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Effect of cationic polymer on the separation of phenols by capillary electrophoresis

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Abstract

The capillary electrophoretic separation of substituted phenols was examined using high-molecular-mass polyethyleneimine (PEI) as a buffer additive. The added PEI to the running buffer showed a strong interaction with the phenols and affected the electrophoretic mobilities and migration order of phenols. The influence of pH, organic solvent and concentration of polyelectrolyte on the electrophoretic mobilities of analytes was investigated.

Keywords: Buffer composition; Phenols; Chlorophenols; Nitrophenols; Polyethyleneimine

1. Introduction

Substituted phenols are of great environmental concern owing to their high toxicity. In the last years, CE has become a suitable alternative to high-performance liquid chromatography for the analysis of phenolic compounds. Separation of phenols has been well achieved by CE [1–3] or by micellar electrokinetic chromatography (MEKC) [4–9]. Selectivity control has been performed via the change of the pH of the buffer, the use of anionic [4–8] and neutral surfactants [9] at concentrations higher than the critical micelle concentration (CMC) or organic solvents together with cationic surfactants at below CMC [3].

An alternative way to change the selectivity is to add ionic polymers to the buffer. The use of cationic polymers for this purpose was first reported by Terabe and Isemura. They introduced this approach as a new separation mode of CE, namely ion-exchange electrokinetic chromatography [10,11]. Stathakis and Cassidy [12,13] reported the use of cationic polymers for the separation of inorganic and organic ions. Recently we introduced high-molecu-

lar-mass polyethyleneimine (PEI) as a buffer additive for the selectivity control of basic proteins and peptides [14]. The addition of PEI to the running buffer caused changes in the electrophoretic mobilities of the proteins and peptides through a possible electrostatic interaction occurring between the negative groups of biopolymers and the positive groups from PEI. It is interesting to use this approach for the control of the selectivity of phenols.

The changes of electrophoretic mobilities of phenols as a function of added polyelectrolyte concentration and pH were investigated. The results of this investigation are presented in this paper.

2. Experimental

2.1. Instrumentation

Separations were carried out with a commercial CE injection system (Prince Technologies, Emmen, Netherlands) in combination with an on-column variable-wavelength UV–visible detector (Lambda 1000, Bishoff, Leonberg, Germany). The wavelength

was set at 210 nm. The fused-silica capillaries used for separation experiments were 75 μm I.D. and were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total and effective lengths of capillaries are indicated in the figures.

Automated capillary rinsing, sample introduction and execution of the electrophoretic runs were controlled by a personal computer. Data processing was carried out with a commercial CE software (Caesar 1995, Roman Scientific). A washing step of 2 min with buffer between runs was applied. Three replicate runs were performed for all conditions.

2.2. Chemicals

PEI (molecular mass range $6 \cdot 10^5$ – $1 \cdot 10^6$) was purchased from Fluka (Fluka, Buchs, Switzerland). Phenols, Tris, 2-(N-morpholino)ethanesulfonic acid (MES) and acetic acid were from Merck (Darmstadt, Germany). All other chemicals were analytical grade. Deionized water was used for the preparation of solutions. Solutes were dissolved in water.

2.3. Coating procedure

The PEI molecule has many positive charges and interacts strongly with negatively charged silanol groups on the surface of the fused-silica capillary. This irreversible adsorption creates a PEI layer on the capillary wall. For this reason, the experiments were performed in the capillaries coated with PEI as reported before [15]. The experiments on this coating showed that the polymer layer has a positive charge over a wide pH range, which results in an electroosmotic flow towards the anode. The reproducibility and long term stability of this coating was investigated and reported [15]. Briefly the method involves the following procedure:

The fused-silica capillary was first etched by flushing the capillary with a solution of 1 M sodium hydroxide for 30 min at $1 \cdot 10^{-1}$ MPa and with water for 15 min at the same pressure. Then the capillary was flushed with a solution of 10% PEI in water at $1.5 \cdot 10^{-1}$ MPa for 10 min and the PEI solution left in the capillary for 1 h. Next the polymer solution was pressed out of the capillary with air at $1.5 \cdot 10^{-1}$ MPa. Finally the capillary was rinsed with water for 15 min.

3. Results

The migration behaviors of test phenols were investigated as a function of the concentration (w/v) of PEI in the buffer. The experiments were performed with the PEI containing buffers at the concentration range between 0–0.75% PEI. The effect of pH of the running buffer on the resolution of the phenols was examined using electrolyte systems consisting of 20 mM acetate for pH 5; 20 mM MES for pH 6.2 and 7.15 and 20 mM Tris for pH 8.3. The pK_a values of selected phenols for this work were given in Table 1.

The migration time of negative water peak was used for the calculation of electroosmotic mobility (EOM). The sign of EOM in figures was selected arbitrary as negative when directed toward the positive electrode. In the same manner the sign of electrophoretic mobilities of solutes in the figures shows their co- or counter-migration to the electroosmotic flow (EOF).

3.1. Polyelectrolyte effect

As can be seen from Table 1, at pH 5, all the phenols are neutral. In the PEI free solution, they migrate with the velocity of the EOF and coelute in one major peak. When PEI is added to the running buffer, the direction of electrophoretic migration of all phenols appears to reverse at 0.25% PEI concentration. Four phenols migrate with the same mobility and one, 4-nitrophenol, migrates faster, in the opposite direction to EOF. The electrophoretic mobilities of all solutes increase with increasing PEI concentration.

At pH 6.2, approximately 10% of 4-nitrophenol is dissociated. In the absence of PEI in the buffer, 4-nitrophenol migrates with an electrophoretic mobility to the direction of anode. The other phenols migrate just in front of the negative EOF peak on the electropherogram. With 0.1% PEI in the buffer, the

Table 1
 pK_a values of the phenols used in this study

	Phenols				
	4-Nitro	3-Nitro	2-Chloro	3-Chloro	4-Chloro
pK_a	7.15	8.28	8.49	8.85	9.18

direction of electrophoretic migration of all phenols reverse. 4-Nitrophenol is separated from four phenols which migrate close to the EOF as one peak. A further increase of the concentration of PEI causes an increase in mobilities and between 0.5–0.75% PEI concentration, the prolonged retention time of 2-chlorophenol is observed and this phenol is completely separated from others, appearing as a separate peak in front of 4-nitrophenol peak. 3- and 4-chlorophenols and 3-nitrophenol migrate together.

Fig. 1 shows the electrophoretic mobilities of phenols vs. PEI concentration at pH 7.15. In the free PEI solution complete co-electroosmotic separation occurs only for 4-nitrophenol. 3-Nitro and 2-chlorophenol migrate as partly resolved peaks and 3- and 4-chlorophenol migrate as one peak on the electropherogram. In the 0.1% PEI concentration, the prolonged migration time of 4-nitrophenol before the EOF marker is observed. Other four phenols migrate just after the EOF peak on the electropherogram. Between 0.25–0.75% PEI concentration, all phenols migrate in the direction opposite to that of the EOF. The largest effect is observed for 4-nitrophenol. A complete separation of five phenol substances can be achieved with 0.5% PEI. The electropherograms showing the separation of phenols in PEI free and 0.5% PEI containing solutions are given in Fig. 2. For the PEI containing buffer, the separation efficiency of 4-nitrophenol is 58 000 theoretical plates and for other phenols the efficiency ranges between 80 000–95 000 theoretical plates.

As can be expected, at pH 8.3, all phenols can be completely separated in a PEI free solution (see Fig.

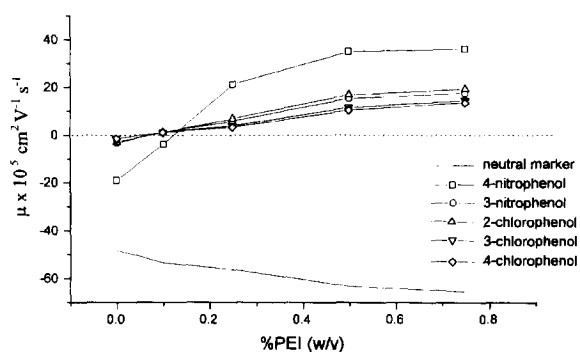


Fig. 1. Plot of electrophoretic mobility vs. PEI concentration at pH 7.15.

3). The same trend with the preceding pH conditions, is observed for this pH with increasing concentration of PEI. The migration velocities of the phenols to the anode direction decrease, and after 0.5% PEI addition, the electrophoretic migration direction of all reverse. The increase in the values of electrophoretic mobilities to the cathode direction with further increase in polyelectrolyte concentration is smaller at this pH compared to those for beforehand examined conditions. Also the resolution between 4-nitrophenol and the following one is not sharp as observed at other pH buffers. Another observation at this pH is the inversion of the migration order. In this pH, 3-nitrophenol migrates first, then 3- and 4-chlorophenol come together followed by 2-chlorophenol and as the last 4-nitrophenol. The separation electropherograms are given in Fig. 3 for PEI free and 0.5% PEI containing solutions and Fig. 4 illustrates the migration behaviors of the phenols at this pH.

With the increasing concentration of PEI, an increase of EOF between 20–35% is observed. This increase is comparatively smaller for pH 5 and 8.3 and higher for 6.2 and 7.15.

3.2. pH effect

The changes of the electrophoretic mobilities of the phenols in PEI free and 0.5% PEI containing buffer vs. pH are given in Fig. 5 and Fig. 6 respectively. In PEI free solution, increase in pH causes increase in the dissociation of phenols and as expected more negatively charged solutes migrate faster to the anode direction. Since their migration and EOF is to the same direction in PEI coated capillary, phenols migrate according to the order of their pK_a values, e.g., 4-nitrophenol comes first and 4-chlorophenol last.

In 0.5% PEI containing buffers, for pH values studied, all phenols migrate to the opposite direction of EOF. Between pH 5 and 6.2, the electrophoretic mobilities of 4-nitro-, 3-nitro- and 2-chlorophenol increase but a slight decrease in the mobilities of 3- and 4-chlorophenol is observed. Between pH 6.2 and 7.15, the mobilities of all phenols increase. Between pH 7.15 and 8.3 the electrophoretic mobilities of the phenols decrease. The same behavior was found with other PEI concentrations.

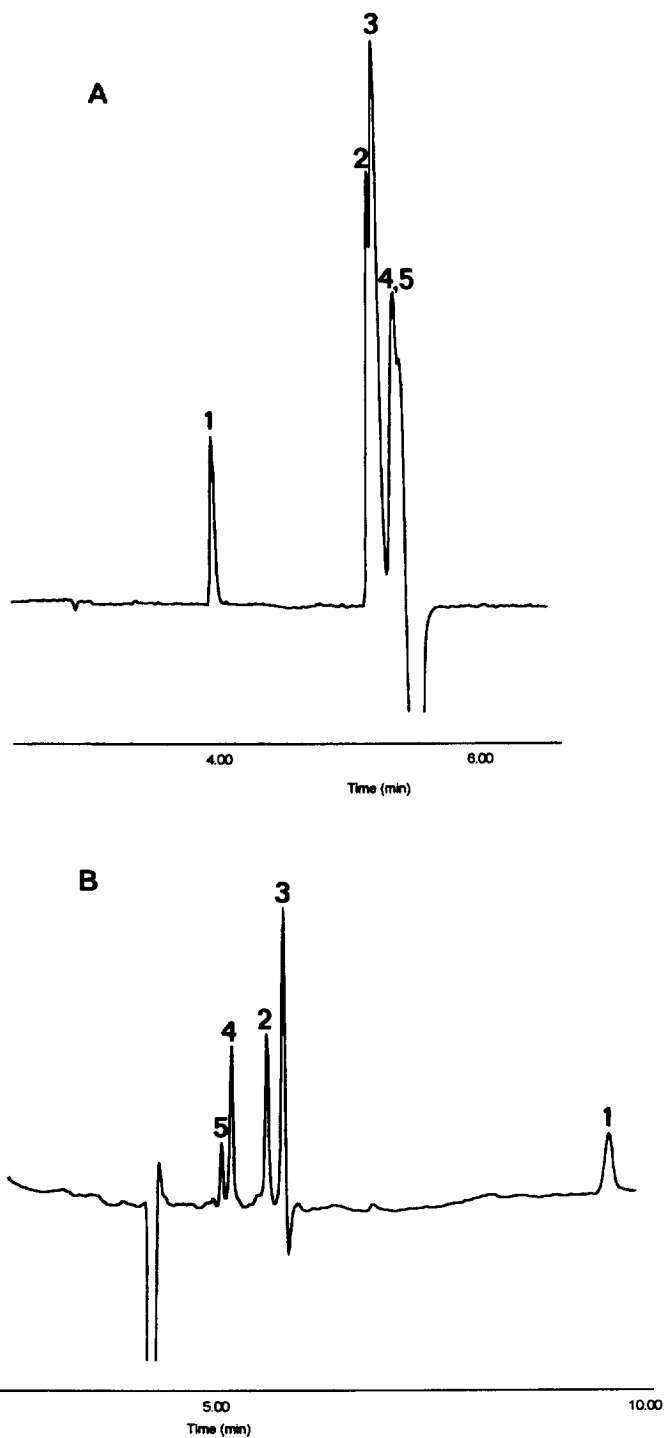


Fig. 2. Electropherogram for five phenols at pH 7.15. A, 0% PEI and B, 0.5% PEI containing buffer. Peaks: 1=4-nitrophenol, 2=3-nitrophenol, 3=2-chlorophenol, 4=3-chlorophenol, 5=4-chlorophenol. Buffer: 20 mM MES for A and 20 mM MES and 0.5% PEI for B. pH 7.15. Injection $4 \cdot 10^{-3}$ MPa, 3 s. Run voltage -28 kV. PEI coated capillary, 75 cm (effective length 60 cm) \times 75 μ m I.D. Direct UV detection at 210 nm.

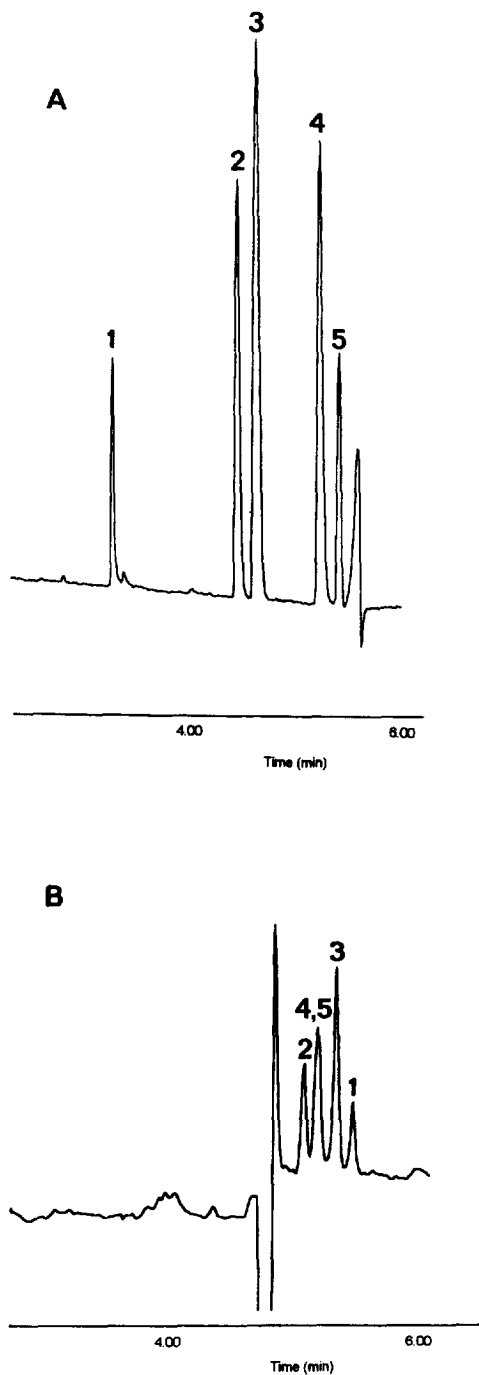


Fig. 3. Electropherogram for five phenols at pH 8.3. A, 0% PEI and B, 0.5% PEI containing buffer. Buffer: 20 mM Tris for A and 20 mM Tris and 0.5% PEI for B, pH 8.3. Other conditions and peak identifications are the same as in Fig. 2.

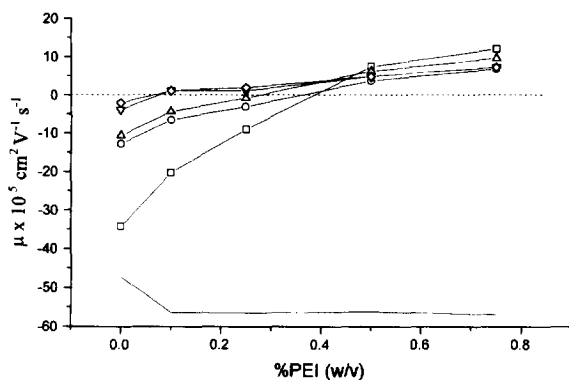


Fig. 4. Plot of electrophoretic mobility vs. PEI concentration at pH 8.3. The symbols are the same as in Fig. 1.

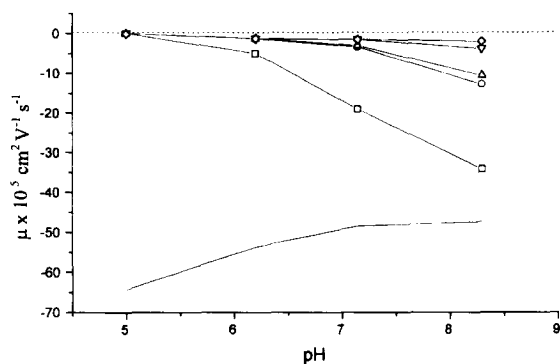


Fig. 5. Plot of electrophoretic mobility vs. pH for the PEI free buffer. The symbols are the same as in Fig. 1.

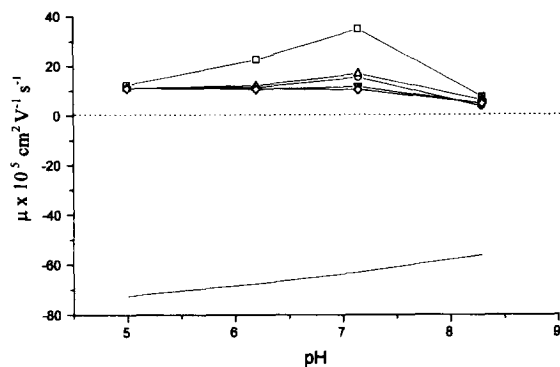


Fig. 6. Plot of electrophoretic mobility vs. pH for 0.5% PEI containing buffer. The symbols are the same as in Fig. 1.

In Figs. 5 and 6, it can be seen that the EOM decrease with increasing pH. This is mainly due to a decrease in the protonation of the imine groups of

PEI adsorbed on the surface with increasing pH, which cause that the wall is less positively charged and hence results in a decrease in EOM. The magnitude of the decrease is almost the same for PEI free buffers and PEI containing buffers and is about 25% at pH 8.3.

3.3. Organic solvent effect

The effect of methanol on the migration of phenols was also investigated. The addition of methanol causes a decrease in the electrophoretic mobilities and the resolution. Moreover, the addition of methanol appears to decrease significantly the EOM. The decrease is about 40% when adding 30% (v/v) methanol to the buffer.

4. Discussion

The results of experiments show that the electrophoretic mobilities of phenols are significantly influenced by the presence of PEI in the buffer and also by the pH of the buffer. The coated PEI layer on the silica surface has a positive charge due to protonation of the imine groups resulting in a reversed electroosmotic flow towards the anode. In this case anionic electrophoretic mobilities of the solute anions and EOM are in the same direction. When the phenols are partially dissociated, they gain a negative charge and migrate with the direction of the EOF and the peaks appear before the EOF marker on the electropherogram or they migrate with the EOF in the case that they are undissociated. This migration behavior was found to occur in the experiments in PEI free solutions. However, the addition of PEI to the buffer appears to cause a reversal of the direction of electrophoretic mobilities of the phenols over a wide pH range. This indicates a strong interaction of the phenols with the positively charged PEI polymer in solution which migrates against the electroosmotic flow.

As can be seen in Fig. 6, for 0.5% PEI concentration, all phenols interact with PEI molecules between pH 5 and 8.3.

Partial dissociation of 4-nitrophenol begins between pH 5 and 6.2, and the other phenols can be

assumed to be neutral. A sharp increase is observed in the curve of 4-nitrophenol, while the other phenols remain the same. At pH 7.15, 50% of 4-nitrophenol is dissociated, and 3-nitro, 2 chloro and 3-chlorophenol are partially dissociated. Again a sharp increase in the electrophoretic mobility of 4-nitrophenol to the same direction of the polyelectrolyte is found. Also a change in the mobilities of three phenols takes place and complete baseline separation is achieved under this condition. At pH 8.3, most phenols are partially dissociated, but at the same time the protonation of the imine groups on PEI decreases. This decrease might be the explanation of the decrease in the interaction between the phenols and PEI.

The observations suggest that the association between phenols and cationic polyelectrolyte is driven by charge attraction but other interactions, probably mainly hydrophobic, seem to be a contributing factor for the buffer solutions in which the phenols are undissociated.

Another observation from this experiment is that the separation selectivity is not strictly dependent on the negative charge density of phenols. Though the pK_a value of 2-chlorophenol is higher than that of 3-nitrophenol, it seems that the interaction between PEI and this phenol is stronger than that of 3-nitrophenol for all the experimental conditions studied. Also at pH 8.3, another selectivity change is observed. In that case 3-nitrophenol migrates first, indicating less interaction with polyelectrolyte. This might be explained by some configuration change of the polymer with increasing pH. At the lower pH range, polyelectrolyte is highly protonated and because of electrostatic repulsion among the charged hydrophilic groups, it will be in the buffer as an extended coil configuration as described by Voycheck and Tan [16]. The decrease in the charge of the polymer with increasing pH results in the formation of a more compact coil structure. This probable structure change might be the explanation of weaker association and selectivity change at pH 8.3.

More experiments are now being undertaken in our laboratory to elucidate the possible interaction mode between PEI and both aromatic and small inorganic solutes including the study of the influence of buffer composition and molecular mass of polymer.

5. Conclusion

The use of cationic polymers for the manipulation of selectivity of aliphatic and aromatic acids was reported by Terabe and Isemura [10,11]. They introduced this method as ion-exchange electrokinetic chromatography and explained the interaction between polyelectrolyte and negatively charged ions as ion pair formation. Stathakis and Cassidy [12] used cationic polymers for the separation of inorganic anions and reported the changes in separation selectivity. The same authors applied their electrolyte systems to the separation of aliphatic and aromatic acids [13]. Both inorganic anions and aliphatic acids migrated before the neutral marker, but they observed a change in the order of migration with respect to neutral marker for two of the five aromatic acids studied. This type of change was not reported by Terabe and Isemura [10,11] though they also examined aromatic acids in the presence of cationic polymer in the buffer. We observed a selectivity change between basic proteins and peptides using of polyethyleneimine as a buffer additive [14].

In the present study, the phenols exhibit strong interaction with the polyethyleneimine. The strong association between the investigated phenols and the polyelectrolyte can even be observed at the buffer conditions in which the phenols do not possess any negative charge. Moreover, the separation order of analytes appeared contrary to the charge density of the phenols, mostly at pH 8.3.

Under the observations of this study, the interaction between the polyelectrolyte and the phenols can not be explained only with the ion pair formation. Besides the electrostatic interaction, some other effects including hydrophobic interactions should be the reason for this association. Maybe the best way is to name it as the formation of polymer complexes covering all kinds of interaction.

As a result, ionic polymers promise an alternative future in CE for the manipulation of selectivity, not only of ionic solutes but also hydrophobic compounds containing hydrophilic groups.

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