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## Open-tubular capillary electrochromatography using a polymeric surfactant coating

A stable polyelectrolyte multilayer (PEM) coating was investigated for use in open-tubular capillary electrochromatography (o-CEC). In this approach, the PEM consisted of the cationic polymer of a quaternary ammonium salt, poly(diallyldimethylammonium chloride) and the anionic polymeric surfactant, poly(sodium undecylenic sulfate). Both the cationic and anionic polymers were physically adsorbed to the surface of a fused-silica capillary by use of a simple coating procedure. This procedure involved an alternate rinse of the positively and negatively charged polymers. The performance of the PEM coating as a dynamic stationary phase was evaluated by use of electrochromatographic experiments and showed good selectivity for both phenols and benzodiazepines. Reproducibility of the PEM coating was also evaluated by calculating the relative standard deviations (RSDs) of the electroosmotic flow (EOF). The run-to-run and capillary-to-capillary RSD values of the EOF were less than 1.5%. The endurance of the coating was more than 100 runs. The importance of the PEM coating was illustrated by comparing separations on a bare uncoated capillary with the coated capillary. In addition, the chromatographic performance using o-CEC and micellar electrokinetic chromatography (MEKC) was compared for the separation of benzodiazepines.

**Keywords:** Benzodiazepine / Open-tubular capillary electrochromatography / Phenol / Polymeric surfactant  
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### 1 Introduction

In recent years, there has been a growing interest in the use of capillary electrochromatography (CEC) due to its high separation efficiency [1, 2] and its compatibility with mass spectrometry [3, 4]. This versatile technique provides a suitable alternative to capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE) and micellar electrokinetic capillary chromatography (MEKC). CEC is a hybrid microcolumn electroseparation technique that combines the selectivity of high-performance liquid chromatography (HPLC) and the efficiency in capillary electrophoresis (CE) [5–7]. High separation selectivity is achieved by combining the electrophoretic mobility and the partitioning coefficients between the stationary phase and the mobile phase of the analytes [8]. As an electrically driven approach, CEC yields a plug-like profile for analyte movement across the capillary with reduced dispersion giving high peak efficiency [9–11].

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**Abbreviations:** o-CEC, open-tubular CEC; p-CEC, packed-column CEC; PDADMAC, poly(diallyldimethylammonium chloride); PEM, polyelectrolyte multilayer; poly-SUS, poly(sodium undecylenic sulfate)

CEC encompasses different modes of operation, two of which are packed-column capillary electrochromatography (p-CEC) and open-tubular capillary electrochromatography (o-CEC) [8, 12]. In p-CEC, the stationary phase is packed into the silica capillaries while in o-CEC the stationary phase is coated onto the inner surface of the capillary. There are a number of disadvantages associated with p-CEC which limit its practical application. Fabrication of stable frits that maintain an unrestricted flow and retain packed material is a major challenge in p-CEC. Difficulties in achieving stable baselines, stable currents, and reproducible migration times arise due to the formation of air bubbles around the packing materials and the frits [10]. Pressurization at both ends of the column is required to prevent bubble formation inside the capillary [13]. Preparation methods of stationary phases used in p-CEC are usually time-consuming and complicated. Although monolithic columns [14–16] have proven to be successful in alleviating the problems associated with bubble formation and frit fabrication, dynamically coated open-tubular columns provide faster and relatively simpler preparation procedures.

In o-CEC the capillary coating may be described as permanent or dynamic depending on the attachment of the coating to the surface of the capillary wall [17]. Permanent coatings are achieved by derivatization of the silanol groups on the capillary wall followed by covalently bond-

ing with a polymeric material [18]. Although covalently modified capillaries are very stable, they are laborious and time-consuming to prepare [19, 20]. A dynamic coating is typically prepared by rinsing the capillary with a solution containing the coating agent or by adding a small amount of the coating agent to the mobile phase [21, 22]. Dynamic coatings are adsorbed to the capillary wall *via* electrostatic interactions and hydrogen bonding. While these interactions are weaker than covalent bonds, multiple electrostatic interactions ensure a stable coating. Horvath and Dolnik [23] reviewed several polymeric coatings in capillary electrophoresis. Decher *et al.* [24, 25] introduced a multilayer procedure that employs electrostatic interaction between oppositely charged macromolecules. Thin films can be constructed on a layer-by-layer basis on a hydrophilic surface, by alternately exposing positive and negative polyelectrolytes on a substrate [26, 27]. Schlenoff *et al.* [28–30] investigated polyelectrolyte multilayer (PEM) properties including the mechanism of formation, thickness, and the surface charge. Recently, simple coating procedures have been developed where the coating material is physically adsorbed to the capillary wall by flushing successive multiple ionic polymer layers (SMIL) across the capillaries [31, 32].

Currently we are interested in o-CEC because of its economical use of polymeric surfactants which are time-consuming to synthesize. Traditionally polymeric surfactants have been used for separations in MEKC [33, 34] where the polymeric surfactant is added to the mobile phase that is constantly replenished. A drawback of this method is that a lot of polymeric surfactant is consumed in the separation. Another advantage of o-CEC is the possibility of coupling o-CEC separation with electrospray ionization-mass spectrometry (ESI-MS) detection because there is little interference of the polymeric surfactant with analyte of interest. The possibility of clogging the ionization source with polymeric surfactant is eliminated.

Our laboratory has recently investigated the use of a polymeric surfactant, using poly(sodium *N*-undecanoyl-L-glycinate) and poly(diallyldimethylammonium chloride) (PDADMAC) in o-CEC [35]. The 10-bilayer PEM coating gave remarkable endurance and stability even at extreme pH values. However, the PEM coating procedure was time-consuming. It should also be noted that the thickness of the PEM coating reduces the inner diameter of the capillary wall, which may lead to frequent blockage of the capillary. In this work, we report the use of a polymeric surfactant, poly(sodium undecylenic sulfate) (poly-SUS) and PDADMAC in a single bilayer PEM coating for o-CEC. To evaluate the performance of the PEM coating, phenols and benzodiazepines analytes were examined. Also, the separation of benzodiazepines analytes using

the polymeric surfactant PEM coating in o-CEC format was compared to the chromatographic performance of a bare silica capillary and MEKC.

## 2 Materials and methods

### 2.1 Chemicals

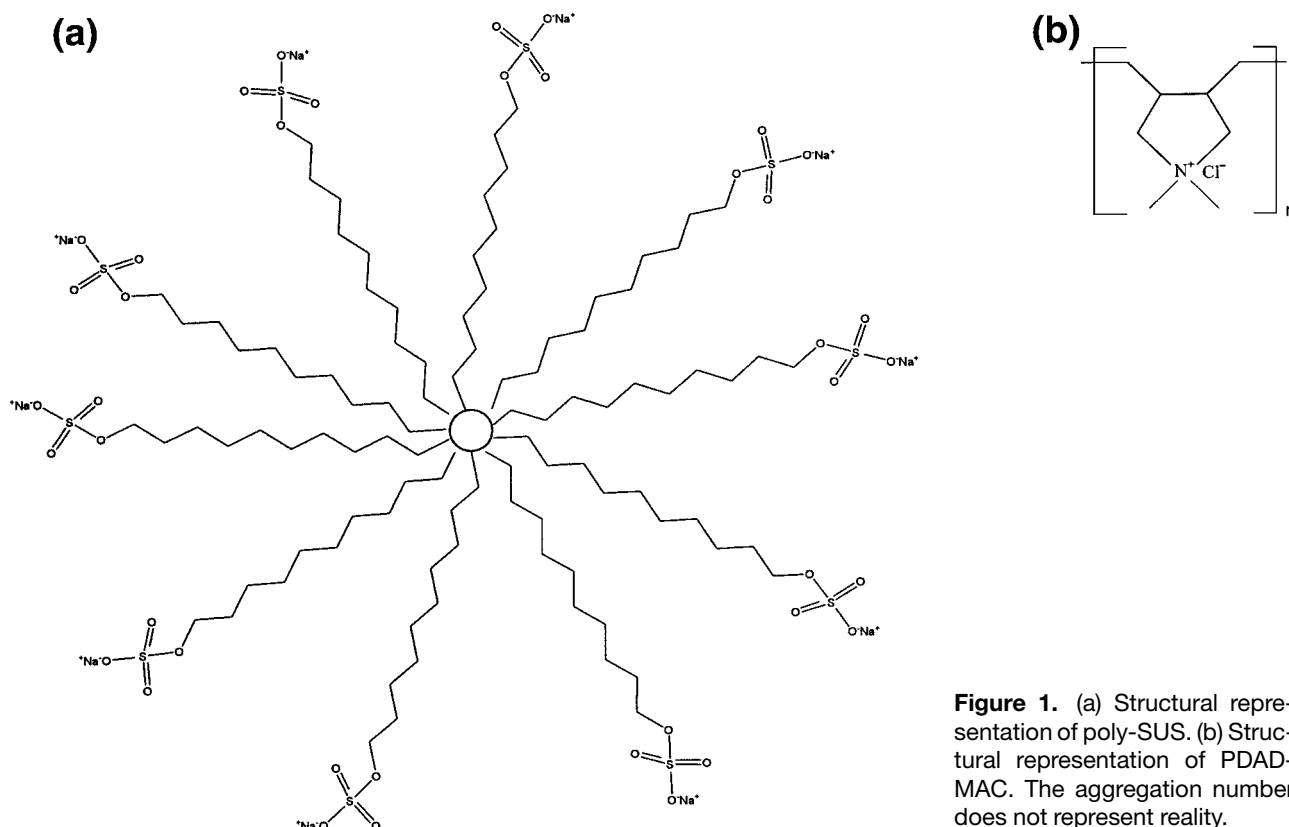
PDADMAC polymer ( $M_r = 200\,000$ – $350\,000$ ) was obtained from Aldrich Chemical (Milwaukee, WI, USA). The monomer of sodium undecylenic sulfate (mono-SUS) was synthesized in our laboratory from chlorosulfonic acid and 10-undecenyl alcohol, according to our previously reported procedure [36]. A 100 mM sodium salt solution of the monomer was then polymerized by use of  $^{60}\text{Co}$   $\gamma$ -radiation to form the poly-SUS. The average molecular weight of the poly-SUS has been determined to be approximately 8780 by use of analytical ultracentrifugation [37]. PDADMAC and poly-SUS (shown in Fig. 1) were used as the PEM coating reagents. The phenol analytes (3,5-dimethylphenol, 4-methylphenol, phenol, 4-fluorophenol, 4-chlorophenol, 3-chlorophenol, 3-bromophenol) and the benzodiazepine analytes (flunitrazepam, temazepam, nitrazepam, diazepam, oxazepam, clonazepam, lorazepam) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium chloride (NaCl) and the buffer solutions composed of sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_4$ ) and sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7$ ) were purchased from Fischer Scientific (Fair Lawn, NJ, USA).

### 2.2 Instrumentation

All experiments were performed on an HP  $^{30}\text{CE}$  capillary electrophoresis system (Hewlett-Packard, Walbronn, Germany) equipped with a diode array detector (DAD). The UV detector was set at 200 nm for phenol detection and 254 nm for benzodiazepine detection. All experimental data was collected and integrated using the HP Chemstations software. Fused-silica capillaries (58 cm total length, 50 cm effective length  $\times$  50  $\mu\text{m}$  ID) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Samples were injected by pressure at 3 kPa for 3 s. The temperature of the capillary cassette was maintained at 20°C and the applied voltage ranged from 15 to 20 kV. The EOF,  $\mu_{\text{eo}}$ , was calculated using the equation

$$\mu_{\text{eo}} = L_d L_t V t_o \quad (1)$$

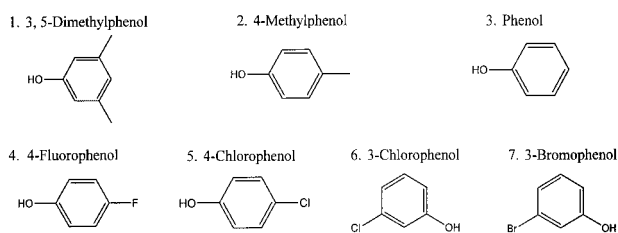
$L_d$  is the effective column length,  $L_t$  is the total capillary length,  $V$  is the applied voltage, and  $t_o$  is the migration time of the electroosmotic flow marker (methanol was used as the electroosmotic flow marker). The migration time ( $t_o$ ) of methanol was used in calculating the RSD values in evaluation of reproducibility.



**Figure 1.** (a) Structural representation of poly-SUS. (b) Structural representation of PDADMAC. The aggregation number does not represent reality.

### 2.3 Sample and buffer preparation

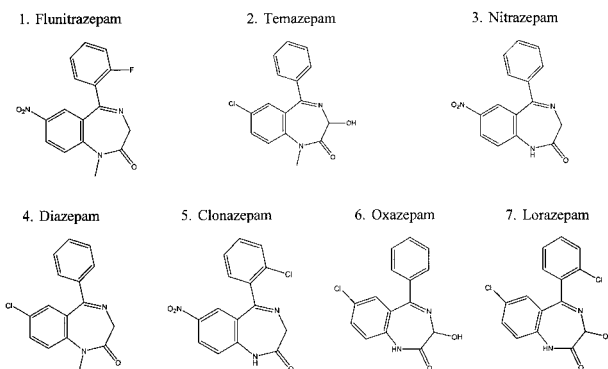
The analyte structures of the seven phenols and seven benzodiazepines used in this study are shown in Figs. 2 and 3, respectively. Standard stock solutions of the analytes were prepared in methanol at concentrations ranging from 0.1 to 0.5 mg/mL. The mobile phase consisted of a mixture of sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7$ ) in the ratio of 1:1, buffered at pH 8.0 to pH 10.0. The pH was adjusted by titrating each buffer solution with either 1 M phosphoric acid ( $\text{H}_3\text{PO}_4$ ) or 1 M sodium hydroxide (NaOH). Finally, the buffer solution was sonicated for 10 min and filtered through a 0.45  $\mu\text{m}$  polypropylene nylon filter (Nalgene, Rochester, NY, USA). The concentration of the mobile phase solution was varied from 15 mM to 50 mM.



**Figure 2.** Structures of the phenol analytes.

### 2.4 Procedure for polyelectrolyte multilayer coating

First, a detection window of 0.5 cm was prepared by burning off the external polyimide capillary coating. The polymer solutions were deposited on the inner capillary surface by using the flush function on the HP <sup>3D</sup>CE instrument. Initially the capillary was flushed with 1 M NaOH for 45 min and then with deionized water for 15 min. Next, the capillary was flushed with 0.5% w/v PDADMAC in 0.2 M NaCl solution. The capillary was flushed with deionized water for 5 min. Finally, the capillary was flushed



**Figure 3.** Structures of the benzodiazepine analytes.

with 1% w/v poly-SUS for 20 min, and then with deionized water for 5 min. The total PEM coating procedure took less than 2.5 h. The temperature of the cassette was maintained at 25°C. After coating, the capillary was conditioned with buffer until a stable baseline and current was achieved. The MEKC and bare fused-silica experiments were performed by first deprotonating the capillary with 1 M NaOH for 45 min and then conditioning with phosphate buffer for 20 min.

### 3 Results and discussion

#### 3.1 Reproducibility

Intra- and inter-capillary reproducibility is an important factor in evaluation of column performance. The relative standard deviation (RSD) values of the electroosmotic flow (EOF) were obtained from three replicate analyses of the phenol analytes. Table 1 reports the calculated RSD values for the migration times of the seven phenol analytes obtained by use of five consecutive runs. Figure 4 illustrates the electropherograms obtained from the separation of the phenol analytes on the first and fifth run. The EOF values from the first and the fifth runs were  $2.932 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  and  $2.943 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ , respectively. These electropherograms also demonstrate the excellent run-to-run reproducibility with respect to the EOF and the migration times of each analyte. Table 2 reports the run-to-run and the column-to-column reproducibilities of the same.

**Table 1.** Migration time reproducibilities of seven phenol analytes

Peak No.	Analyte	Average migration time $t_m$ (min)	%RSD ( $n = 5$ )
1	3,5-Dimethyl phenol	8.95	0.92
2	4-Methyl phenol	9.19	0.92
3	Phenol	9.50	0.95
4	4-Fluorophenol	9.87	0.94
5	4-Chlorophenol	12.08	0.52
6	3-Chlorophenol	16.09	1.06
7	3-Bromophenol	17.02	0.91

The experiments were all carried out in the same capillary.  $n$  = total number of runs. Conditions same as in Fig. 4.

#### 3.2 Column stability

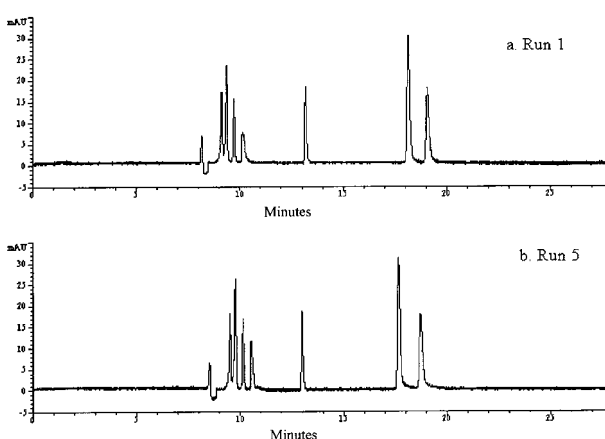
Another important consideration of the PEM coating is the lifetime of the stationary phase. The endurance of the coating was tested in a PEM-coated capillary by setting the instrument to perform a series of runs over a period of

**Table 2.** Run-to-run and column-to-column reproducibilities of PEM capillary coating

Capillary No.	EOF (average) $t_m$ (min)	Run-to-run % RSD ( $n = 3$ )	Column-to-column <sup>a)</sup> % RSD ( $n = 4$ )
1	8.20	0.79	0.95
2	7.99	0.45	
3	7.93	0.08	
4	8.00	0.08	

a) Column-to-column RSD values were computed from the average EOF values obtained from the four capillary columns.

$n$  = number of runs. Conditions same as in Fig. 4.

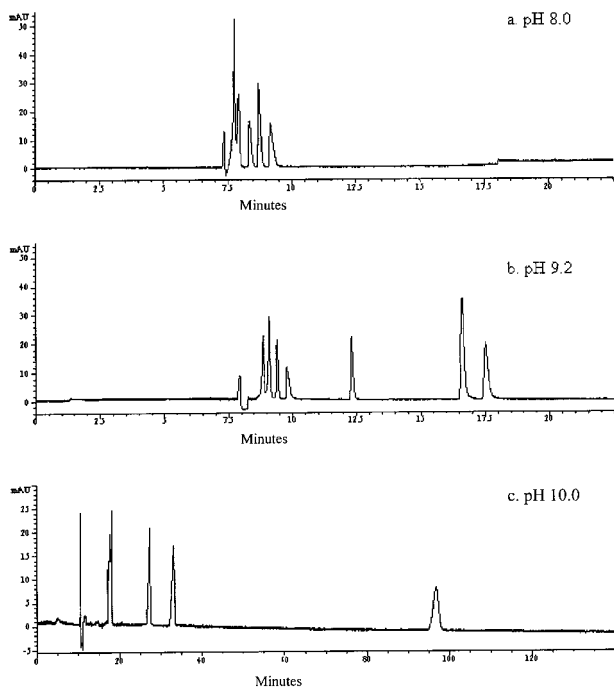


**Figure 4.** Run-to-run reproducibilities. (a) Run 1, (b) Run 5. Conditions: PEM coating, 0.5% w/v PDADMAC dissolved in 0.2 M NaCl and 1% w/v poly-SUS; mobile phase, 20 mM sodium phosphate (dibasic) and sodium borate at pH 9.2; temperature, 20°C; injection, 3 s at a pressure of 3 kPa; applied voltage, 20 kV; capillary, 58 cm total length, 50 cm effective length, 50  $\mu\text{m}$  ID; detection, 200 nm.

five days. The mobile phase was replenished after every 20 runs in order to maintain current stability. All separations were performed at 20°C using 20 mM phosphate/borate buffer (pH 9.2). The endurance of the coating was found to be more than 100 runs. After 120 runs there was a significant drift in EOF and in the migration times of the phenol analytes. This was potentially due to the detachment of the PEM coating from the capillary wall.

#### 3.3 Separation of phenols

o-CEC has a number of parameters that can be varied in order to optimize the separation of particular analytes. One factor that influenced the separation of the phenols was the pH of the buffer. The pH changes the net charge

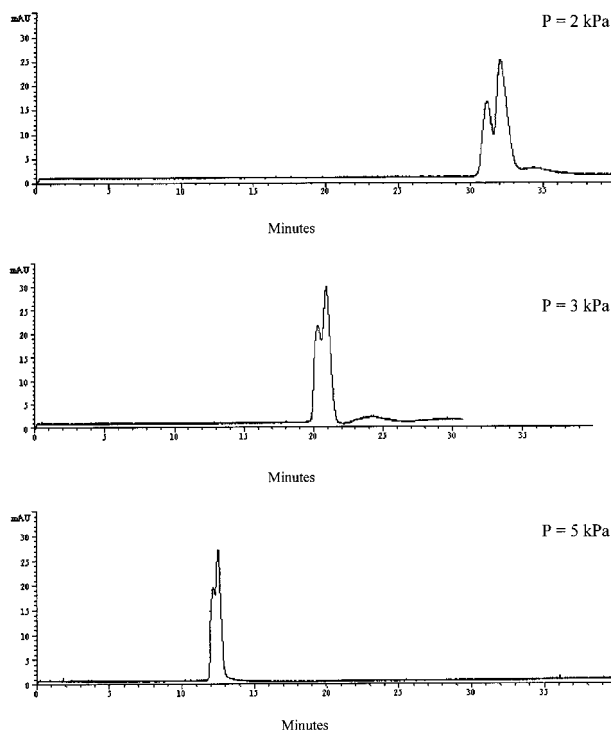


**Figure 5.** Effect of buffer pH on the o-CEC on separation of phenols. Conditions same as in Fig. 4, except mobile phase at pH 8.0–10.0.

of the analyte and hence the electrophoretic mobility of the analytes. Figure 5 is an illustration of the separation of phenols at pH 8.0, pH 9.2 and pH 10.0, respectively. At the intermediate pH 8.0 a partial resolution of the analytes is observed (Fig. 5a). At pH 9.2 (Fig. 5b) a baseline separation is achieved. At pH 10.0 (Fig. 5c), there was a significant increase in the EOF and hence increase in migration time of the analytes. The change of the EOF with change in pH values indicates the EOF's dependency on the surface charge. The migration of phenol analytes in this case was influenced by the EOF, the electrophoretic mobility of each analyte, and the degree of interaction of analytes with the PEM coating.

### 3.4 Pressure studies

The PEM coating in o-CEC column is analogous to the stationary phase of HPLC. To better understand the partitioning behavior of the analytes with the PEM coating, the separation of phenols in the coated capillary was investigated by use of pressure only. Thus, the separation mechanism of the o-CEC without an applied voltage was similar to that of HPLC. The phenol analytes were injected into the PEM-coated capillary at various pressures and the applied voltage was zero. Figure 6 demonstrates the separation of seven phenols at varying pressures 2, 3 and 5 kPa, respectively. The partial resolution of seven

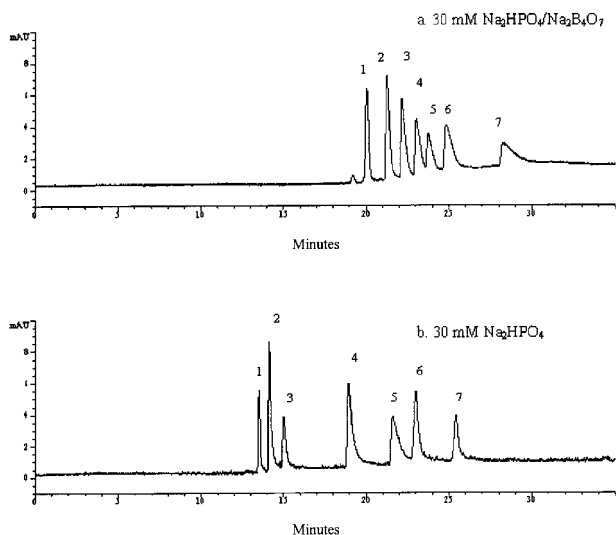


**Figure 6.** Effect of pressure in the separation of phenols. Conditions: PEM coating, 0.5% w/v PDADMAC dissolved in 0.2 M NaCl and 1% w/v poly-SUS; applied voltage, 0 kV; applied pressure, 2, 3 and 5 kPa, respectively. Mobile phase,  $\text{Na}_2\text{HPO}_4/\text{Na}_2\text{B}_4\text{O}_7$  (pH 9.2); temperature, 20°C; injection, 3 s at pressure of 3 kPa; capillary, 58 cm total length, 50 cm effective length, 50  $\mu\text{m}$  ID; detection, 200 nm.

phenols indicates that there was slight interaction between the analytes and the coating. As expected, the increase in pressure resulted in a decrease in the migration time and the resolution of the analytes. From the results it can be concluded that not only does electrophoretic mobility enhance the separation, but also the hydrophobic interaction of the analytes with the coating as well.

### 3.5 Separation of benzodiazepines

The separation of benzodiazepine analytes was also explored by use of the PEM coating. A baseline separation of the seven analytes was achieved in less than 30 min under optimized conditions (Fig. 7). Both the resolution and elution time of the analytes changed by varying the buffer system. The separation of benzodiazepine mixture using two different buffers, 30 mM borate/phosphate (pH 9.2) and 30 mM phosphate (pH 9.2) is shown in Fig. 7. A slight tailing of benzodiazepine analytes was observed due to the presence of positively charged amine groups

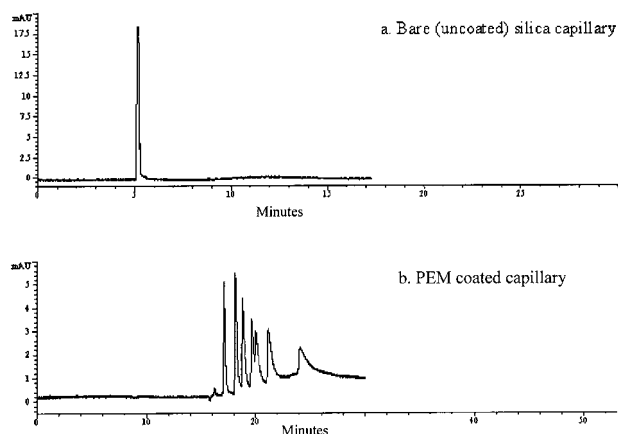


**Figure 7.** Separation of benzodiazepines using (a) 30 mM  $\text{Na}_2\text{HPO}_4/\text{Na}_2\text{B}_4\text{O}_7$ , pH 9.2; (b) 30 mM  $\text{Na}_2\text{HPO}_4$ , pH 9.2. Conditions: PEM coating, 0.5% w/v PDADMAC dissolved in 0.2 M NaCl and 1% w/v poly-SUS; temperature, 20°C; injection, 3 s at pressure of 3 kPa; applied voltage, 15 kV; capillary, 58 cm total length, 50 cm effective length, 50  $\mu\text{m}$  ID; detection, 254 nm.

that are attracted by the sulfonate groups on the coating. Better resolution and shorter elution times of the analytes were observed with phosphate buffer.

### 3.6 Comparison between bare silica and PEM coating in o-CEC

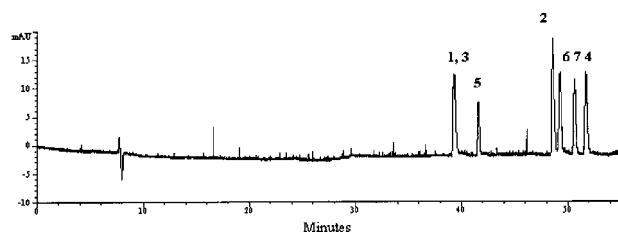
Previous reports have illustrated the usefulness of o-CEC capillaries in the separation of some analytes. The importance of the PEM coating in optimizing separation was demonstrated by comparing separations on a bare silica capillary and a coated capillary. First, the importance of the PEM coating was tested using the benzodiazepine analytes. Figure 8a illustrates the separation of the benzodiazepines on a bare silica capillary. No separation of the benzodiazepines was achieved when the bare capillary was used. In comparison, separation of a seven benzodiazepines was observed when a PEM-coated capillary was used in Fig. 8b. The PEM coating acts as a stationary phase and the hydrophobic interaction between the hydrophobic polymer core and the nonpolar moiety of each analyte enhanced separation. Also, the importance of the coating was tested using phenols. The separation of phenol analytes was carried out on a bare capillary and even though the elution time of the analytes was shorter, only six out of the seven phenol analytes were resolved. These results demonstrate the importance of the PEM coating in the resolution of some analytes.



**Figure 8.** Separation of benzodiazepines. (a) Bare fused-silica capillary, (b) PDADMAC/poly-SUS PEM-coated capillary. Conditions: mobile phase, 20 mM sodium phosphate (dibasic) and sodium borate at pH 9.2; temperature, 20°C; injection, 3 s at a pressure of 3 kPa; applied voltage, 20 kV; capillary, 58 cm total length, 50 cm effective length, 50  $\mu\text{m}$  ID; detection, 254 nm.

### 3.7 Separations of benzodiazepines using MEKC and o-CEC

A comparative study of the separation of benzodiazepine analytes in MEKC and o-CEC was performed. Figure 9 illustrates the separation of benzodiazepines using MEKC. In this study poly-SUS was added to the mobile phase. All other separation conditions were similar to those used in o-CEC (Fig. 7a). In Fig. 7a, o-CEC gave a baseline separation of the seven benzodiazepines and a shorter elution of all seven benzodiazepines. In MEKC, flunitrazepam and nitrazepam (peak 1, 3) coelute and longer retention times were observed. The elution order of the analytes changed when MEKC experiments were performed and this may imply a change in the separation mechanism. The separation mechanism for o-CEC and MEKC is based on the electrophoretic mobility of the analytes and hydrophobic interactions with the stationary phases. However, in MEKC the analytes partition in the pseudo-stationary phase while in o-CEC analytes partition in the stationary phase coating.



**Figure 9.** Separation of benzodiazepines using MEKC. Conditions: mobile phase, 1% w/v poly-SUS in 20 mM sodium phosphate (dibasic). Other conditions as in Fig. 8.

In conclusion, the use of PEM-coated capillaries has been shown to be a viable approach to o-CEC. The PEM coating procedure is simple and takes a relatively short time (less than 2.5 h). The PEM coating shows good selectivity towards the phenol and benzodiazepine analytes. The PEM coating exhibits excellent reproducibility producing RSD values of less than 1.5%. The stability of the PEM coating has been shown to more than 100 runs. The coating has demonstrated superiority over MEKC and CZE in the separation of benzodiazepines.

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