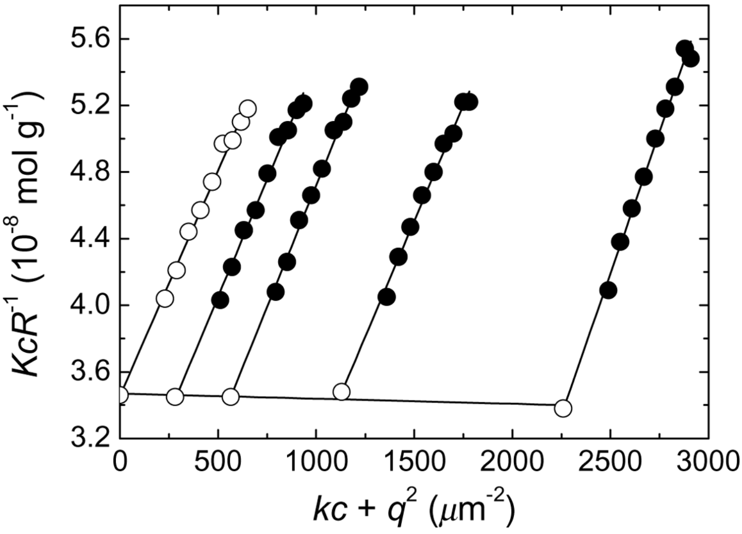


Figure 1. Zimm-plot of PSPMA-micelles in a buffer of pH 7.52. Concentrations of the solutions are 1.128, 0.564, 0.282 and 0.141 mg/ml. The circles represent the extrapolated dependence of Kc/R^{cor} ($q = 0$, c) on the copolymer concentration c and $Kc/R^{\text{cor}}(q, c = 0)$ on the scattering vector q. The constant k is $2000\,\mu\text{m}^{-2}\text{lg}^{-1}$.

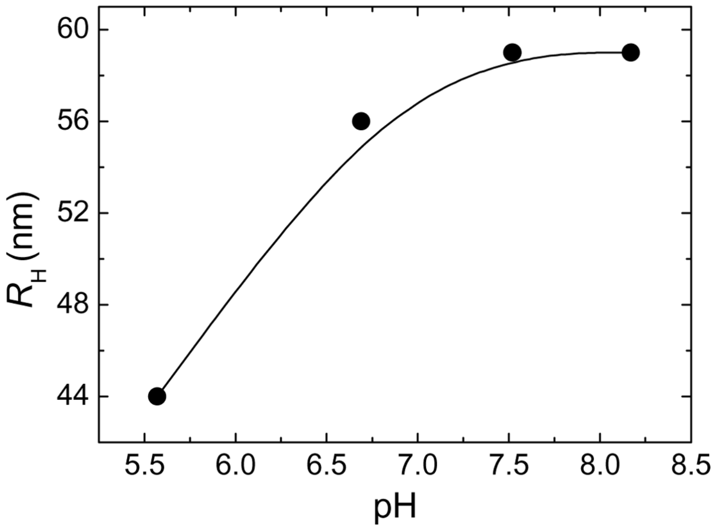
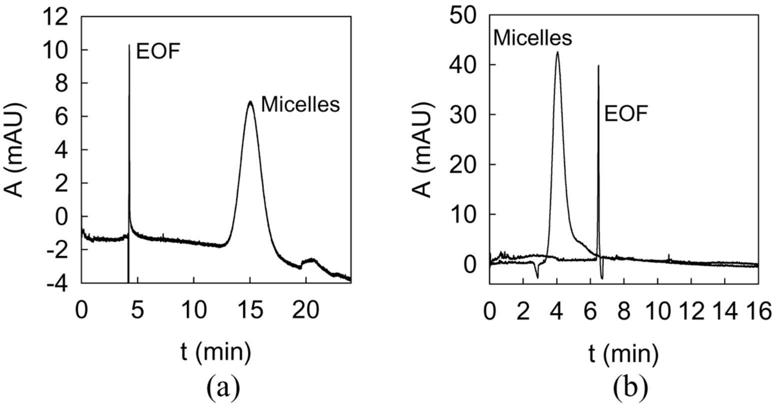


Figure 2. The hydrodynamic radius R_H measured as a function of pH by dynamic light scattering.

seems that CZE is a very sensitive method for polydispersity evaluation. The electrophoretic mobility of micelles, μ_M, as a function of pH is shown in Figure 4. The mobility increases with the degree of ionization of PMA, i.e., with pH. In the region of fairly low pH close to the effective pK_A of PMA, it rises quickly and at high pH it levels off. It is necessary to keep in mind that the effective dissociation constant of PMA is lower than that of



(a) (b)

Figure 3. Electrophoretic curves measured in two different buffers of pH 5.49 (a) and pH 6.66 (b). The absorbance A is measured in milli-absorption-units (mAU).

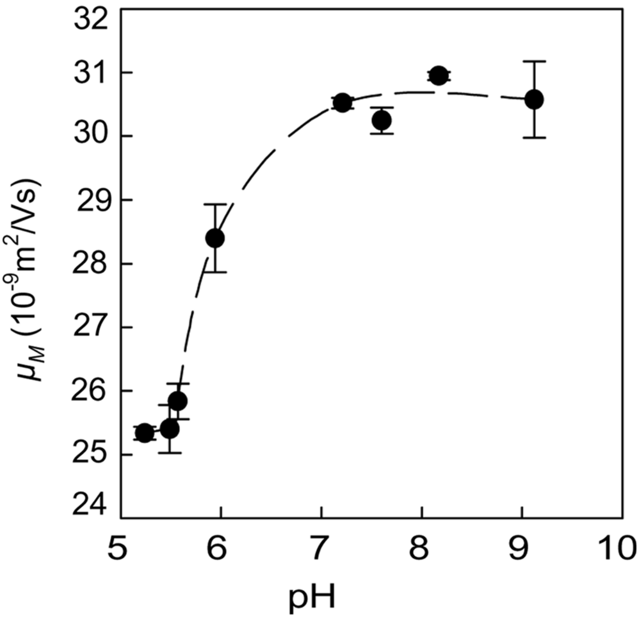


Figure 4. Electrophoretic mobility of PSPMA-micelles as a function of pH. The error bars correspond to the standard deviation as evaluated from ca. 6 sequential experiments.

monomeric methacrylic acid and depends on the degree of dissociation. In micellar shells, the dissociation is further suppressed due to the Donnan equilibria.[17] The mobility of micelles is fairly high for their high molar mass—it is comparable to that of small inorganic ions. This finding looks slightly surprising, but it is necessary to keep in mind that micelles are compact and fairly small objects (for their molar mass) and their net charge is not negligible and plays important role despite the strongly suppressed dissociation of carboxylic groups in PMA shells and efficient electrostatic screening. Even more surprising is the observation that the measured mobility is quite high at a low pH of approximately 5, i.e., in the pH region fairly close to the precipitation limit. This observation can be explained by the fact that water is a marginal solvent (slightly worse than the θ-solvent at ambient temperature) for non-dissociated PMA,[18,19] and hence some ionization is a necessary condition for the stabilization of PMA solutions.

Because electrophoretic mobility is related to the diffusion coefficient via the Einstein-Smoluchowski formula, the combination of electrophoretic and DLS data directly yields the net charge of micelles as a function of pH (Table I). As expected on the basis of studies by other authors, the net charge is fairly low in comparison with the high number of potentially dissociable –COOH groups per micelle. It increases with pH because the overall dissociation increases. As the electrostatic screening by small ions is efficient, the changes of the effective charge with pH are not dramatic. Nevertheless, the negative charge in the range of 136 to 212 *e* per micelle is important on the short-range scale and affects not only the electrophoretic mobility, but also the local properties of the shell and properties of micelle-solubilized compounds. Because polymeric micelles are studied as carriers of drugs in living organisms, it is necessary to take into account that the short-range interaction of charged micelles with charged biological structures can influence the behavior of micelles in living cells.

We also studied the dependence of the electrophoretic mobility of micelles on the ionic strength, I. The measurements were performed in buffers with I from 5 to 20 mM. No significant changes in the mobility were observed.

The electrophoresis allows for estimation of the zeta potential. In the case of spherical particles, this potential can be expressed, in the first approximation, as $\zeta = \mu\eta/\varepsilon$, where ε is the dielectric permittivity and the other symbols have been already defined. The values of ζ-potential are interesting because they can also be evaluated by other techniques. The calculated values are given in Table I. They compare reasonably well with data on similar systems measured by other authors by electrophoretic light scattering.[20]

CONCLUSIONS

The performed experiments show that CZE is a suitable technique for studying the polyelectrolyte behavior of micelles. The mobility is pH dependent, but depends very little on the ionic strength. The combination of CZE with DLS enables the determination of the charges of micelles.

The study shows that the effective charge of micelles is low as compared both with the high number of potentially dissociable and with the number of really dissociated carboxylic groups. The charge is reduced due to strongly hindered escape of counterions from the shell and efficient electrostatic screening. However, it is certainly non-negligible and strongly affects local properties of micelles at the short-range scale and results in a high electrophoretic mobility of micelles.

REFERENCES

[1] Kataoka, K., A. Harada, and Y. Nagasaki. (2001). Block copolymer micelles for drug delivery: Design, characterization and biological significance. *Adv. Drug Deliv. Rev.* **47**, 113–131.

[2] Hadjichristidis, N., S. Pispas, and G. A. Floudas. (2003). *Block Copolymers: Synthetic Strategies, Physical Properties and Applications*. Hoboken: Wiley-Interscience.

[3] Zhulina, E. B. and O. V. Borisov. (1998). Absorption of a polyelectrolyte brush into an oppositely charged layer. *Macromolecules* **31**, 7413–7422.

[4] Borisov, O. V., F. A. M. Leermakers, G. J. Fleer, and E. B. Zhulina. (2001). Polyelectrolytes tethered to a similarly charged surface. *J. Chem. Phys.* **114**, 7700–7712.

[5] Guo, X. and M. Ballauff. (2001). Spherical polyelectrolyte brushes: Comparison between annealed and quenched brushes. *Phys. Rev. E* **64**, 51406–51415.

[6] Štěpánek, M., K. Procházka, and W. Brown. (2000). Time-dependent behavior of block polyelectrolyte micelles in aqueous media studied by potentiometric titrations, QELS and fluorometry. *Langmuir* **16**, 2502–2507.

[7] Matějíček, P., K. Podhájecká, J. Humpolíčková, F. Uhlík, K. Jelínek, Z. Limpouchová, K. Procházka, and M. Špírková. (2004). Polyelectrolyte behavior of polystyrene-block-poly(methacrylic acid) micelles in aqueous solutions at low ionic strength. *Macromolecules* **37**, 10141–10154.

[8] Uhlík, F., Z. Limpouchová, K. Jelínek, and K. Procházka. (2004). Polyelectrolyte shells of copolymer micelles in aqueous solutions: A Monte Carlo study. *J. Chem. Phys.* **121**, 2367–2375.

[9] Křivanková, L. and P. Boček. (1998). Continuous free-flow electrophoresis. *Electrophoresis* **19**, 1064–1074.

[10] Padarauskas, A. (2006). CE determination of small ions: Methods and techniques. *Anal. Bioanal. Chem.* **384**, 132–144.

[11] Nishi, H. (1997). Pharmaceutical applications of micelles in chromatography and electrophoresis. *J. Chromatogr. A* **780**, 243–264.

[12] Štěpánek, M., K. Podhájecká, E. Tesařová, K. Procházka, Z. Tuzar, and W. Brown. (2001). Hybrid polymeric micelles with hydrophobic cores and mixed polyelectrolyte/nonelectrolyte shells in aqueous media. 1: Preparation and basic characterization. *Langmuir* **17**, 4240–4244.

[13] Morel, A., H. Cottet, M. In, S. Deroo, and M. Destarac. (2005). Electrophoretic behavior of amphiphilic diblock copolymer micelles. *Macromolecules* **38**, 6620–6628.

[14] Ramireddy, C., Z. Tuzar, K. Procházka, S. E. Webber, and P. Munk. (1992). Styrene-tert-butyl methacrylic acid block copolymers: Synthesis and characterizartion. *Macromolecules* **25**, 2541–2545.

[15] Brandup, J. and E. H. Immergut. (1989). *Polymer Handbook*. New York: Wiley Interscience.

[16] Karymov, M. A., K. Procházka, J. M. Mendenhall, T. J. Martin, P. Munk, and S. E. Webber. (1996). Chemical attachment of polystyrene-block-poly(methacrylic acid) micelles on a silicon nitride surface. *Langmuir* **12**, 4748–4753.

[17] Munk, P., Z. Tuzar, and K. Procházka. (1997). Donnan equilibria in polymeric micellar systems with weak polyelectrolyte shells: The lowering of the pH value. *Collect. Czech. Chem. Commun.* **62**, 1730–1736.

[18] Bednář, B., J. Trnĕná, P. Svoboda, S. Vajda, V. Fidler, and K. Procházka. (1991). Time-resolved fluorescence study of chain dynamics. 1: Poly (methacrylic acid) in dilute water solutions. *Macromolecules* **24**, 2054–2059.

[19] Košovan, P., Z. Limpouchová, and K. Procházka. (2006). Molecular dynamics simulation of time-resolved fluorescence anisotropy decays from labelled polyelectrolyte chains. *Macromolecules* **39**, 3458–3465.

[20] Brown, W. and J. X. Zhao. (1994). Interaction between sodium dodecyl sulfate and styrene-butadiene copolymer and styrene-butadiene copolymer latex particles with carboxyl groups studied using dynamic light scattering and electrophoretic mobility measurements. *Langmuir* **10**, 3395–3401.