

# Poly(sodium-4-styrenesulfonate) as the pseudostationary phase in capillary electrokinetic chromatography

**Birutė Pranaitytė,  
Audrius Padarauskas\***

*Department of Analytical and  
Environmental Chemistry,  
Vilnius University,  
Naugarduko 24,  
LT-2006 Vilnius, Lithuania*

A water-soluble anionic polymer (poly(sodium-4-styrenesulfonate) or PSS) was investigated as new pseudostationary phase in capillary electrokinetic chromatography for the separation of anionic, cationic and neutral compounds. It was shown that the use of a polymer-containing electrolyte generated a significantly faster cathodic electroosmotic flow, especially at lower pHs. A comparison of the mobilities measured in the presence and absence of PSS showed that the migration of the organic anions (aromatic sulfonates) was unaffected by the polymer. In contrast, even relatively low concentrations (0.01–0.03%) of the polymer dramatically decreased the migration velocity of cationic compounds (aromatic amines). However, the peak resolution was only slightly improved due to a significant peak broadening and tailing when PSS was included in the electrolyte. The system studied was shown to be suitable for the separation of uncharged analytes possessing hydrophobic and/or aromatic groups. For the system with 0.4% PSS as a pseudostationary phase, four neutral compounds (benzoic acid, methyl, ethyl, and propyl 4-hydroxybenzoates) were completely resolved. Analyte hydrophobicity was found to have a major influence on the migration behaviour of neutral compounds.

**Key words:** poly(sodium-4-styrenesulfonate), capillary electrokinetic chromatography, pseudostationary phase

## INTRODUCTION

Capillary electrophoresis (CE) is a powerful technique for the determination of charged species where separation occurs due to differences in electrophoretic mobilities [1]. CE offers the advantages of very high efficiency, reduced analysis time and small sample volume. However, the ability to control the separation selectivity is limited since mobilities are primarily physical properties of the analytes and are not easily varied. Separation selectivity can be modified to some extent by varying such parameters as pH, ionic strength or using organic modifiers, but generally these approaches lead to small changes in mobility [2]. Moreover, separation of uncharged compounds is impossible with a conventional CE technique.

The use of additives to CE system has proven to be a popular way to control selectivity for a wide variety of analytes including neutral ones. This technique is based on the differing interactions of the additive (often termed a pseudostationary phase)

with the analytes of interest, leading to mobility changes and hence an ability to control the selectivity. This approach has been termed capillary electrokinetic chromatography (EKC), since it combines the electro-driven separation of CE with a chromatographic component brought about by the addition of the pseudostationary phase [3]. The commonly used pseudostationary phases are micelles formed dynamically from low molecular mass surfactants such as sodium dodecylsulfate [4, 5]. However, the critical micelle concentration of such conventional surfactants varies with the ionic strength, pH, temperature [6, 7], etc. Under higher organic solvent concentration, the micelle structure could be changed dramatically, making separation impossible [8].

In the recent years there has been a great deal of interest in the use of polymeric pseudostationary phases. Water-soluble polymers have several advantages over conventional micelles, *e.g.*, they have no critical micelle concentration and are stable in the presence of a high content of organic solvents. The use of polymeric phases, the structures employed and their advantages have been reviewed in detail elsewhere [9, 10].

\* Corresponding author.

In the present study, we investigated a commercially available, water-soluble anionic polymer (poly(sodium-4-styrenesulfonate) or PSS) as a pseudostationary phase for EKC separations.

## EXPERIMENTAL

Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments Inc., Fullerton, CA, USA) equipped with a UV detector with wavelength filters (200, 214, 230 and 254 nm). A fused silica capillary (Polymicro Technology, Phoenix, AZ, USA) of 75  $\mu\text{m}$  i.d. (375  $\mu\text{m}$  o.d.) and 57 cm total length (50 cm to the detector) was used. Separations were performed in a positive polarity mode, *i.e.* with migration of both the analytes and the electroosmotic flow toward the negative electrode. Samples were injected in the hydrodynamic mode by overpressure ( $3.43 \times 10^3$  Pa). The system Gold software was used for data acquisition. All experiments were conducted at 25  $^{\circ}\text{C}$ .

All electrolyte and standard solutions were prepared using double-distilled water. Poly(sodium-4-styrenesulfonate) (PSS, average molecular mass  $\sim 70000$  g/mol) was purchased from Aldrich (Milwaukee, WI, USA). All other reagents, of analytical-reagent grade, were obtained from Merck (Darmstadt, Germany).

All electrolyte solutions were filtered through a 0.45  $\mu\text{m}$  membrane filter. The capillary was rinsed with 1.0 mol/l sodium hydroxide and water for 5 min and then equilibrated with a carrier electrolyte for 10 min at the beginning of each day. Between all electrophoretic separations the capillary was rinsed for 2 min with a carrier electrolyte.

The values of electroosmotic flow (EOF) mobility ( $\mu_{\text{eo}}$ ) were calculated by the following equation:

$$\mu_{\text{eo}} = L_{\text{d}} \cdot L_{\text{t}} / t_{\text{eo}} \cdot U,$$

where  $L_{\text{d}}$  is the effective length of the capillary,  $L_{\text{t}}$  is the total length of the capillary,  $t_{\text{eo}}$  is the migration time of neutral marker (acetone),  $U$  is the voltage applied.

## RESULTS AND DISCUSSION

### Effect of polymer on EOF

In order to investigate the capillary surface behaviour, the electroosmotic flow (EOF) generated in both the PSS-containing electrolyte and the same electrolyte without PSS was measured at different pH values. The results obtained from this experiment are shown in Fig. 1. As expected, the EOF measured in the electrolyte without the polymer

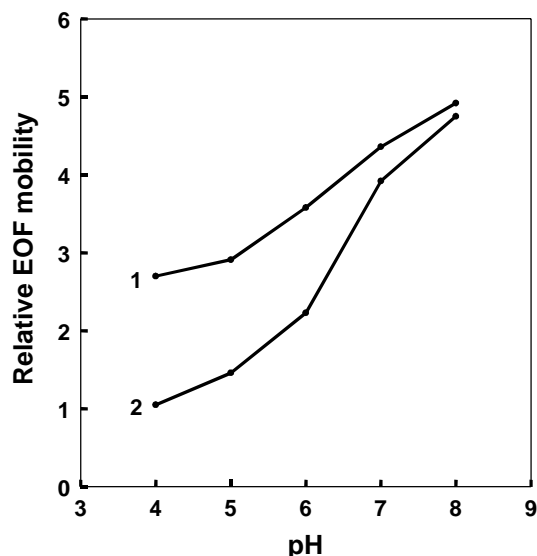


Fig. 1. EOF mobility as a function of pH in (1) PSS-containing electrolyte and (2) the same electrolyte without PSS. Electrolytes: (1) 10 mmol/l  $\text{Na}_2\text{SO}_4$ , 2 mmol/l  $\text{H}_3\text{PO}_4$  and 0.2% (w/v) PSS; (2) 10 mmol/l  $\text{Na}_2\text{SO}_4$  and 2 mmol/l  $\text{H}_3\text{PO}_4$ . Conditions: voltage, 25 kV, temperature, 25  $^{\circ}\text{C}$ , neutral marker, acetone

showed a typical dependence on the pH, *i.e.* increasing the pH resulted in an increased deprotonation of the capillary surface silanols, thereby increasing the zeta potential and the EOF mobility. The use of a PSS-containing electrolyte under identical separation conditions gave a significantly faster EOF, especially at lower pHs. This suggests that PSS increases the negative charge density on the capillary wall most likely due to physical adsorption on the capillary surface. Under alkaline conditions the capillary wall silanols are completely ionized, *i.e.* negatively charged, preventing the adsorption of the anionic PSS due to electrostatic repulsion. As a result, at pH above 7, the EOF in the both electrolytes was generated only by the ionized silanols and, consequently, in the both cases similar EOF mobilities were observed. When the electrolyte pH was lowered, the native negative charge of the capillary wall decreased causing a stronger interaction of the PSS with the less charged surface. This increased the number of negative charges (the sulfonate groups of the polymer are fully deprotonated in the pH range studied) on the surface, and the EOF in the presence of PSS became significantly faster than that obtained in the electrolyte without PSS.

The stability of the capillary wall coating with PSS was investigated by monitoring the EOF after removal of the polymer from the electrolyte. Specifically, the capillary was first rinsed with a PSS-containing electrolyte (pH 3.0) for 10 min. The excess of PSS was then removed from the capillary by

a 1 min rinse of the same electrolyte in the absence of the polymer. Ten successive EOF measurements were then made by injection of acetone. Additionally, the same measurements were performed using a PSS-containing electrolyte. Figure 2 illustrates a gradual increase in the EOF migration time (or a decrease in the EOF mobility) observed when PSS was omitted in the electrolyte. This indicates that the adsorption of PSS onto the capillary surface was not strong enough and the coating was depleted from the capillary wall. In contrast, addition of PSS to the electrolyte resulted in a substantial enhancement of EOF stability. Over the entire experimental space, the EOF varied by less than 2.3%.

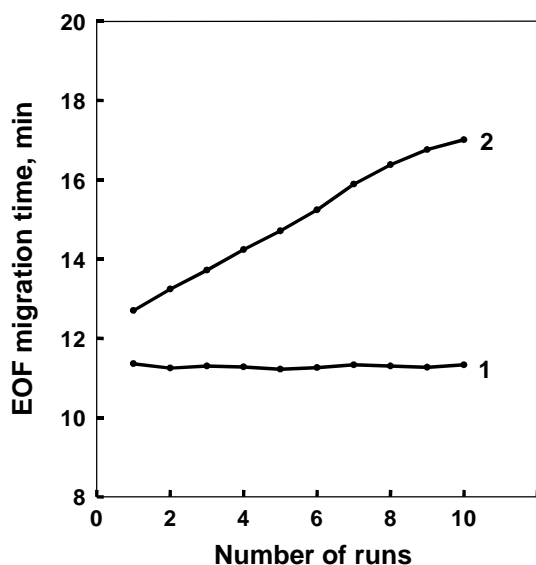


Fig. 2. Stability of EOF in (1) PSS-containing electrolyte and (2) the same electrolyte without PSS (pH 3.0). Other conditions as in Fig. 1

### Separation ability of PSS

The primary purpose of this study was to examine PSS as a pseudostationary phase in EKC separations. A series of organic analytes was therefore chosen, which exhibited different charges and/or hydrophobicities as well as different affinities for PSS. Benzene, toluene and ethylbenzene sulfonates were used as a test mixture of anionic analytes. A comparison of the mobilities measured in the presence and absence of PSS showed that the migration of the sulfonates was unaffected by the polymer. Since the analytes and PSS are negatively charged, there was no interaction between them due to electrostatic repulsion.

As a test mixture of the cationic analytes we chose four amines, namely singly charged aniline, 4-aminophenol, 2-aminopyridine and doubly charged

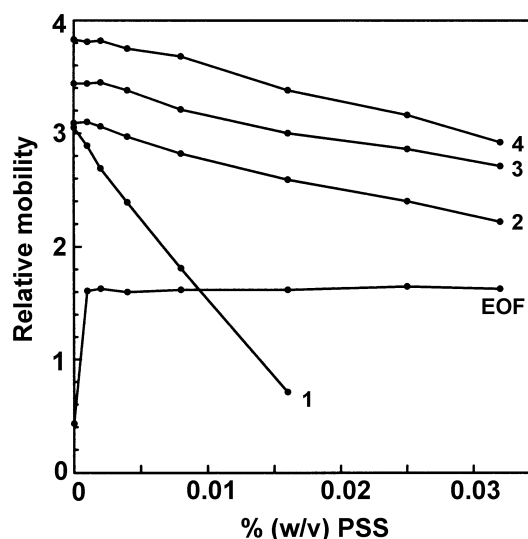


Fig. 3. Effect of PSS concentration on the mobilities of cationic analytes. Electrolyte: 10 mmol/l  $\text{Na}_2\text{SO}_4$ , 2 mmol/l  $\text{H}_3\text{PO}_4$  and PSS (pH 3.0). 1 - 1,10-phenanthroline; 2 - 4-aminophenol; 3 - aniline; 4 - 2-aminopyridine

1,10-phenanthroline. The migration behaviour of the amines was investigated as a function of the PSS concentration present in the electrolyte at pH 3.0 (Fig. 3). One can see that even relatively low concentrations of the polymer dramatically influenced the migration velocity of cationic compounds. In all cases the mobilities of the analytes were decreased when the concentration of PSS was increased. Figure 3 also shows that varying the concentration of PSS had a very significant effect on the mobility of the doubly charged analyte (1,10-phenanthroline) but a less pronounced effect on the mobilities of the singly charged cations. For example, the PSS concentration between 0 and 0.016%, the mobility for 1,10-phenanthroline was decreased by about 80%, while those for other amines dropped by 8–12%. As the percentage of PSS increased further, the electrophoretic mobility for 1,10-phenanthroline continued to decrease, however, identification of the analyte was not possible due to the very long migration time. These results indicate that the electrostatic interaction between the analyte and the PSS was the major driving force in the separation of cationic compounds. However, the analytes also showed a significant peak broadening and tailing when PSS was included in the electrolyte. Moreover, this effect became stronger when the percentage of PSS in the electrolyte increased. As an example, Fig. 4 compares the peaks of 1,10-phenanthroline cation obtained in the electrolyte without PSS and in the same electrolyte containing 0.016% (w/w) PSS. Such a decrease in peak efficiency was most probably caused by the very strong electrostatic interaction (ion pairing) between the analyte cations and PSS.

The third group of analytes represented neutral compounds differing in their hydrophobicity expressed by a different number of  $\text{CH}_2$  groups in their molecules. Benzoic acid, methyl, ethyl and propyl 4-hydroxybenzoates were used as a test mixture. Under the applied conditions (pH 3.0) all of these esters are completely uncharged, whereas only about 6.5% of total benzoic acid ( $\text{pK}^a = 4.19$ ) are present as an anion. The plot of the mobility values for the four analytes *versus* the polymer concentration is shown in Fig. 5. A definite increase in the mobility for all the analytes is noted when the concentration of PSS was increased. Electrophoregrams showing separation of these compounds without and with PSS are given in Fig. 6. Without introduction of PSS into the electrolyte all analytes, except slightly char-

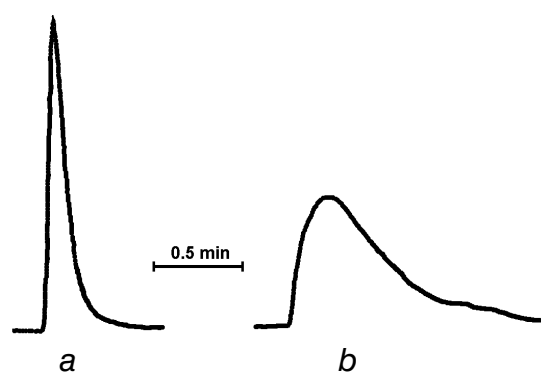


Fig. 4. Effect of PSS on the peak shape of the 1,10-phenanthroline. Electrolytes: (a) 10 mmol/l  $\text{Na}_2\text{SO}_4$ , 2 mmol/l  $\text{H}_3\text{PO}_4$  (pH 3.0); (b) 10 mmol/l  $\text{Na}_2\text{SO}_4$  and 2 mmol/l  $\text{H}_3\text{PO}_4$  and 0.016% (w/v) PSS (pH 3.0)

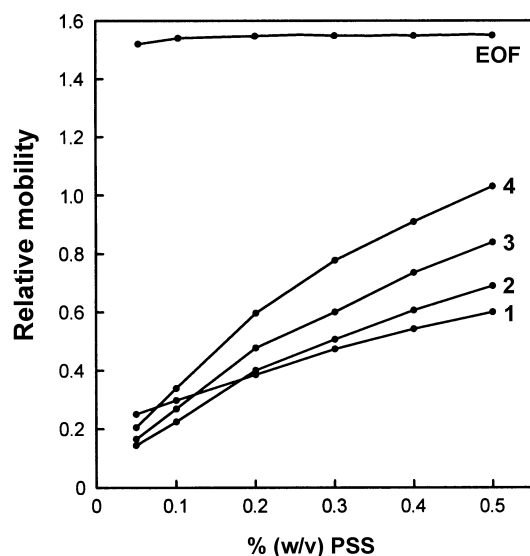


Fig. 5. Effect of PSS concentration on the mobilities of neutral analytes. Electrolyte: 10 mmol/l  $\text{Na}_2\text{SO}_4$ , 2 mmol/l  $\text{H}_3\text{PO}_4$  and PSS (pH 3.0). 1 – benzoic acid; 2 – methyl 4-hydroxybenzoate; 3 – ethyl 4-hydroxybenzoate; 4 – propyl 4-hydroxybenzoate

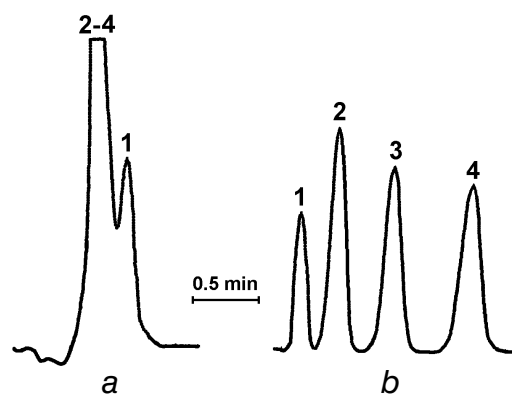


Fig. 6. Separation of four neutral analytes (a) in the electrolyte without PSS and (b) in the same electrolyte containing 0.4% (w/v) PSS. 1 – benzoic acid; 2 – methyl 4-hydroxybenzoate; 3 – ethyl 4-hydroxybenzoate; 4 – propyl 4-hydroxybenzoate. Other conditions as in Fig. 1

ged benzoic acid, migrated like a neutral marker (acetone) with a velocity of the EOF and thus produced a single peak. When a 0.4% PSS concentration was added to the electrolyte, the analytes were completely separated. Their migration times (mobilities) increased with increasing the number of methylene groups in the analyte molecule in the sequence benzoic acid < methyl 4-hydroxybenzoate < ethyl 4-hydroxybenzoate < propyl 4-hydroxybenzoate. This is in agreement with the elution order obtained for these analytes on a conventional octadecylsilylica stationary phase [11]. Hence the separation in this case is based on the different distribution of

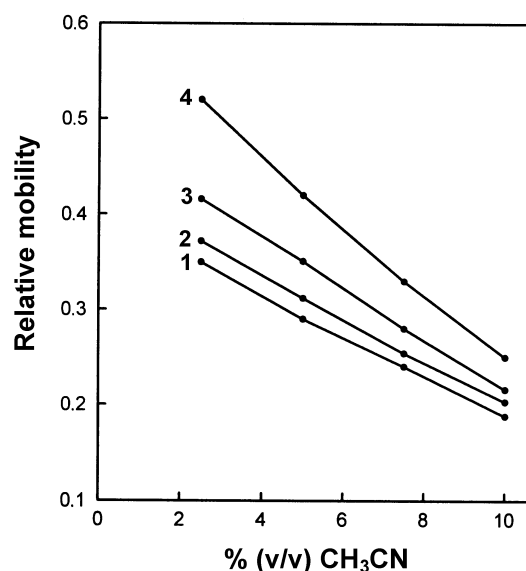


Fig. 7. Effect of acetonitrile concentration on the mobilities of neutral analytes. Electrolyte: 10 mmol/l  $\text{Na}_2\text{SO}_4$ , 2 mmol/l  $\text{H}_3\text{PO}_4$ , 0.4% (w/v) PSS and  $\text{CH}_3\text{CN}$  (pH 3.0). 1 – benzoic acid; 2 – methyl 4-hydroxybenzoate; 3 – ethyl 4-hydroxybenzoate; 4 – propyl 4-hydroxybenzoate

neutral analytes between the free electrolyte and the hydrophobic backbone of the polymer: less hydrophobic compounds interact less strongly with the pseudostationary phase and migrate with a lower mobility. The magnitude of this interaction could be directly controlled by addition to the electrolyte of organic solvents to suppress hydrophobic interactions with PSS. A plot of the mobility values *versus* the acetonitrile concentration in the electrolyte is shown in Fig. 7. We can see that the mobilities for all the analytes decreased over the whole range of CH<sub>3</sub>CN concentrations almost linearly to as much as a 10% CH<sub>3</sub>CN concentration. This provided a confirmation that in the presence of an organic modifier hydrophobic attraction of the neutral analytes and the PSS is decreased.

In conclusion, this report demonstrates that poly(sodium-4-styrenesulfonate) can be successfully employed as a pseudostationary phase for separation of neutral compounds in a capillary electrokinetic chromatography system, similarly to micellar systems.

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#### References

1. R. Kuhn and S. Hoffstetter-Kuhn, *Capillary Electrophoresis: Principles and Practice*, Springer-Verlag, Berlin (1993).
2. C. A. Lucy, *J. Chromatogr. A*, **850**, 319 (1999).
3. J. P. Quirino and S. Terabe, *J. Chromatogr. A*, **856**, 465 (1999).
4. U. Pyell, *Fresenius J. Anal. Chem.*, **371**, 691 (2001).
5. M. Molina and M. Silva, *Electrophoresis*, **23**, 3907 (2002).
6. S. Terabe, T. Katsura, Y. Akada, Y. Ishihama and K. Otsuka, *J. Microcol. Sep.*, **5**, 23 (1993).
7. J. H. Knox and K. A. MacCormack, *Chromatographia*, **38**, 207 (1994).
8. M. F. Emerson and A. Holzer, *J. Phys. Chem.*, **71**, 3220 (1967).
9. C. P. Palmer, *Electrophoresis*, **23**, 3993 (2002).
10. I. Peric and E. Kenndler, *Electrophoresis*, **24**, 2924 (2003).
11. K. Kuprovskytė, B. Pranaitytė and A. Padarauskas, *Chemija*, **13**, 160 (2002).

#### Birutė Pranaitytė, Audrius Padarauskas

#### POLI(NATRIO-4-STIRENSULFONATAS) – PSEUDOSTACIONARI FAZĖ KAPILIARINEI ELEKTROKINETINEI CHROMATOGRAFIJAI

#### S a n t r a u k a

Ištirtos vandenyje tirpus anijoninio polimero (poli(natrio-4-stirensulfonato), arba PSS) panaudojimo pseudostacionaria faze kapiliarinėje elektrokinetinėje chromatografijoje galimybės. Nustatyta, kad elektrolite su polimeru (pH 3–6) elektroosmosinis srautas yra 2–2,5 karto greitesnis nei analogiškame elektrolite be polimero. Polimeras visiškai neturi įtakos anijoninių junginių (aromatinių sulfonatų) judriams. Tuo tarpu netgi labai mažos polimero koncentracijos (0,01–0,03%) smarkiai sumažina katijonų (aromatinių aminių) judrius. Tačiau katijonų atskyrimo atrankumas pagerėja nežymiai, nes polimeras sukelia analičių smailių išsiplėtimą ir asimetriškumą. Tyrimų rezultatai parodė, kad PSS tinkamiausias neutraliems junginiams su hidrofobinėmis ir/arba aromatinėmis funkcinėmis grupėmis atskirti. Pridėjus į elektrolitą 0,4% PSS buvo atskirti keturi neutralūs junginiai: benzenkarboksirūgštis, metil, etil ir propil 4-hidroksibenzenkarboksirūgšties esteriai. Pagrindinis veiksnys, sąlygojantis neutralių junginių migravimo greitį elektrolite su PSS, yra jų hidrofobiškumas.