



ELSEVIER

Journal of Chromatography A, 939 (2001) 109–121

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Capillary electrophoresis of associative diblock copolymers[☆]

H. Cottet^{a,1}, P. Gareil^{a,*}, P. Guenoun^b, F. Muller^b, M. Delsanti^c, P. Lixon^c,
J.W. Mays^d, J. Yang^d

^aLaboratoire d'Electrochimie et de Chimie Analytique, UMR CNRS 7575, Ecole Nationale Supérieure de Chimie de Paris,
11, rue Pierre et Marie Curie, 75231 Paris, Cedex 05, France

^bService de Physique de l'Etat Condensé, CEA Saclay, Orme des Merisiers, 91191 Gif-sur-Yvette, Cedex, France

^cService de Chimie Moléculaire, CEA Saclay, 91191 Gif-sur-Yvette, Cedex, France

^dDepartment of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Received 18 July 2001; accepted 1 October 2001

Abstract

Water soluble diblock copolymers composed of a long poly(styrene sulfonate) chain (between 200 and 400 monomers) and a short poly(ethylene propylene) or poly(*tert.*-butylstyrene) hydrophobic end (20–50 monomers) are highly associative and form micelles in aqueous solution. The micelles are composed of a small hydrophobic core and a polyelectrolyte corona, the dimensions of which can be estimated by neutron and light scattering. These physical techniques are, however, not amenable to discriminate easily between the free copolymer and the copolymer micelle. Capillary electrophoresis was implemented in this work as a new and effective tool to investigate the behaviour of such associative copolymer systems. Since the rate of exchange between the micellised and free states is very slow in comparison with the time scale of the electrophoretic process, the electropherograms of the diblock copolymers obtained in plain aqueous borate buffers exhibit two peaks assigned to the two states mentioned above. The identification of the two peaks was first made on the basis of the retention orders of the two peaks equally obtained in similar conditions by size-exclusion chromatography. The copolymer micelles appeared to have a smaller electrophoretic mobility than the free copolymers. This peak assignment is also consistent with the observed ratio of the time-corrected peak areas and peak dispersions. The effects of the copolymer concentration, electric field, temperature and hydroorganic composition of the medium was also studied. Such systems do not exhibit a defined concentration threshold equivalent to a classical critical micelle concentration. Adding methanol to the electrolyte resulted in the progressive loss of baseline return between the two peaks, which might be attributed to a slight increase in the rate of exchange between the two states. Finally, adding a neutral surfactant to the electrolyte at a concentration in excess of its critical micelle concentration resulted in a decrease in the electrophoretic mobility of the peak attributed to the free copolymer, while the electrophoretic mobility of the copolymer micelle remained unperturbed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Micelles; Polymers; Poly(styrene sulfonate); Poly(ethylene propylene); Poly(*tert.*-butylstyrene)

[☆]Early results of this work were presented as a poster at the 13th International Symposium on High-Performance Capillary Electrophoresis and Related Microscale Techniques (HPCE 2000), Saarbrücken, February 20–24, 2000.

*Corresponding author.

E-mail address: gareil@ext.jussieu.fr (P. Gareil).

¹Present address: Organisation Moléculaire Évolution et Matériaux Fluorés, UMR CNRS 5073, Université de Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France.

1. Introduction

Capillary electrophoresis (CE) in gels or hydrophilic polymer solutions has become a well-established analytical technique for the separation of a variety of biomolecules (nucleic acids, proteins) according to their sizes and for a number of application purposes. A few authors have shown more recently that this approach is also applicable for the size separation and characterisation of uniformly charged synthetic polymers [1–7]. The possibility of separating polyelectrolytes in more open media than gels or polymer solutions by attaching a neutral moiety to at least one of its ends has been theoretically investigated [8–12] and experimentally demonstrated for nucleic acids [11–13] and polysaccharides [14,15]. Concurrently to these trends, the potential of free solution CE for the separation of charged composite edifices has emerged from concepts based on modelling the electrophoretic mobility according to the distribution of the charge and friction coefficient over the building blocks [16–18]. Experimentally, separations of statistical copolymer polyelectrolytes according to their charge have also been investigated by CE [19–21].

Up to now, very few experimental studies concerning the electrophoresis of charged composite objects have been reported in the literature. In a previous study [22], we showed that hydrophobically modified polyacrylic acids obtained by random copolymerisation of acrylic acid and *N*-alkylacrylamides could be separated according to their content of alkyl pendant chains (in the 1–10 molar% range), by allowing interactions with neutral surfactant micelles to take place in the electrolyte, resulting in a necklace structure. More recently, Wijnen et al. [23] also demonstrated the potential of CE to characterise swellable latex beads composed of a compact polystyrene core and a negatively charged shell of varying methacrylic acid content. As an example of another industrially relevant class of complex composite edifices, charged associative diblock copolymers appear to be good candidates for physicochemical studies by CE.

Water soluble diblock copolymers composed of a long poly(styrene sulfonate) chain and a short poly(ethylene propylene) or poly(*tert*-butylstyrene) chain at one end are highly associative and form micelles

in aqueous solution. The micelles are composed of a small hydrophobic core and a polyelectrolyte corona, the dimensions of which can be estimated by neutron and light scattering [24]. These physical techniques are, however, not amenable to discriminate easily between the free copolymer and the copolymer micelle. The purpose of this work was to evaluate capillary electrophoresis as a new and complementary tool for investigating the behaviour of such associative copolymer systems in aqueous media and to confront the results with those accessible through additional experiments performed in size-exclusion chromatography (SEC).

First, the influence of the sample concentration, temperature and electric field on the electrophoretic behaviour of these diblock copolymers in free aqueous solution was investigated. Next, the impact of the presence of an organic modifier in the electrolyte on the rate of exchange between the micellised and free forms of the copolymer was studied. Furthermore, neutral surfactant micelles were implemented in the electrolyte as a means of altering the structure of the free diblock copolymers. The effect of the electric field on the electrophoretic mobility of the copolymer–surfactant micelle complex was also considered to check for a possible change in the conformation of these composite objects.

2. Materials and methods

2.1. Reagents

Borax (disodium tetraborate decahydrate) and methanol were purchased from Prolabo (Paris, France). Mesityl oxide and the neutral surfactants polyoxyethylene 23 dodecyl ether (Brij 35) and poly(oxyethylene) 20 octadecylether (Brij 78) were obtained from Aldrich (Milwaukee, WI, USA). Sodium poly(styrene sulfonate) PSS 430 (M_r $88 \cdot 10^3$, polymerisation degree, $n = 430$; sulfonation degree: 0.9) was obtained from American Polymer Standards (Mentor, OH, USA). Two sets of sodium poly(styrene sulfonate) standards of M_r 10^5 , $5 \cdot 10^5$ and $8 \cdot 10^3$, $35 \cdot 10^3$ were used in the SEC calibration. The first set was purchased from Sopares (Paris, France) and the second one from Polymer Labs. (Shropshire, UK). The water used to prepare all buffers and

standard solutions was delivered by an Alpha-Q system (Millipore, Molsheim, France). The 40 mM borate buffer, pH 9.2, was directly prepared by dissolving the appropriate amount of borax in water or in the specified water–methanol mixtures.

2.2. Diblock copolymers

The diblock copolymers studied in this work were composed of a sodium poly(styrene sulfonate) (PSS) polyelectrolyte chain and either a poly(ethylene propylene) $\{-[\text{CH}_2-\text{CH}(\text{CH}_3)\text{CH}_2-\text{CH}_2]_n-$, PEP} or a poly(*tert.*-butyl styrene) (PtBS) hydrophobic tail. Both parts of the polymeric skeleton were synthesised by anionic polymerisation and were thus obtained with very low polydispersity. The polystyrene block of the copolymer was sulfonated after the polymerisation. The details of the synthesis procedure were reported elsewhere [25]. The chemical composition, the dimensions of the hydrophobic core and of the whole micelle in pure water and the aggregation numbers of the different copolymers (estimated by neutron and light scattering) are gathered in Table 1. For CE experiments, 3 g/l stock sample solutions were prepared by dissolving the solid product in water. Unless otherwise specified, this solution was further diluted ten times with the separation electrolyte. For SEC experiments, the stock sample solutions were prepared between 2.0 and 2.5 g/l in a mixture of aqueous 40 mM sodium borate buffer, pH 9.2, and methanol (45%, v/v).

2.3. Capillary electrophoresis

The CE experiments were performed using a HP^{3D}CE (Hewlett-Packard, Waldbronn, Germany) capillary electrophoresis system. The separation

capillaries (Supelco, Bellefonte, PA, USA) were of bare fused silica, 33.5 cm (25 cm to the detector) \times 50 μm I.D. New capillaries were conditioned by performing the following flushes: 1 M NaOH for 30 min, 0.1 M NaOH for 10 min and water for 5 min. Samples were introduced hydrodynamically (approximately 4 nl) by application of a positive pressure on the inlet side of the capillaries. Mesityl oxide (approximately 0.1% (v/v) in the borate buffer) was injected as a neutral marker to determine the electroosmotic mobility. The voltages applied are given in the figure captions. The polarity on the inlet side of the capillaries was positive. Copolymers were monitored spectrophotometrically by UV absorbance at 225 nm. The temperature of the capillary cartridge was set at 27°C, unless otherwise specified.

2.4. Size-exclusion chromatography

The SEC measurements were made by using a Shimadzu LC-9A pump (Tokyo, Japan) and a Knauer differential refractometer (Berlin, Germany) as detector. The exclusion column set was made up of three Toyo Soda columns TSK-Gel G6000PW, G5000PW, G4000PW (Japan), each 30 cm \times 7.5 mm I.D., connected in series. As usually, to protect the exclusion column, a precolumn TSK-Gel PWH (7.5 cm \times 7.5 mm I.D.) was used. Samples were introduced into the exclusion column with a Rheodyne 7125 valve injector (Cotati, CA, USA) equipped with a 200- μl loop.

The eluents were mixtures of aqueous 40 mM sodium borate buffer, pH 9.2, and methanol in various proportions. The flow-rate was set at 1.0 ml/min and measurements were performed at room temperature. The molecular mass vs. retention volume calibration of this exclusion column was estab-

Table 1

Chemical compositions and physicochemical characteristics of the diblock copolymers studied. The radii of the hydrophobic cores and of the whole micelles were estimated by neutron diffusion and light scattering measurements in pure water, respectively [26]

Copolymer designation	Number of monomers in each block	Molecular mass	Radii (nm)		Aggregation number
			Hydrophobic core	Whole micelle	
PSS 227–PEP 48	227–48	50 200	5.2 \pm 0.2	42 \pm 1	80–90
PSS 257–PEP 21	257–21	54 500	n. d.	n.d.	n.d. ^a
PSS 404–PtBS 25	404–25	87 300	2.6 \pm 0.1	80 \pm 8	30–40

^a n.d., not determined.

lished by injection of four low polydispersity PSS standards ranging in molecular mass from $8 \cdot 10^3$ to $5 \cdot 10^5$.

3. Results and discussion

Owing to their high ratio of polyelectrolyte to hydrophobic polymer chain lengths, the amphiphilic diblock copolymers studied (see Table 1) are easily soluble in aqueous media. They are, however, highly associative and form micelles at very low concentrations. As depicted in Fig. 1, these micelles were previously described as being built of a small hydrophobic core and of a thick corona made up of the stretched polyelectrolyte chains [24]. For both copolymers, it has been shown that the aggregation number was rather insensitive to ionic strength whereas the overall radius is weakly contracting on increasing ionic strength in this domain [24].

3.1. Electrophoretic behaviour of the diblock copolymers

The electrophoretic behaviour of the diblock copolymers under study was first investigated in a 40 mM borate buffer, pH 9.2, using a bare fused-silica capillary. For such an ionic strength, the Debye length (about 2 nm) is much smaller than the

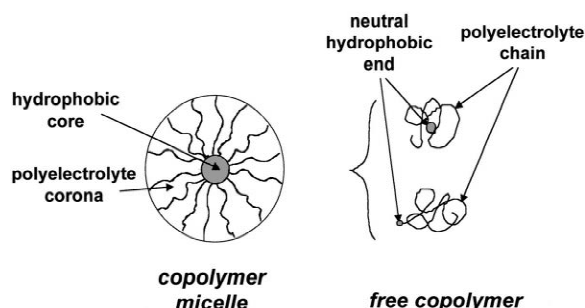


Fig. 1. Schematic outline of the conformation of amphiphilic diblock copolymers composed of a long sodium poly(styrene sulfonate) polyelectrolyte chain and of a short hydrophobic [either poly(ethylene propylene) or poly(*tert*-butyl styrene)] tail in their micellised and free forms. In the case of the free form, two putative conformations are represented: one showing the hydrophobic end fully wrapped by the PSS chain, the other showing the collapsed hydrophobic tail more exposed to the solvent.

copolymer contour length or the dimension of the whole micelles (see Table 1). Representative electropherograms of each copolymer analyte and of an almost fully sulfonated polystyrene of similar molecular mass, used as an evenly charged reference polyelectrolyte, are shown in Fig. 2. Apart from the neutral marker signal, the copolymer electropherograms display two peaks of very different time-corrected areas, numbered 1 and 2 in the order of increasing migration times. The migration times of peaks 2 (the minor one) appear to be close to that of the PSS standard, which, considering its chain length and sulfonation degree [21], should undergo a classical free draining migration under these electrolyte conditions. The electrophoretic mobilities corresponding to all these peaks are given in Table 2. The possibility of assigning one of the two peaks associated to the copolymer samples to the copolymer micelle and the other to the free copolymer comes first to the mind. For such an hypothesis to hold, the rate of exchange between the two forms should be very slow with respect to the time scale of the electrophoretic process. As a matter of fact, such a low rate of exchange is expected, taking into account the strong interaction between the hydrophobic blocks.

To ascertain this point, the SEC behaviour of PSS 404–PtBS 25 copolymer was also investigated with an eluent of composition similar to the electrophoretic electrolyte. This copolymer and the PSS polymers cannot be eluted from the TSK-Gel column using eluents without adding methanol, due to the affinity of the polymers for the stationary phase. As shown in Fig. 3, the resulting chromatograms also exhibit two peaks, the minor one corresponding to a molar mass of $7 \cdot 10^4$ close to that of the free diblock copolymer. The molecular mass corresponding to the major peak (about $6 \cdot 10^5$) is much under the expected one for the micelle (about $3 \cdot 10^6$). However, this discrepancy is probably due to the strong difference in the structure between the linear PSS standards and the micelles. The SEC experiment, associated to the fact that the micelle is expected to be predominant invited us to assign the major peaks of both the chromatograms and electropherograms to the copolymer micelles and the minor peaks to their free forms. Additional experimental confirmations of this attribution from CE results are given below

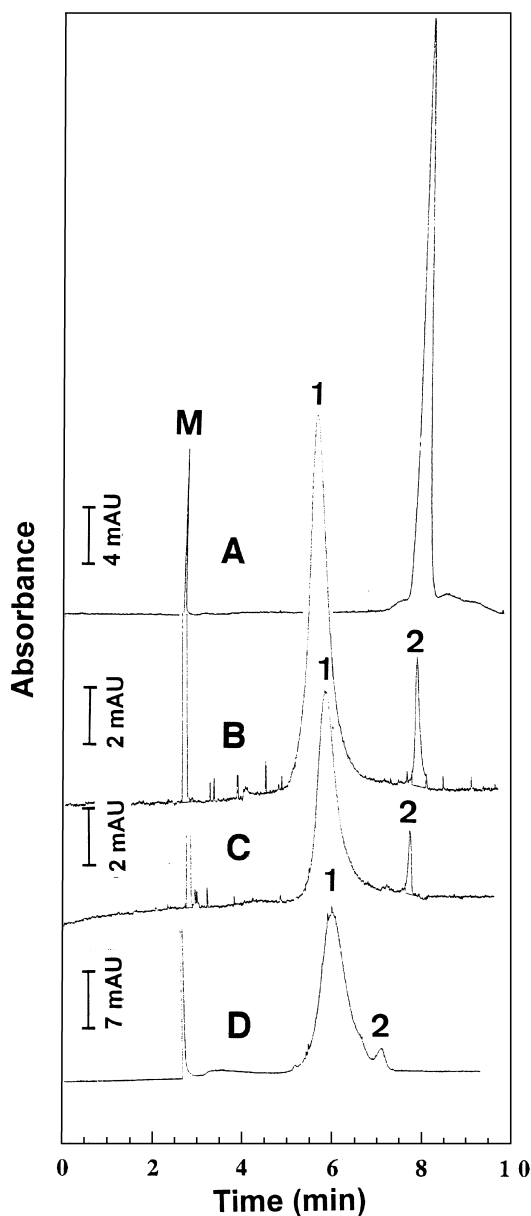


Fig. 2. Electropherograms of the diblock copolymers studied and of a PSS standard in free aqueous solution. (A) PSS 430; (B) PSS 227–PEP 48 copolymer; (C) PSS 257–PEP 21 copolymer; (D) PSS 404–PtBS 25 copolymer. Operating conditions: fused-silica capillary, 33.5 cm (25 cm to the detector) \times 50 μ m I.D. Electrolyte: 40 mM sodium borate buffer, pH 9.2. Applied voltage: 7.5 kV (220 V/cm). Hydrodynamic injection: 50 mbar, 3 s. Samples: 0.3 g/l for the diblock copolymers and 0.5 g/l for PSS 430, all dissolved in water. Detection: UV absorbance at 225 nm. Temperature: 27°C. Peaks: 1=copolymer micelles; 2=free copolymer; M=mesityl oxide (electroosmotic marker).

Table 2

Electrophoretic mobility values for the diblock copolymers in their micellised and free forms. The electrophoretic mobility of a free draining poly(styrene sulfonate) of similar molecular mass (PSS 430) is also given for the sake of comparison; operating conditions: see Fig. 2

Analytes	Electrophoretic mobilities ($10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$)	
	Micellised form (peak 1)	Free form (peak 2)
PSS 227–PEP 48	–34	–43
PSS 257–PEP 21	–35	–43
PSS 404–PtBS 25	–37	–41
PSS 430		–42

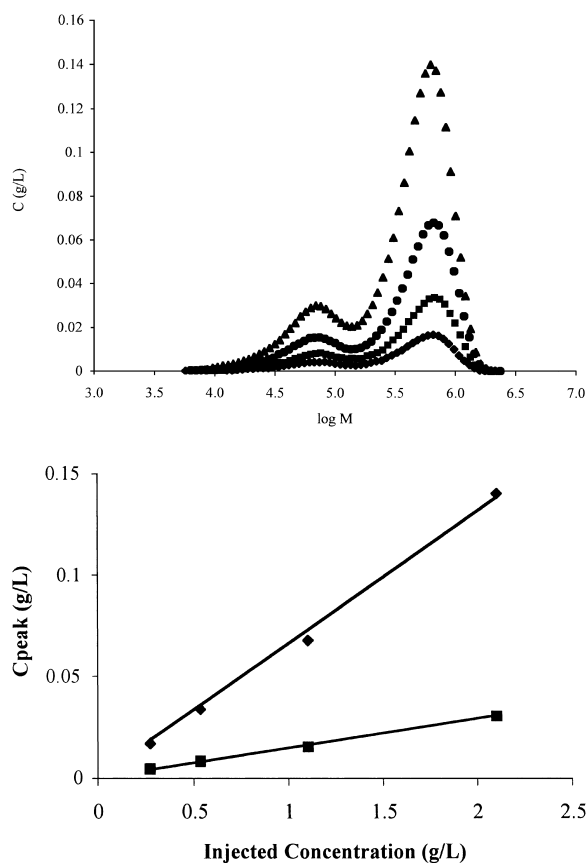


Fig. 3. Top: SEC chromatograms of PSS 404–PEP 25 sample. The eluent is a mixture of aqueous 40 mM sodium borate buffer, pH 9.2, and 40% (v/v) methanol. Influence of the injected concentration on the chromatograms from top to the bottom: 2.1, 1.1, 0.54, 0.27 g/l. When renormalised in weight fractions, all the results presented collapse onto a single curve (not shown). Bottom: variation of the peak heights with the injected concentration.

1. the peak corresponding to the micelles should be broader than that of the free, almost monodisperse copolymers, since the scatter in the aggregation numbers induces some dispersion in the micellar mobilities
2. peak 2 cannot be assigned to small ionic impurities because the peak 1 to peak 2 time-corrected area ratio was not modified after the sample had been dialysed for 1 week. Finally, the hypothesis that peak 2 might be due to PSS originating from a cleavage between the two blocks was also discarded from the electrophoretic behaviours observed in the presence of neutral surfactant micelles in the electrolyte (see Section 3.3 below).

Taking for granted this peak assignment, the micelle copolymer appears to have a smaller electrophoretic mobility (in absolute value) than the free copolymer, which could be explained by the fact that the density of the PSS chains in the corona may not be high enough to screen the hydrodynamic friction on the neutral core. In the limiting case where there were no hydrodynamic coupling at all between the neutral micellar core and the polyelectrolyte corona, the copolymer micelle would be equivalent to a free draining PSS bound to a non-free draining impervious particle, so that its electrophoretic mobility should be smaller (in absolute value) than that of a free draining PSS. On the contrary, the fact that the electrophoretic mobility of the free copolymer is about equal to that of a free draining PSS suggests that the hydrophobic segment in the free copolymer form is too small, due to its number of monomers and collapsed conformation in a strong nonsolvent, to alter the friction of the polyelectrolyte block (see Fig. 1). Another explanation may be a high hydrodynamic coupling between the short hydrophobic end and the long PSS chain, the latter being able to take a conformation allowing to fully wrap the hydrophobic tail.

3.2. Influence of the injected concentration of the copolymer

The distribution equilibrium between the copolymer micelle and the free copolymer was further investigated by studying the influence of the injected concentration of PSS 227–PEP 48 copolymer on the

time-corrected areas of peak 1 and peak 2. Fig. 4 shows that the time-corrected areas of the two peaks associated to the copolymer system increase linearly on increasing the sample concentration. In the hypothetical case of a classical monomer–micelle equilibrium having a very fast kinetics, the corrected peak area of the free monomer should remain constant and proportional to the critical micellar content (CMC). The results observed can then be explained by considering that the copolymer micelle-free copolymer system had not reached its thermodynamic equilibrium owing to its slow kinetics, which is consistent with the possibility of separating the two forms. Fig. 4 also gives a value of ~ 14 for the peak 1 to peak 2 time-corrected area ratio. Assuming that the mass absorption coefficients are equal for the copolymer micelles and the free copolymer, this value then corresponds to the mass proportion of micelles to free copolymer present in the sample stock solution.

Similar SEC experiments were performed with PSS 404–PEP 25 copolymer with an eluent containing 40% methanol. The injected copolymer con-

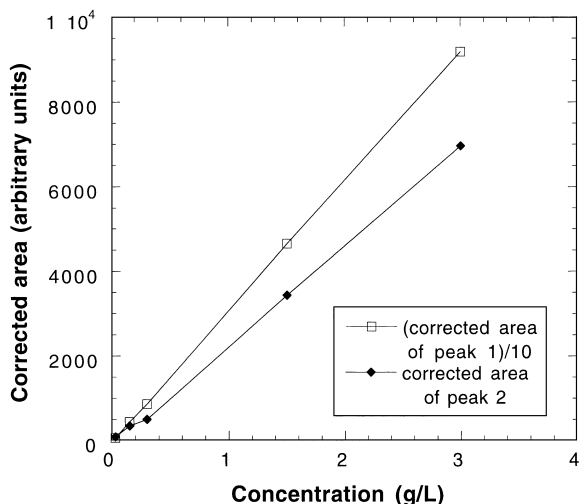


Fig. 4. Influence of the injected concentration of PSS 227–PEP 48 copolymer on the time-corrected peak areas obtained by CE experiments. Operating conditions: fused-silica capillary, 60 cm (50 cm to the detector) \times 50 μ m I.D. Applied voltage: 13.5 kV. For other conditions and peak numbering see Fig. 2. The samples were prepared at the stated concentrations by diluting the stock copolymer solution at 3 g/l in water with the electrolyte, just prior to be injected. No variation of the measured time-corrected areas was observed, however, for samples injected 24 h after dilution.

centration was varied from 0.27 to 2.1 g/l (Fig. 3) and in accordance with the CE experiments, weight fraction was constant, confirming the slow kinetics of the micelle–copolymer equilibrium [27].

3.3. Influence of the temperature

The influence of the temperature on the distribution equilibrium between the micellised and free forms was next studied by CE between 27 and 55°C. For this purpose, the samples were kept in a water bath at the temperature stated for 1 h, next loaded onto the carousel and finally injected into the capillary, both of these parts being thermostated at the same temperature. Fig. 5 shows that the electropherograms of PSS 227–PEP 48 and PSS 257–PEP 21 copolymers still exhibited two peaks when the temperature was raised to 55°C, but the resolution between peaks 1 and 2 was slightly impaired. The variations of the electrophoretic mobilities corresponding to peaks 1 and 2 and of the peak 1 to peak 2 time-corrected area ratio are displayed as a function of the temperature for PSS 227–PEP 48 copolymer in Fig. 6. These mobilities were normalised to the electroosmotic mobility to compensate for the viscosity variation. A slight decrease (in absolute value) of the mobility corresponding to peak 2 can be noticed, while that of peak 1 and the corrected peak area ratio do not exhibit significant variations. Identical results were obtained for PSS 257–PEP 21 (not shown). It appears that the kinetics of the distribution between the free copolymer and the copolymer micelle is hardly (if any) modified in the temperature range of 27–55°C. No attempt was made, however, to work at higher temperatures because of the limitations arising from the CE instrument specifications.

3.4. Influence of the electric field

The influence of the electric field, E , on the electrophoretic behaviour of the diblock copolymers was next studied in the range of 30–900 V/cm. This latter value was obtained by applying the highest voltage available (30 kV) to the shortest capillary that can be mounted in the cartridge (33.5 cm). Fig. 7 shows the electropherograms of PSS 227–PEP 48 and PSS 257–PEP 21 copolymers and of PSS 430

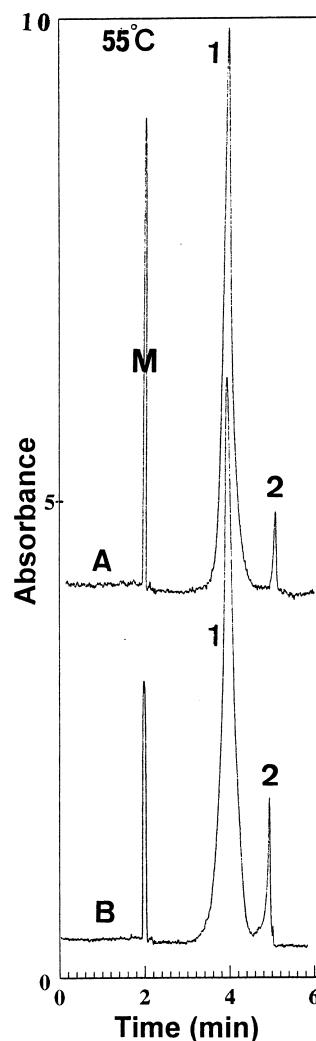


Fig. 5. Electropherograms of (A) PSS 227–PEP 48 and (B) PSS 257–PEP 21 diblock copolymers at 55°C. Operating conditions as in Fig. 2, except for the temperature.

for the extreme values of voltages applied, 1 and 30 kV. Interestingly for both copolymers, when the electric field is reduced from 900 to 30 V/cm, the separation efficiency increases tremendously for peak 2 and only to a much lesser extent for peak 1. For example, the calculated plate numbers for the second peak of PSS 227–PEP 48 copolymer were ~ 5000 and 660 000, respectively, at 900 and 30 V/cm electric field. This behaviour suggests that peak efficiency is not controlled by molecular diffusion at least over part of this range of electric field. On the

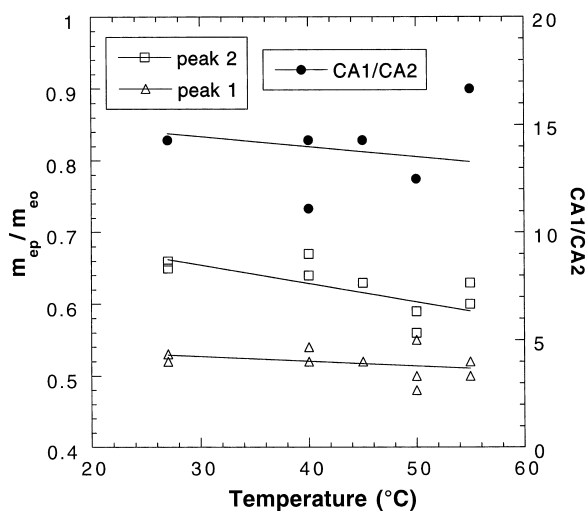


Fig. 6. Influence of the temperature on the electrophoretic mobilities corresponding to peaks 1 and 2 (normalised to the electroosmotic mobility) and on the peak 1 to peak 2 time-corrected area ratio (CA) for PSS 227–PEP 48 diblock copolymer. Operating conditions as in Fig. 2, except for the temperature. Straight lines are just guidelines for eyes.

other hand, the high plate count attained at low electric field is indeed reminiscent of what can be reached by monodisperse high-molecular-mass analytes in free draining behaviour under diffusion-controlled band broadening. It is thereby also consistent with the previous assignment of peak 2 to the free copolymer. The much lower apparent plate count determined for peak 1 under the same low electric field conditions (~ 700) is in agreement with a complex, more polydisperse and non free draining structure. Fig. 8 shows that the electric field seems to have no significant effect in this range on the electrophoretic mobilities corresponding to the peaks 1 and 2 of the diblock copolymers, as for PSS 430, used as an evenly charged reference polyelectrolyte. This result demonstrates that conformational changes such as field-oriented stretching are not to be contemplated in this range of electric field.

3.5. Influence of the presence of methanol

Since it was shown previously that temperature has little effect on the kinetics of the diblock copolymer systems in aqueous media below 55°C , the influence of adding methanol to the electrolyte

was tested. For this series of experiments, the diblock copolymer samples were prepared by ten-fold diluting an aqueous 3 g/l stock solution into aqueous 40 mM borate buffer–methanol (55:45, v/v). The corresponding electropherograms of PSS 227–PEP 48 and PSS 404–PtBS 25 diblock copolymers are presented in Fig. 9. Upon increasing the methanol content up to 40% (v/v), the resolution between peaks 1 and 2 decreases down to the point for which return to baseline is lost. Such a result can be explained by a slight increase in the rate of exchange between micellised and free copolymers.

3.6. Influence of the presence of neutral surfactant micelles

As already mentioned in Section 3.1, the presence of neutral surfactant micelles can be another way to discriminate between the free amphiphilic diblock copolymers and fully sulfonated polystyrenes. To test this idea, two surfactants, both of the alkoxy poly(oxyethylene) type (Brij 35 and Brij 78), were successively added to a 40 mM aqueous borate buffer at a concentration of 5 mM, which is much greater than the CMC values (0.1 mM for Brij 35, unavailable in the literature for Brij 78, but expected to be still lower). Fig. 10 enables us to compare the electropherograms of PSS 227–PEP 48 and PSS 404–PtBS 25 copolymers and of PSS 430 obtained in the presence and in the absence of Brij micelles in the electrolyte. Table 3 gives the electrophoretic mobilities calculated from the peaks observed in this figure. The PSS electrophoretic mobility is just slightly decreased in the presence of Brij 35 micelles, likely because of the increase in viscosity of the electrolyte. For PSS 227–PEP 48 and PSS 404–PtBS 25 copolymers, an additional peak (numbered 2*) can be noticed in front of the copolymer micelle peak (peak 1), when Brij micelles are present (Fig. 10C–E), seemingly at the expense of peak 2, which fully disappears in Fig. 10C. This new peak, corresponding to a lower mobility (in absolute value) than that of the copolymer micelle, might be attributed to an hydrophobic association between the free copolymer and Brij micelles. This experiment shows that, as anticipated in Section 3.1, peak 2 cannot be assigned to a PSS fragment resulting from a cleavage between the two blocks, since it does not behave like

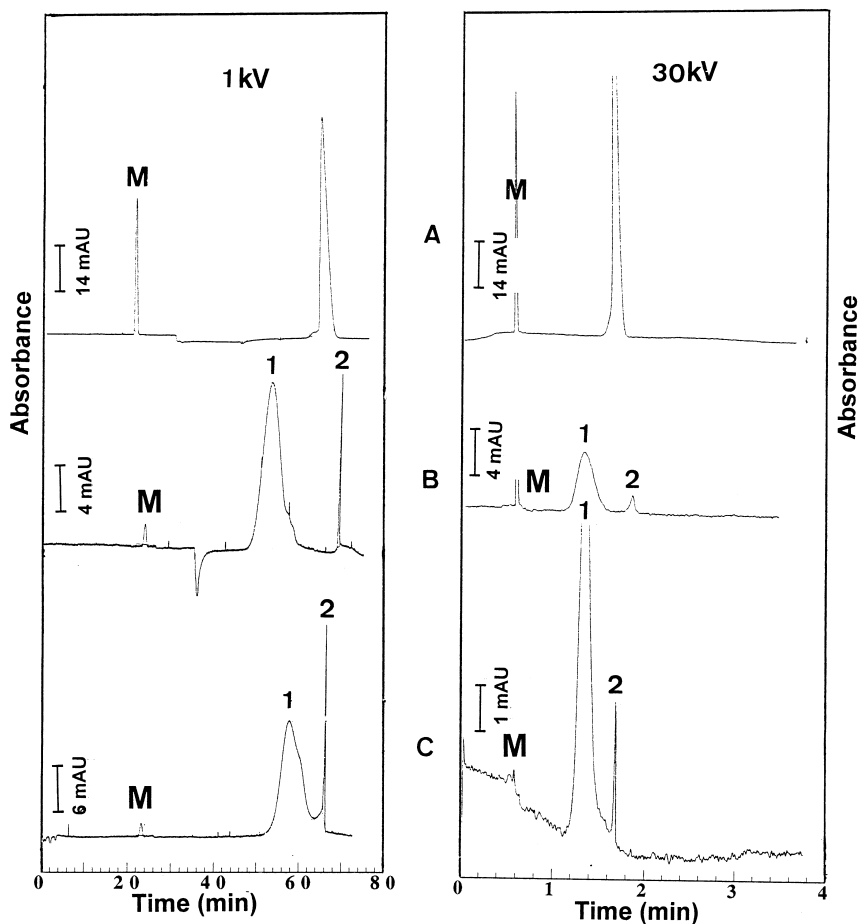


Fig. 7. Effect of the electric field on the electrophoretic behaviour of (A) PSS 430, (B) PSS 227–PEP 48 and (C) PSS 257–PEP 21 diblock copolymers. Applied voltages: left panel: 1 kV (30 V/cm); right panel: 30 kV (900 V/cm). Operating conditions as in Fig. 2, except for the applied voltage.

the PSS peak in the presence of neutral Brij micelles. Moreover, the fact that peak 2 is still observed in the case of PSS 227 PEP 48 with Brij 78 and of PSS 404–PtBS 25 with Brij 35, while it totally disappears in the case of PSS 227–PEP 48 in the presence of Brij 35, might be due to kinetic effects (unreached equilibrium). Besides, the observed decrease in the electrophoretic mobility of the copolymer micelle (peak 1) in the presence of the Brij micelles can be explained both by the increase in viscosity of the electrolyte and the insertion of a few neutral surfactant molecules into the copolymer micelles.

In order to obtain better insight into the structure of the free copolymer–surfactant micelle complex,

we attempted to apply the model for the electrophoretic mobility of composite objects that was developed by Long et al. [16]. For this model to apply, the following assumptions were initially considered: (i) the hydrophobic block of the copolymer was associated to a single Brij micelle; (ii) the hydrodynamic friction on this block is fully shielded by the friction on the Brij micelle; (iii) there is no hydrodynamic coupling between the PSS block and the Brij micelle. The electrophoretic mobility of the free copolymer–Brij micelle complex can then be expressed as the average of the electrophoretic mobilities of the two parts building the complex (PSS chain and Brij micelle), weighted by their

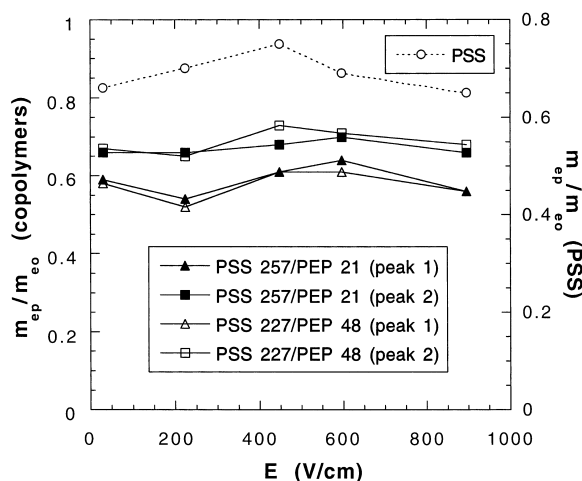


Fig. 8. Influence of the applied electric field, E , on the electrophoretic mobilities of peaks 1 and 2 of the diblock copolymers and of PSS 430 (electrophoretic mobilities were normalised to the electroosmotic mobility to compensate for viscosity variations induced by Joule heating). For operating conditions and peak numbering see Fig. 7.

respective hydrodynamic friction coefficients, which can be written as:

$$m_{\text{copo-Brij}} = m_{\text{PSS}} \frac{R_{\text{PSS}}}{R_{\text{PSS}} + R_{\text{Brij}}} \quad (1)$$

m_{PSS} represents the free draining mobility of PSS, the value of which is reported in Table 3 ($-40 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$). R_{PSS} is the radius of gyration of the PSS block in the copolymer. It was estimated from the Mark–Houwink relationship determined for the same electrolyte in a previous work [3] (8 nm for PSS 227 and 13 nm for PSS 404). R_{Brij} is the micelle radius, the value of which (4 nm) was taken from Ref. [22]. Finally, the mobility values for a one-to-one free copolymer–Brij micelle complex that can be estimated with these data using Eq. (1) were $-27 \cdot 10^{-9}$ and $-31 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ in the cases of PSS 227–PEP 48 and PSS 404–PtBS 25 copolymers, respectively. These estimations were much higher than the experimental values attributed to these complexes ($-13 \cdot 10^{-9}$ and $-14 \cdot 10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, respectively, see Table 3). This discrepancy may indicate that the hydrophobic block of the copolymers interacts with more than one Brij micelle, possibly four to six of them. Alternatively, the hydrophobic block may cause larger, perhaps non-

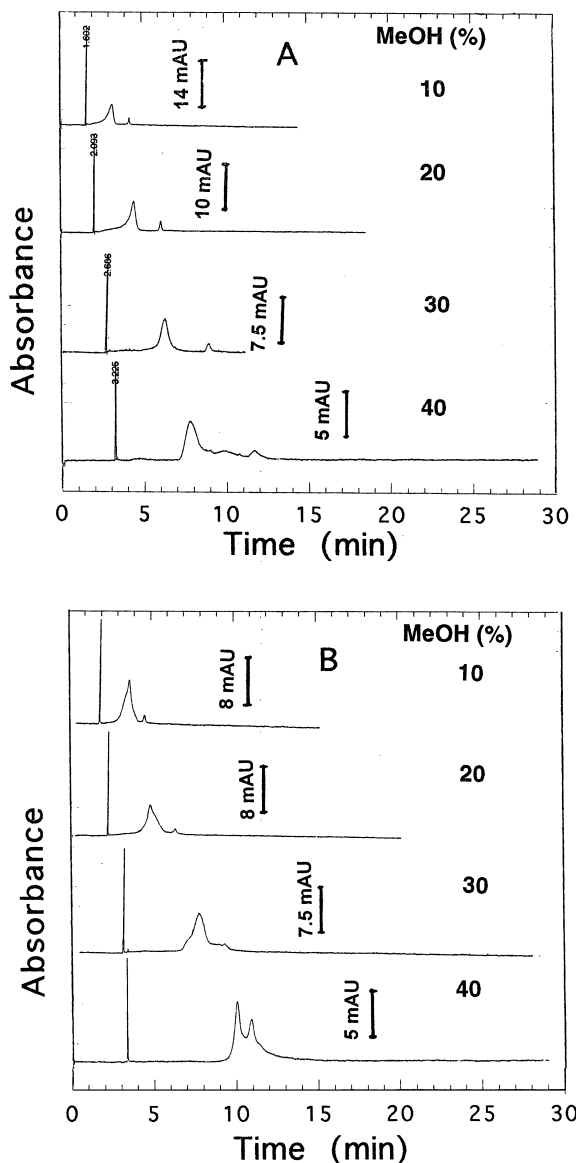


Fig. 9. Influence of the methanol content in the electrolyte on the electrophoretic behaviour of (A) PSS 227–PEP 48 and (B) PSS 404–PtBS 25 copolymers. Operating conditions as in Fig. 2, except electrolyte: 40 mM sodium borate buffer in water–methanol mixtures of varying compositions, as specified on the graph (constant ionic strength); applied voltage: 20 kV (600 V/cm); hydrodynamic injection: 50 mbar, 2 s; sample preparation: ten-fold dilution into aqueous 40 mM borate buffer–methanol (55:45, v/v), just prior to be injection, of an aqueous 3 g/l stock solution.

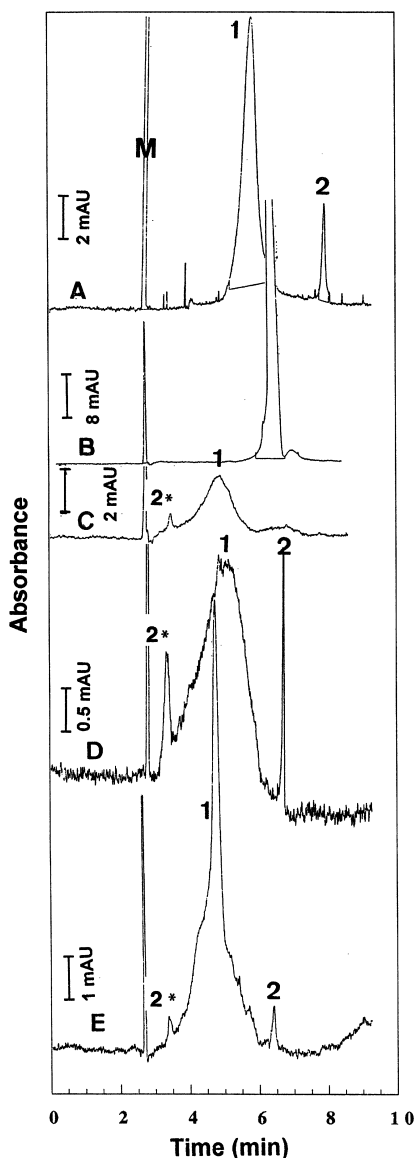


Fig. 10. Comparison of the electropherograms of PSS 227–PEP 48 and PSS 404–PtBS 25 diblock copolymers and PSS 430 obtained in the presence or in the absence of neutral Brij micelles in the electrolyte. (A) PSS 227–PEP 48 copolymer, 40 mM sodium borate buffer, pH 9.2, electrolyte without surfactant. (B) PSS 430, borate buffer containing 5 mM Brij 35. (C) PSS 227–PEP 48 copolymer, borate buffer containing 5 mM Brij 35. (D) PSS 227–PEP 48 copolymer, borate buffer containing 5 mM Brij 78. (E) PSS 404–PtBS 25 copolymer, borate buffer containing 5 mM Brij 35. The samples were prepared from an aqueous 3 g/l stock solution that was ten-fold diluted in the separation electrolyte containing the surfactant. Other operating conditions as in Fig. 2. Peaks: 2* = free copolymer–Brij micelle complex; other peaks as in Fig. 2.

spherical micelles of Brij to form. It is important to note that the existence of an hydrodynamic coupling between the PSS chain and the Brij micelle cannot account for this discrepancy, since it would result in an increase in the mobility of these complexes, with respect to the value given by Eq. (1).

The influence of the electric field, E , was also considered with the electrolyte containing Brij micelles, to check for possible stretching of the PSS coil and hence for a possible increase in their electrophoretic mobilities. During its migration motion, this coil is submitted to thermal cohesive forces, F_T , given by:

$$F_T = \frac{kT}{R_{PSS}} \quad (2)$$

where T is the absolute temperature and k the Boltzmann constant, but it also undergoes a drag force F_D on the part of the Brij micelle, which can be expressed by:

$$F_D = 6\pi\eta R_{PSS} E(m_{PSS} - m_{\text{copo-Brij}}) \quad (3)$$

where η is the electrolyte viscosity. From a theoretical point of view, it can be anticipated that the electric field will induce an elongation of the PSS coil above a threshold value E_{th} corresponding to the point for which the thermal cohesive forces are balanced by the drag force, so that:

$$E_{th} = \frac{kT}{6\pi\eta R_{PSS}^2 (m_{PSS} - m_{\text{copo-Brij}})} \quad (4)$$

Using the experimental data of Table 3 for the mobility values and the previously given values for R_{PSS} , the threshold values E_{th} were estimated to 1200 V/cm for PSS 227–PEP 48 and 550 V/cm for PSS 404–PtBS 25. Using the current design of the CE apparatus, we were able to vary the electric field between 30 and 900 V/cm. As shown in Fig. 11, no significant variation of the electrophoretic mobility corresponding to peak 2* was noticed over this range for PSS 404–PtBS 25. The mobility of PSS 430 was also plotted for comparison. No variation was disclosed either for PSS 227–PEP 48 copolymer (not shown). The calculated threshold values of the electric field might be underestimated in the case of an hydrodynamic coupling between the PSS block and the hydrophobic block bound to the Brij micelle(s).

Table 3

Electrophoretic mobilities ($10^{-9} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$) calculated from the peaks associated to PSS 227–PEP 48 and PSS 404–PtBS 25 diblock copolymers and to PSS 430 in the presence and in the absence of neutral surfactants (Brij 35 or Brij 78)

Analytes	Peaks	40 mM borate buffer, pH 9.2		
		No additive	+ 5 mM Brij 35	+ 5 mM Brij 78
PSS 227–PEP 48	1	–34	–28	–31
	2	–43	n.d.	–40
	2*		–13	–11
PSS 404–PtBS 25	1	–37	–30	
	2	–41	–39	
	2*		–14	
PSS 430		–42	–40	

n.d., not detected; operating conditions and peak numbering as in Fig. 10.

4. Conclusion

This work is, to our knowledge, the first attempt to use CE for the elucidation of the physicochemical behaviour of water soluble associative diblock copolymers. For each diblock copolymer studied, two peaks were detected. In spite of the inherent limitations of on-line UV detection for structure elucidation and peak assignment, these peaks were attrib-

ted to the free copolymer and the copolymer micelle forms thanks to additional experiments implemented in SEC, which provided a means for obtaining molecular mass information. This peak assignment was consistent with the whole physicochemical behaviour of these diblock copolymers, as observed from all the performed CE and SEC experiments. The separation of the two forms confirms the very slow rate of exchange that can be expected from previous investigations employing physical techniques. CE has been demonstrated to provide further insight into the distribution equilibrium between the two forms of the copolymers. In an aqueous solution at ambient temperature, these systems were shown to be out of equilibrium and no classical CMC value can be determined. Temperature has practically no effect below 55°C and addition of methanol up to 40% hardly increases the rate of exchange between the two forms. Finally, the unexpectedly low mass ratio between the micellised and free forms (~ 14) should reflect the early stages of the copolymer dissolution, or even perhaps copolymer elaboration. This point remains to be clarified. Mobility measurements have also provided additional interesting physicochemical insight into these systems. Especially, the mobility of the copolymer micelle appears to be smaller (in absolute value) than the mobility of the free copolymer, suggesting that the polyelectrolyte chains forming the micelle corona do not fully screen the hydrodynamic friction on the hydrophobic core. Furthermore, it has been shown that neutral surfactant micelles likely interact with the hydrophobic block of the free copolymers. The

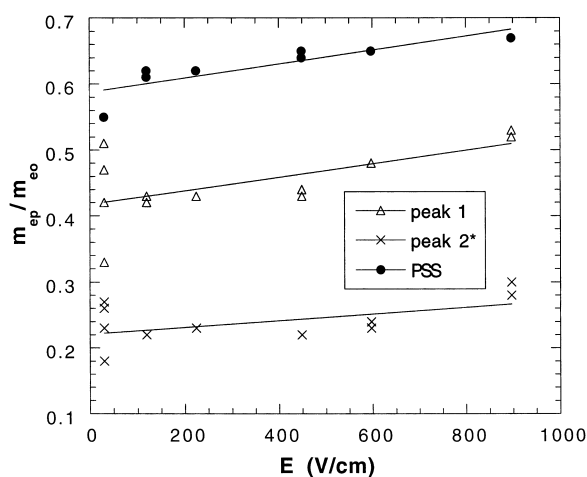


Fig. 11. Influence of the applied electric field E on the electrophoretic mobilities corresponding to the peaks of PSS 404–PtBS 25 copolymer and of PSS 430 in the presence of Brij 35 in the electrolyte. (Electrophoretic mobilities were normalised to the electroosmotic mobility to compensate for viscosity variations induced by Joule heating). All the experimental measurements were plotted. Operating conditions and peak numbering as in Fig. 10. Straight lines are just guidelines for eyes.

comparison between the mobilities experimentally attributed to the free copolymer–surfactant micelle complex and the values estimated from modelling of these composite objects lends support to the idea that several surfactant micelles might be bound to the same hydrophobic block. It can be anticipated that the wider availability of CE–MS coupling in the near future will bring about even more physicochemical understanding of such complex systems in solution.

Acknowledgements

A. Ajdari and D. Long are gratefully acknowledged by the authors for stimulating and fruitful discussions.

References

- [1] J.B. Poli, M.R. Schure, *Anal. Chem.* 64 (1992) 896.
- [2] M. Minarik, B. Gas, E. Kenndler, *Electrophoresis* 18 (1997) 98.
- [3] H. Cottet, P. Gareil, *J. Chromatogr. A* 772 (1997) 369.
- [4] H.N. Clos, H. Engelhardt, *J. Chromatogr. A* 802 (1998) 149.
- [5] O. Grosche, J. Bohrisch, U. Wendler, W. Jaeger, H. Engelhardt, *J. Chromatogr. A* 894 (2000) 105.
- [6] J. Bohrisch, O. Grosche, U. Wendler, H. Engelhardt, *Macromol. Chem. Phys.* 201 (2000) 447.
- [7] M.E. Starkweather, D.A. Hoagland, M. Muthukumar, *Macromolecules* 33 (2000) 1245.
- [8] J. Noolandi, *Electrophoresis* 13 (1992) 394.
- [9] P. Mayer, G.W. Slater, G. Drouin, *Anal. Chem.* 66 (1994) 1777.
- [10] S.J. Hubert, G.W. Slater, *Electrophoresis* 16 (1995) 2137.
- [11] A.R. Volkel, J. Noolandi, *Macromolecules* 28 (1995) 8182.
- [12] G.W. Slater, T.B.L. Kist, H. Ren, G. Drouin, *Electrophoresis* 19 (1998) 1525.
- [13] C. Heller, G.W. Slater, P. Mayer, N. Dovichi, D. Pinto, J.-L. Viovy, G. Drouin, *J. Chromatogr. A* 806 (1998) 113.
- [14] J. Sudor, M.V. Novotny, *Anal. Chem.* 67 (1995) 4205.
- [15] J. Sudor, M.V. Novotny, *Anal. Chem.* 69 (1997) 3199.
- [16] D. Long, J.-L. Viovy, A. Ajdari, *J. Phys. Condens. Matter* 8 (1996) 9471.
- [17] D. Long, A. Ajdari, *Electrophoresis* 17 (1996) 1161.
- [18] D. Long, A.V. Dobrynin, M. Rubinstein, A. Ajdari, *J. Chem. Phys.* 108 (1998) 1234.
- [19] D.A. Hoagland, D.L. Smisek, D.Y. Chen, *Electrophoresis* 17 (1996) 1151.
- [20] B. Staggemeier, Q.R. Huang, P.L. Dubin, Y. Morishima, T. Sato, *Anal. Chem.* 72 (2000) 255.
- [21] H. Cottet, P. Gareil, O. Theodoly, C.E. Williams, *Electrophoresis* 21 (2000) 3529.
- [22] J. Collet, C. Tribet, P. Gareil, *Electrophoresis* 17 (1996) 1202.
- [23] P.H. Wijnen, J.W.Th. Lichtenbelt, K.S. Williams, in: Presented as a poster at the 13th International Symposium on High Performance Capillary Electrophoresis, Saarbrücken, 2000.
- [24] P. Guenoun, H.T. Davis, M. Tirrell, J.W. Mays, *Macromolecules* 29 (1996) 3965.
- [25] L. Valint, J. Bock, *Macromolecules* 21 (1988) 175.
- [26] F. Muller, PhD Thesis, University of La Rochelle, 2000.
- [27] Z. Tuzar, P. Kratochvil, in: E. Matijevic (Ed.), *Surface and Colloid Science*, Vol. 15, 1993, pp. 1–83, See especially pp. 50–55.