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Capillary Electrokinetic Chromatography with Charged Linear Polymers as a Nonmicellar PseudoStationary Phase: Determination of Capacity Factors and Characterization by Solvation Parameters

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A permanent polycation, polydiallyldimethylammonium (PDADMA), is applied as a linear, polymeric, replaceable, and nonmicellar pseudostationary phase for the separation of neutral analytes by capillary electrokinetic chromatography. It is shown that this polymer used in the background electrolyte is able to separate the analytes even if it does not form micelles under the given conditions. The most favorable aspect for practical use lays in the simple replacement of the separation media after each run, thus generating highly reproducible conditions. To determine the capacity factors of the analytes, a new method, based on an isotachophoretic regime, has been introduced for the measurement of the electrophoretic mobility of the polymeric pseudo-stationary phase. The capacity factors in the separation system, derived from the mobilities of the polymer, the electroosmotic flow, and the mobilities of 15 individual aromatic analytes, range between 0.3 and 1.2 for the given separation media (aqueous solution of acetate buffer, pH 5.2, with 4% w/w PDADMA). The type of interaction in the pseudochromatographic system was clarified from solvation parameters based on the linear free energy relationship model. It was found that π and n electron interactions and hydrogen-bond basicity of the polymer, as compared with the aqueous bulk phase, are the main cause of retention of the analytes.

Although electromigration separation methods are generally considered to exhibit better separation efficiency than high-performance liquid chromatography (HPLC), there is still a substantial advantage of the latter method: HPLC has many means for varying the separation selectivity, because a wide display of stationary and mobile phases allows an optimal choice for the system. While capillary zone electrophoresis (CZE) in its basic mode has some abilities to influence the mobility of the charged compounds, the presence of additional separation media is necessary when separating neutral analytes. In addition to well-established micellar electrokinetic chromatography (MEKC),[1,2] capillary electrochromatography (CEC)[3,4,5,6,7] also appears to become attractive. The separation process in CEC is based on partitioning between two phases similar to HPLC. In contrast to the latter technique, analytes are transported in CEC by electroosmosis with an almost flat radial velocity profile. Nevertheless, some technical problems arise during reproducible fabrication of packed capillaries.

Polymers have been successfully used as an alternative to a packed bed. Fujimoto[8,9,10,11] and Hjertén[12] utilized a cross-linked, charged, acrylamide-based gel attached to the capillary wall. The charged groups of the polymer generate the electroosmotic flow while its hydrophobic parts are supposed to interact with the analytes to influence their retardation. Though the efficiency of separation is good in these continuous-bed columns, the polymerization of the gel inside the capillary might be problematic.

Recently Svec et al.[13,14,15] applied charged polymeric monoliths as separation media in CEC. Monolithic columns show very good separation properties, and their preparation is easier and more reproducible than those packed with small particles. Polymeric monoliths can be prepared with both well-controlled porous properties and different surface performances.

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Different amphophilic polymers with both hydrophilic and hydrophobic regions were used as separation media in CEC[16,17,18,19] as well. These polymers are considered to form micelles from a single molecule. The polymer micelles are very stable as they have a size and structure fixed by covalent bonds. The critical micellar concentration of the polymer surfactants is practically zero. Unlike that of conventional low-molecular micelles, the formation of polymer micelles is not sensitive to organic modifiers or other additives used with the mobile phase.[20,21] Yang et al.[22] applied a linear solvatochromic model to characterize the triblock copolymer surfactant Elvacite 2669. Tanaka et al.[23] controlled the migration time window and the separation by utilizing a mixture of two polyallylamine-based surfactant polymers.

Charged polymers can be successfully used to influence the migration of both charged or neutral analytes even if they are not chemically cross-linked or present in the form of polymer micelles. Several authors reported the use of cationic polymers to influence selectivity in capillary electrophoresis of ions.[24,25,26,27] The separation principle is based on ion-pair formation of the ionic analyte with a charged polymer ion. Erim[28] demonstrated the electrophoretic separation of substituted phenols using cationic polyethyleneimine as a buffer additive. Potoček et al.[29] and Maichel et al.[30] performed the separation of neutral compounds, mostly phenols, in solutions of both negatively and positively charged polymers, namely partially hydrolyzed polyacrylamide and polyethyleneimine, respectively. Maichel et al.[31] investigated the influence of organic solvents (methanol, acetonitrile) on the separation of neutral compounds in systems containing polyethyleneimine.

The charged cationic polymers can also serve as dynamic noncovalent coatings of the inner capillary wall preventing the adsorption of large positive analytes such as basic proteins at the capillary wall. Among various charged polymers the use of poly(diallyldimethylammonium) cation, PDADMA, which consists of a diallyl backbone with strongly basic quaternary ammonium groups was described for this purpose.[32,33,34]

The present paper deals with solutions of a charged, nonmicellar linear polymer (PDADMA) used as separation media for electrically driven separation of neutral analytes. In addition to implementing separation selectivity, the polymer forms a stable layer on the capillary wall, thus being the cause of an electroosmotic flow (EOF) toward the anode. Samples injected at the cathodic end of the capillary are drifted with this electroosmotic flow to the anode while the charged polymer moves in the opposite direction, acting as a pseudostationary phase. In this way an electrochromatographic system is established.

Besides the investigation of the principal ability of PDADMA to separate neutral analytes, there are two other main topics of this paper. The first is the introduction of a method for the determination of the migration velocity and, therefore, the mobility of the charged polymer. This method thus enables the determination of the (chromatographic) capacity factors of the solutes, which are most important for the discussion of selectivity in pseudochromatographic systems. The knowledge of these capacity factors is a prerequisite for the second topic—the application of the linear free-energy relationship model. This model allows for the characterization of interactions between the solutes and the pseudostationary phase applied, expressed by the solvation parameters.

THEORETICAL

Capacity Factor and Mobility in Systems with a Polymeric Pseudophase. The system proposed in this paper consists of a solution of a linear charged polymer in a low molecular electrolyte buffer. This polymer serves as the pseudo-stationary phase and the rest of the solution, i.e., water with the low molecular electrolytes, (hereafter termed bulk), acts as the mobile phase. The charged polymer migrates under the influence of the electric field in the direction opposite to the solutes, which are moved by the EOF.

Neutral low-molecular solutes are separated if they have different distribution constants between the polymer and the bulk solution. According to the model of chromatography, the distribution of the analyte can be expressed by the capacity factor k_i

$$k_i = \frac{n_i^{(\mathrm{poly})}}{n_i^{(\mathrm{bulk})}} = K_i q \qquad (1)$$

where $n_i^{(\mathrm{poly})}$ and $n_i^{(\mathrm{bulk})}$ are the mole numbers of the ith analyte attached to the polymer (poly) and present in the bulk solution (bulk), respectively. K_i is the distribution constant expressed as the ratio of the molar fractions, $x_i^{(\mathrm{poly})}$ and $x_i^{(\mathrm{bulk})}$, of the solute bound to the polymer and that in the bulk liquid phase, respectively.

$$K_i = \frac{x_i^{(\mathrm{poly})}}{x_i^{(\mathrm{bulk})}} = \frac{\dfrac{n_i^{(\mathrm{poly})}}{n_i^{(\mathrm{poly})} + n_{\mathrm{poly}}}}{\dfrac{n_i^{(\mathrm{bulk})}}{n_i^{(\mathrm{bulk})} + n_{\mathrm{bulk}}}} \approx \frac{\dfrac{n_i^{(\mathrm{poly})}}{n_{\mathrm{poly}}}}{\dfrac{n_i^{(\mathrm{bulk})}}{n_{\mathrm{bulk}}}} \qquad (2)$$

Here n_{poly} and n_{bulk} are the total mole numbers of the polymeric pseudophase and the bulk liquid in the separation column, respectively. The phase ratio, q, is expressed for such systems instead of the volume ratio as the molar ratio of the two

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pseudophases:

$$q = \frac{n_{poly}}{n_{bulk}} \quad (3)$$

It can be assumed that the interaction of the analyte with the polymer can be related to the monomeric entities rather than to the entire molecule of the polymer. Then it is more appropriate to take formally n_{poly} as the mole number of the monomer units.

In analogy to MEKC, the migration velocity of the neutral solute, v_i, is the weighted average of its velocity in the bulk solution, given by the velocity of the EOF, v_{eof}, and its velocity when associated with the charged polymer. The latter can be approximated by the velocity of the polymer, v_{poly}.

$$v_i = \frac{1}{1 + k_i} v_{eof} + \frac{k_i}{1 + k_i} v_{poly} \quad (4)$$

All velocities are related here to the coordinate system connected with the capillary. The capacity factor k_i can be determined from velocities that are, in principle, experimentally available. As the velocities in eq 4 depend on the field strength in the same way, they can be replaced by the respective mobilities, μ_i, μ_{eof}, μ_{poly}, and the capacity factor can be calculated as:

$$k_i = \frac{\mu_{eof} - \mu_i}{\mu_i - \mu_{poly}} \quad (5)$$

We also introduce here the electrophoretic mobility of the polymer related to the bulk solution, μ^0_{poly}, which is connected by definition to the mobility of the polymer related to the capillary, μ_{poly}, as

$$\mu^0_{poly} = \mu_{poly} - \mu_{eof} \quad (6)$$

Solvation Parameters. An analogy of the proposed system with MEKC implies that a suitable description of the retention properties of a separation system can be made in the framework of the linear free-energy relationship (LFER) model[35,36,37] by solvation parameters. In this paper we utilize this model, which is based on the following expression for the capacity factor k_i:

$$\log k_i = c + mV_{x,i} + rR_i + s\pi_i + a\alpha_i + b\beta_i \quad (7)$$

The solute descriptors are the McGowan's characteristic volume $V_{x,i}$ (in $cm^3mol^{-1}/100$), the excess molar refraction R_i (in $cm^3/10$), the solute polarizability/dipolarity, π_i, the solute's effective hydrogen-bond acidity, α_i, and hydrogen-bond basicity, β_i. The system constants m, r, s, a, b are defined by their complementary interactions with the solute descriptors. The regression constant c does not reflect any type of interaction. For the present case, the constant m is a measure of the relative ease of cavity formation and general dispersion interactions for the solute with the charged polymer and the bulk solution (the mobile phase), respectively. The constant r determines the difference in capacity of the charged polymer and the bulk solution to interact with n or π electrons of the solute. Similarly, the constant s expresses the difference between both phases taking part in dipole−dipole and dipole−induced dipole interactions. Constants a and b are measures of the difference in hydrogen-bond basicity and hydrogen-bond acidity, respectively. The system constants can be obtained by multiple linear regression analysis of the experimentally obtained capacity factors from various solutes with known descriptors. Those used in the present work are given in Table 1.

Table 1. Solute Descriptors in LFER Model (Data From refs 43−44)

sample	V_x	R	π	α	β
acetophenon	1.0139	0.818	1.01	0.00	0.48
benzylalcohol	0.9160	0.803	0.87	0.33	0.56
methylbenzoate	1.0726	0.733	0.85	0.00	0.46
benzonitrile	0.8711	0.742	1.11	0.00	0.33
toluene	0.8573	0.601	0.52	0.00	0.14
benzylacetate	1.2135	0.798	1.06	0.00	0.65
benzylaldehyde	0.8730	0.820	1.00	0.00	0.39
nitrobenzene	0.8910	0.871	1.11	0.00	0.28
benzene	0.7164	0.610	0.52	0.00	0.14
anisol	0.9160	0.708	0.75	0.00	0.29
p-nitrophenol	0.9493	1.050	1.57	0.79	0.23
m-cresol	0.9160	0.822	0.88	0.57	0.34
phenol	0.7751	0.805	0.89	0.60	0.30
resorcinol	0.8340	0.980	1.00	1.10	0.58
α-naphthol	1.1440	1.520	1.05	0.61	0.37

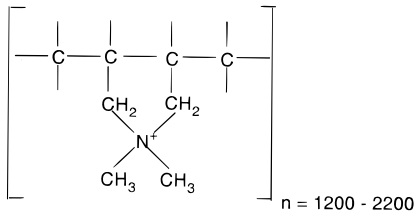

Figure 1. Structural formula of poly(diallyldimethylammonium cation) used as a pseudostationary phase.

EXPERIMENTAL SECTION

Equipment. All CE measurements were performed with a laboratory-built instrument equipped with a UV detector (Thermo Separation Products, Riviera Beach, FL) operating at 214 nm. Separations were carried out at a temperature of 25 °C. A fused-silica capillary 75/220 μm i.d./o.d. with a total length of 45.6 cm and a path to the detector of 8.5 cm was used. Samples were injected electrokinetically at 1 kV, 3−5 s.

The electric conductivity was measured with the Conductivity Meter CDM210, Radiometer, Copenhagen, Denmark.

Chemicals. Twenty percent w/w aqueous stock solution of poly(diallyldimethylammonium chloride), see Figure 1, average M_w 200 000−350 000 (Aldrich, Milwaukee, WI) was dissolved in 20.0 mM acetate buffer, pH = 5.2, in a desired concentration and then degassed under a vacuum. PDADMA is a strong base that is commercially available in aqueous solution. This solution is weakly acidic and has no buffering capacity. The acetate buffer

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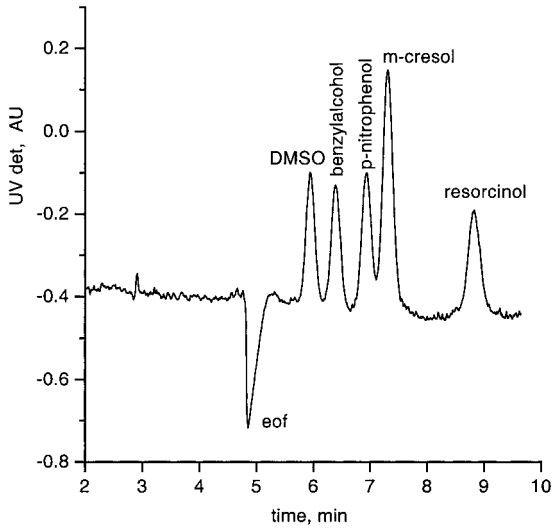

Figure 2. Separation of DMSO, benzyl alcohol, *p*-nitrophenol, *m*-cresol, and resorcinol in acetate buffer (pH 5.2) with 4% w/w PDADMA at 4 kV; injection, 1.3 kV, 3 s; detection at 214 nm, average current 47 μA.

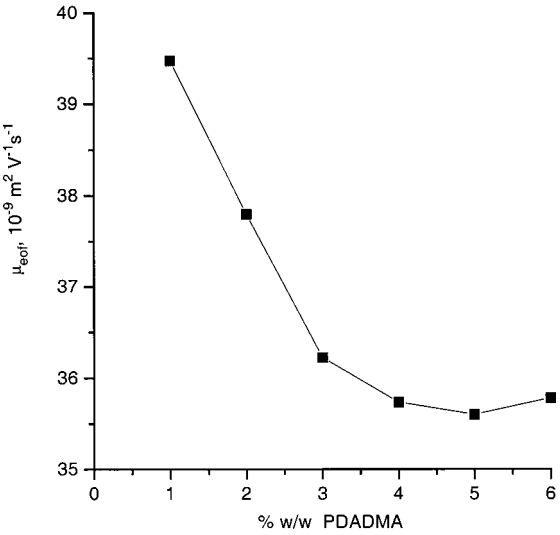

Figure 3. Plot of the electroosmotic mobility vs percent w/w of PDADMA at an applied voltage of 4 kV.

present in the background electrolyte stabilizes the pH value. The pH is low enough to avoid the possible dissociation of all measured phenolic samples.

The substituted aromatic sample compounds (Lachema, Czech Republic) were of an analytical grade. They were dissolved in water in a concentration of 7.5×10^{-3} % (w/v). Dimethyl sulfoxide (DMSO) was in a 7×10^{-2} % (w/v) concentration. Distilled water further purified by Milli-Q RG (Millipore, Bedford, MA) was used for the preparation of all solutions.

Procedures. A new uncoated silica capillary was rinsed for several minutes with water and afterwards with polymer and then left filled with a polymer solution overnight. The capillary was only rinsed with the polymer solution in the respective concentration between experiments.

RESULTS AND DISCUSSION

Separation Ability of the Polymer Solution. An example of the inherent suitability of the system to resolve neutral compounds is shown in Figure 2 for DMSO and different aromatic compounds with a background electrolyte containing 4% w/w PDADMA. Indeed the specific retardation of the neutral analytes due to their individual interactions with the pseudostationary phase forming a counter-flow leads to resolved sample peaks within 9 min. At first glance it might seem that the efficiency of the separation is not as one might expect in electrochromatography. However, it should be noted that the column length to the detector is only L_D = 8.5 cm. The experimental plate height of, say, the benzyl alcohol peak is $H_{exp} = 10.6\ \mu$m. As the retention time of benzyl alcohol is $t_{bal} = 6.3$ min, and its diffusion coefficient is $D_{bal} = 9.3 \times 10^{-10}$ m^{2}s^{-1} in this medium,[38] the theoretical diffusion limit is $H_{dif} = 2D_{bal}\, t_{bal}/L_D = 8.3\ \mu$m. It follows that the separation efficiency is not deteriorated excessively by another dispersion phenomena as it reaches almost 80% of the diffusion limit in this case.

The effect of polymer concentration on separation has been investigated in the range between 1 and 6% w/w polymer. DMSO, phenol, and resorcinol were used as model analytes. Obviously the conductivity of the solution increases substantially with the concentration of the charged polymer, which results in a corresponding growth of temperature inside the capillary due to the production of Joule heat. Even more complicated are phenomena due to the temperature and concentration change of the viscosity of the solution. The macroscopic viscosity increases with the polymer concentration (e.g., viscosity of 4% w/w PDADMA polymer solution is above 10 mPa s at 25 °C), but simultaneously there is an almost exponential decrease in the temperature of the system. According to the Helmholtz-Smoluchowski equation, the electroosmotic velocity is mostly influenced by the viscosity of the solution. The dependence of the electroosmotic velocity on polymer concentration in real experiments is therefore rather complex. Figure 3 shows the influence of these factors on the electroosmotic mobility. The EOF first decreases with increasing concentration of the polymer (apparently due to the increasing viscosity); for higher concentrations, where the temperature effect becomes significant, a slight increase is observed.

Figure 4 shows the dependence of the mobility ratios μ_i/μ_j for the DMSO−resorcinol, DMSO−phenol and phenol−resorcinol couples on the concentration of PDADMA. It can be seen that the mobility ratios grow over the whole range of concentrations of PDADMA, almost linearly, to as much as a 5% polymer concentration. This fact would imply that using higher concentrations of the PDADMA in the background electrolyte is, in principle, preferable for improving resolution. Nevertheless, practical reasons set upper limits on this concentration: first, the ability of polymer replacement in the separation capillary and, second, excessive Joule heat due to the high conductivity of the background electrolyte. Therefore, all of the following measurements were performed using 4% PDADMA solution.

Determination of the Electrophoretic Mobility of the Polymer. The capacity factors of the solutes are quantities of central concern for the discussion of the separation selectivity of the pseudochromatographic system and for the clarification of the type of interaction described by the solvation parameters. It can

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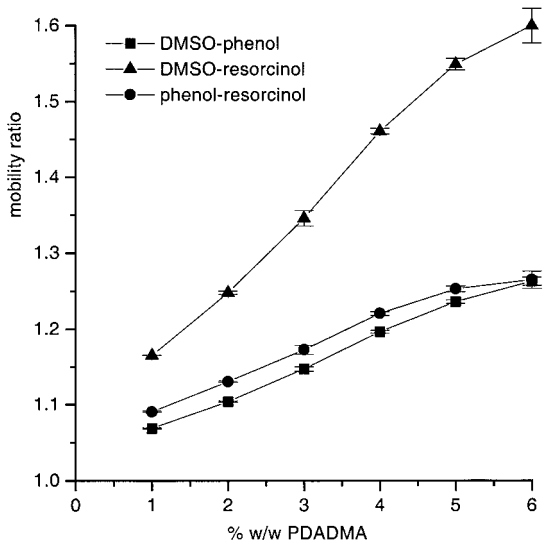

Figure 4. Dependence of the mobility ratio, μ_i/μ_j, for couples of DMSO, phenol, and resorcinol. Acetate buffer (pH 5.2) with varying concentrations of PDADMA; applied voltage 4 kV.

be seen from eq 5 that the electrophoretic mobility of the polymer and the bulk electroosmotic mobility, μ_{poly} and μ_{eof}, respectively, are the variables from which the capacity factors are calculated. The electroosmotic flow can be determined relatively simply in each experiment from the position of the water system peak (note, however, that there can be a problem of additionally migrating concentration boundaries [39,40]). The measurement of the mobility of the polymer, μ_{poly}, is not that trivial. In MEKC the mobility of the micelles is determined by the aid of a marker substance, for which an infinite capacity factor is assumed. No such substance is available a priori for the polymers under consideration. It must be further noted that the electrophoretic mobility of a charged polymer is strongly dependent on its concentration. Consequently, a reliable measurement of the mobility of the polymer has to be performed at the polymer concentration that is at the respective value.

We propose an isotachophoretic regime in a part of the separation capillary for such measurements of the polymer electrophoretic mobility (Figure 5). The capillary was first rinsed with 4% PDADMA to establish a high electroosmotic flow toward the anode. Both the electrode vessels and the capillary were then filled with 21 mM His/His·HCl buffer, pH 6.05. Four percent PDADMA was injected for 90 s by hydrostatic sampling, at a level difference of 7 cm. In this way, a plug of PDADMA polymer solution was introduced. As the PDADMA mobility is higher than that of histidine, PDADMA acts now as the leading electrolyte, and the His/His·HCl solution in the capillary as the terminating electrolyte in this isotachophoretic arrangement. When voltage is applied with the cathode at the injection end, the polymer zone starts to move out of the capillary toward the cathode, but electroosmosis flows in the opposite direction with a velocity high enough to shift the polymer zone into the direction toward the detector. As a result of the higher PDADMA mobility compared

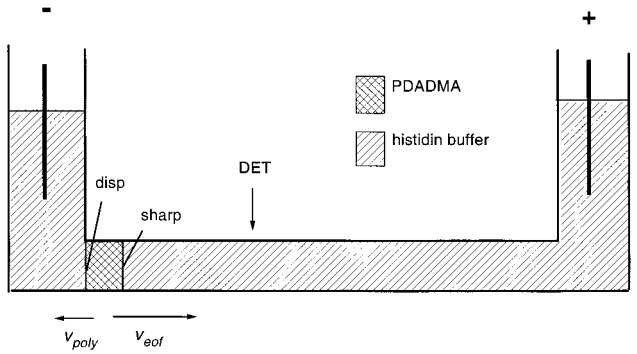

Figure 5. Schematic drawing of the isotachophoretic mode for measurement of the electrophoretic mobility of the polymer. disp, dispersion boundary His/PDADMA; sharp, sharpened boundary PDADMA/His, DET, position of UV detector, v_{poly} – direction of PDADMA zone movement; v_{eof}, direction of electroosmotic flow.

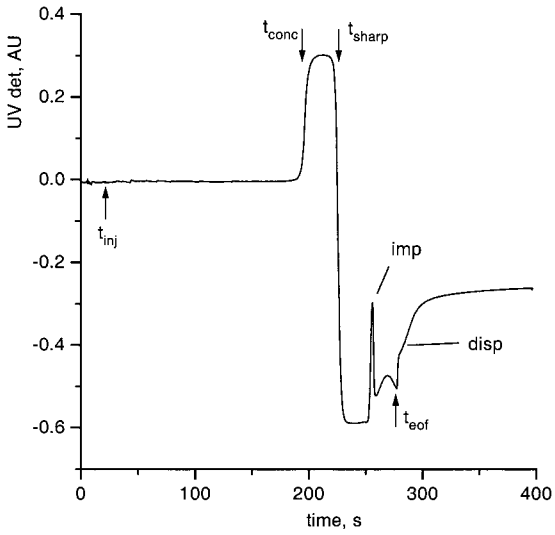

Figure 6. ITP measurement of the mobility, μ^0_{poly}, of 4% w/w aqueous solution of PDADMA at constant current of 7.6 μA. Polymer sampled hydrostatically for 90 s, level difference 7 cm, 21 mM His/His·HCl, pH 6.05 used as terminating electrolyte; t_{conc}, time of occurrence of concentration boundary of histidine cation; t_{sharp}, time of occurrence of the sharpened boundary of PDADMA/histidine; disp, dispersed boundary histidine/PDADMA; t_{eof}, migration time of the water marker of the electroosmotic flow; imp, unknown impurity.

with that of histidine, the boundary closer to the detector is sharpened, and the PDADMA/histidine boundary closer to the cathode is dispersed.

An example of the experimental record of the UV detector is shown in Figure 6. In the system used, only histidine has a significant absorption at 214 nm. Consequently, the concentration boundary of histidine (time t_{conc}), the isotachophoretically sharpened boundary histidine/PDADMA (time t_{sharp}), and the dispersed boundary PDADMA/histidine can be observed in the detector record. If, additionally, a water plug is injected (5 kV/2 s) for 20 s (t_{inj}) after the start of the electrophoretic experiment, the negative "water peak" at time t_{eof} can be detected in the dispersed boundary. This peak can be regarded as a marker of the EOF.

As the specific conductivity, κ, of the polymer solution can be obtained from independent measurements, it is possible to calculate the electrophoretic mobility of the polymer, μ^0_{poly}, related to the solution according to

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(40) Kenndler-Blachkolm, K.; Popelka, Š.; Gaš, B.; Kenndler, E. *J. Chromatogr., A* **1996**, *734*, 351–356.

$$|\mu^0_{poly}| = v_{eof}\frac{\kappa}{j}\left(1 - \frac{t_{conc}}{t_{sharp}}\right) \quad (8)$$

Here j is the current density; v_{eof} is the electroosmotic velocity, which is calculated as $v_{eof} = l_D/(t_{eof} - t_{inj})$, where l_D is the distance to the detector.

Eq 8 can be easily derived when realizing that both the sharpened and concentration boundaries migrate across the same distance to the detector, therefore

$$v_{eof}\, t_{conc} = v_{poly}\, t_{sharp} = (v_{eof} - |v^0_{poly}|)t_{sharp},$$
$$\mu^0_{poly} = v^0_{poly}\,\kappa/j \quad (9)$$

The measurement was carried out at a constant current of 7.6 μA at an ambient temperature of 25 °C. For the specific conductivity, a value of $\kappa = 1.51$ Sm^{-1} was obtained. In this way, a value of 38.4×10^{-9} m^{2}V^{-1}s^{-1} (span 1.9×10^{-9} m^{2}V^{-1}s^{-1}, 4 measurements) was found for μ^0_{poly}, the electrophoretic mobility of PDADMA in the 4% polymer solution under the stated conditions.

Capacity Factors. To determine the capacity factors of 15 analytes according to eq 5, their migration times were measured; electroosmotic mobility, μ_{eof}, was determined in each experiment from the position of the water-system peak. All measurements were carried out at a constant voltage of 4 kV with a corresponding current of about 90 μA. For the electrophoretic mobility of the polymer, μ^0_{poly}, the value of 38.4×10^{-9} m^{2}V^{-1}s^{-1} was used, which was determined by the method described above. The corresponding mobility of the polymer related to the capillary, μ_{poly}, which is necessary for calculating k_i values, was determined in each experiment according to eq 6 using the respective value of the electroosmotic mobility.

The capacity factors are summarized in Table 2. They range between 0.3 and 1.2, indicating a considerable interaction with the pseudostationary phase. To illustrate the extent of this interaction, the corresponding distribution constants, K_i, were calculated from the k_i values and the approximate phase ratios using eqs 1 and 3. Taking the molecular weight of the monomer unit, 157.7 g/mol, the 4% (w/w) aqueous solution of PDADMA has a phase ratio of $q = 0.0048$, resulting in distribution constants between 54 and 257. These values again show even more convincingly that there is a remarkably strong interaction of the analytes with this polymer.

Linear Solvation Model. We have applied the linear solvation model LFER to characterize the separation properties of the pseudochromatographic system, consisting of 4% PDADMA polymer solution, on the basis of the capacity factors of the set of the 15 aromatic compounds. Using the parameters of the individual test analytes given in Table 1, the constants of the solvation equation were obtained with the step multiple linear regression at a level of significance of 0.95. The total correlation coefficient of the regression was 0.964. The parameters of the PDADMA polymer solution resulting from the calculation are given in Table 3.

Although the number of solutes measured (15) is not fully sufficient for a robust fit, some general conclusions can be made when comparing the solvation parameters to analogous separation systems. It was shown by Poole and Poole[41] that buffer composition and concentration do not significantly influence separation properties of MEKC systems as their selectivity is predominantly controlled by the choice of the surfactant type. We presume from that it will be especially useful to make a comparison of the PDADMA solution with both cationic and anionic micelles. The data for hexadecylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) micelles taken from ref 41 are also included in Table 3.

Table 2. Experimentally Determined Capacity Factors, k_i, and Corresponding Distribution Constants, K_i

sample	k_i	$R_{m,i}$[a]	N[b]	K_i
acetophenon	0.258	0.062	4	54
benzylalcohol	0.259	0.054	4	54
methylbenzoate	0.270	0.049	3	57
benzonitrile	0.272	0.018	3	57
toluene	0.281	0.013	3	58
benzylacetate	0.284	0.019	3	59
benzylaldehyde	0.289	0.018	3	60
nitrobenzene	0.293	0.016	3	61
benzene	0.306	0.017	3	64
anisol	0.307	0.010	3	64
p-nitrophenol	0.351	0.045	3	73
m-cresol	0.397	0.046	3	82
phenol	0.415	0.071	5	86
resorcinol	0.705	0.140	5	147
α-naphthol	1.233	0.064	3	257

[a] $R_{m,i}$ is the range of the measured values. [b] N is the number of measurements.

The most significant difference between polymer solution and micelles lays in cohesive properties. While the cavity term, m, is highly positive in micelles (no matter if positively or negatively charged), it is not significant in the polymer solution. This is a consequence of the fact that the transfer of the analyte between a micelle and the buffer is connected with a high energy difference for cavity formation, while this process in polymer solution does not play a role.

The highest impact on the retention behavior with PDADMA has the parameter r. It can be deduced that the charged polymer (and also a charged micelle) has a higher ability to interact with π or n electrons of the solutes than with the mobile phase (a buffer). On the other hand, dipole-type interactions (parameter s) favor solubility of the analyte in the buffer both for charged-polymer and micellar systems.

The value of the parameter a for cationic polymer is positive and confirms a rule observed in micellar systems, where the positively charged micelles (CTAB) are strong hydrogen-bond bases and thus have a positive a constant, while negatively charged micelles (SDS) have a negative a constant.

While for micellar systems water in the buffer is a strong acid in comparison with micelles, this is not so in the case for charged polymer systems. Here, the parameter b (hydrogen-bond acidity) is not statistically significant at the level of 0.95. Therefore, this type of interaction is not contributing to the retention in the system with the cationic polymer.

Inspecting the results in Table 3, it comes out that a range of values of all the solvation constants is higher in micellar systems as they vary here from about −3 to 3, while only from about −0.35

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Table 3. Solvation Parameters of the PDADMA Polymer Solution Obtained by Linear Multiple Regression

parameter	PDADMA	CTAB[b]	SDS[b]	type of interaction
c	−0,81 (0,07)	−1.67 (0.11)[c]	−1.82 (0.07)	none; regression constant
m	*a*	3.40 (0.10)	2.99 (0.07)	cavity formation
r	0,75 (0,09)	0.61 (0.06)	0.46 (0.05)	interaction with *n* or π electrons
s	−0,35 (0,07)	−0.55 (0.07)	−0.44 (0.05)	dipole-dipole and dipole-induced dipole
a	0,19 (0,05)	0.58 (0.06)	−0.30 (0.05)	hydrogen bond basicity
b	*a*	−3.08 (0.10)	−1.88 (0.08)	hydrogen bond acidity

[a] Parameters *m* and *b* for PDADMA are not significant at the level of significance of 0.95. [b] Solvation parameters for CTAB and SDS micelles in MEKC systems are taken from ref 41. [c] Values in parenthesis are standard deviations.

to 0.75 for PDADMA. This implies that micellar separation systems generally allow higher selectivity to be obtained than does PDADMA used in this study. However, it must be realized that micellar electrokinetic chromatography has been developed for many years, while here we describe one of the first uses of a charged polymer other than to form micelles for separation of neutral analytes. There is still a potential, in modification of the polymer chain with appropriate substituents, for increasing the selectivity of such systems.

There is also one technical aspect of using charged linear polymers in electrolyte systems. It should be noticed that the relatively high electric current occurring in the experiments causes a temperature rise inside the capillary. Regarding the Joule heat generation in the solution and the heat loss through the capillary wall in the air-cooled capillary, it can be calculated that the mean increase in temperature of the solution is of about 9 degrees in the given experimental conditions.[42] Therefore, the mobility of the polymer in the electrokinetic experiments where capacity factors are measured will be higher than 38.4×10^{-9} $m^2V^{-1}s^{-1}$, the value determined from isotachophoretic measurements performed at low current. Supposing that the temperature dependence of mobility is approximately 2−3% per degree, the polymer mobility in the electrokinetic experiments is about 47×10^{-9} $m^2V^{-1}s^{-1}$. Fortunately, the regression parameters are not very sensitive when changing the mobility of the polymer, μ_{poly}, that influences the capacity factors. If, tentatively, $\mu_{poly} = 47 \times 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$, the significant solvation parameters *r*, *s* and *a* result in values of 0.626, −0.302, and 0.189, respectively. There is a certain shift in the absolute values of the parameters when comparing them with the original values, (see Table 3). However, the qualitative conclusions about the weight of individual interactions remain the same.

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