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Performance of metal complex substituted polysiloxanes in capillary electrophoresis and capillary electrochromatography

Qirong Wu, Milton L. Lee, Roger G. Harrison*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602-5700, USA

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Abstract

Two novel polysiloxanes containing the metal complex, $\text{Co}(\text{TACN})_2^{3+}$ (TACN=1,4,7-triazacyclononane) were used as coatings for capillary electrophoresis (CE) and capillary electrochromatography (CEC). Through crosslinking and covalent bonding, the positively charged polymers were bonded to silica supports. In both CE and CEC, these coatings exhibited strong, pH-independent, and anodic electroosmotic flow (EOF), and had excellent long-term stability. Successful separations of aromatic acids were achieved in CE. In CEC, separation of alkylbenzenes (7 min) and basic compounds (20 min) was achieved with higher resolving power than conventional octadecyl silica packings. These polymers represent a new class of coatings for CE and CEC that generate pH-independent EOF.

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1. Introduction

Capillary electrophoresis (CE) is used extensively in the separation of charged compounds. However, the charged silanol groups on the capillary surface, can cause zone broadening and peak tailing as well as pH-dependent electroosmotic flow (EOF). The pH dependency of EOF, when coupled with the $\text{p}K_a$ values of ionic moieties on the solute, can create complicated pH-resolution profiles that become sensitive to localized electrolyte pH changes. This phenomenon compromises reproducibility, efficiency and sensitivity in CE separations. Ion suppression techniques are often required to enhance sensitivity

[1]. The reproducibility of CE can also be undermined by solute adsorption, which is primarily the result of ionic and hydrogen bonding between the solutes and the charged groups on the capillary wall. Even moderately weak interactions can give rise to zone spreading and peak tailing.

One approach to solve these problems is to permanently coat the capillary wall with a material to which the analytes do not adsorb. Both nonionic and ionic polymers have been used as coatings in CE. Nonionic polymers include covalently bonded or cross-linked polymers, such as cellulose derivatives [2], cross-linked dextrans [3], poly(ethylene glycol) [4], and polyacrylamide [5]. These coatings are used to permanently shield the silanol groups on the capillary wall from the bulk solution. Such coatings can minimize analyte adsorption, but can also result in undesirable EOF suppression. As coating materi-

*Corresponding author. Tel.: +1-801-422-8096; fax: +1-801-422-0153.

E-mail address: roger_harrison@byu.edu (R.G. Harrison).

als, these nonionic polymers are unstable beyond a limited pH range (4–9) and their useful lifetimes are generally short.

Ionic polymers include cationic coatings, such as polyacrylamide resin [6], chitosan [7], polyethylene amine [8] and polyarginine [9], and anionic coatings including phenylboronic acid [10] and polymerized sodium 2-acrylamido-2-methyl-1-propanesulfonate [11]. These ionic coatings employ positively charged groups (quaternary amine) or negatively charged groups (sulfonic acid) on the capillary wall to afford EOF. Since these functional groups have higher ionization constants than surface SiOH, their concentrations do not change with variation in buffer pH within a wide pH range. Thus, stable EOF can be generated over a relatively wide pH range. However, even though these coatings can minimize solute adsorption to the SiO^- groups, the introduced charged groups can interact with analytes. This adsorption behavior can compromise separation efficiency and reproducibility. Recently, coatings composed of alternating positively and negatively charged polymers have shown longer lifetimes and reproducible control of EOF, however, adsorption of analytes is still a problem [12,13].

Capillary electrochromatography (CEC) has brought together CE and HPLC in an attempt to separate not only charged compounds, but neutral compounds as well. One major obstacle in CEC development is the limited availability of specially designed stationary phases with strong EOF and appropriate selectivity to allow compound separation. To date, most of the research on CEC is based on packings that are primarily intended for liquid chromatography. The variety of stationary phases that have been employed for CEC separations include modified silica gel, sol–gel monoliths, organic polymers (continuous bed) and ion-exchange materials [14].

Early investigations of CEC column packings showed that it is important to have both charged groups to support EOF, as well as traditional surface functionality, such as C_{18} or C_8 . However, capillaries packed with silica-based particles often show poor separation efficiency due to the flow-rate dependence on pH and surface adsorption. Ionizable analytes are sensitive to pH and generally require a different pH range for separation compared to neutral

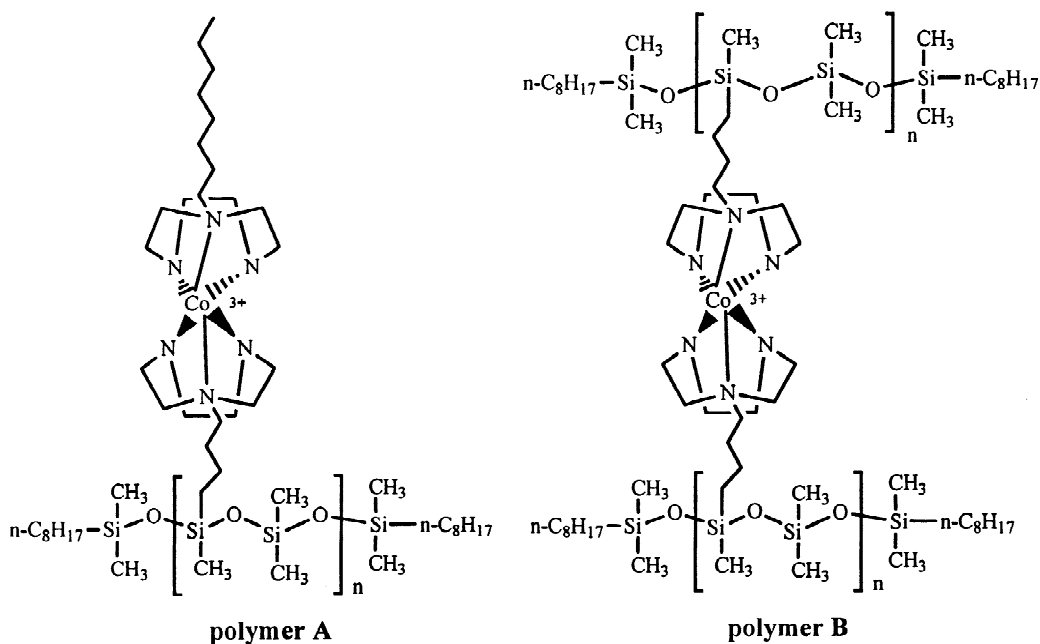
species. Common silica deactivation approaches used in HPLC such as total endcapping and polymer encapsulation cannot be applied in CEC, since a charged surface is needed for EOF. To utilize in CEC the combined separation mechanisms of electromobility and partitioning, packings with pH-independent charged surfaces need to be developed. Several attempts [15–17] have combined charge carrying ion-exchange groups (sulfonic acids or quaternary amines) and interactive groups such as C_{18} , C_8 and phenyl groups to form mixed mode packings for CEC. With the presence of strongly acidic or basic groups on the surface of the packing materials, the EOF remains constant over a wide pH range. However, analyte adsorption due to the presence of charged sites on the packing surface still remains.

Herein, we report a novel approach to “deactivating” charge sites while retaining strong and stable EOF over a wide pH range. Our approach is based on the synthesis of a specially designed material that can be used both as a coating in CE and CEC. Specially designed polymers (Fig. 1) were synthesized and immobilized onto the silica support. Instead of conventional charged SiO^- , $-\text{SO}_3^-$ and $-\text{NR}_4^+$ groups, we have incorporated a transition metal complex ($\text{Co}(\text{TACN})_2^{3+}$) into a polysiloxane to provide positive charge for the generation of strong EOF. The positively charged metal center is surrounded by a cyclic ligand and has been designed to overcome analyte adsorption and produce pH-independent EOF. To the best of our knowledge, this is the first example where a transition metal complex has been successfully used in CE coatings and CEC stationary phases. We report on the stability of this new coating and its ability to afford separations of acidic, neutral and basic compounds in CE and CEC.

2. Experimental

2.1. Materials

Fused silica capillaries of 75 μm I.D. and 365 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) and two types of porous bare silica, 3 μm particle size with 80 Å average pore size (Waters Corporation, Milford, MA, USA) and 3.5 μm particle size

Fig. 1. Structures of polymers A and B ($n \approx 50$).

with 100 Å average pore size (Agilent Technologies, Palo Alto, CA, USA) were used. Trimethylsilyltrifluoromethanesulfonate and 3,5-lutidine (Aldrich, Deerfield, IL, USA), along with their solutions, were stored under nitrogen in a desiccator containing drying agent. Dichloromethane was dried over CaH_2 prior to use. Trizma hydrochloride and trizma base (Sigma, St. Louis, MO, USA) were used to make the Tris buffer. Water was purified by means of a Milli-Q system (Millipore, Milford, MA, USA). Other chemicals mentioned in this paper were all available from Aldrich unless otherwise noted. All buffer, mobile phase and sample solutions were filtered through a 0.2 μm Acrodisc syringe filter (Gelman Science, Ann Arbor, MI, USA). Buffer and mobile phases were thoroughly degassed prior to use via simultaneous ultrasonication and helium purging for 1 h. Thorough degassing of the mobile phases before use was necessary to prevent subsequent bubble formation. Detailed procedures for the synthesis of polymers A and B have been reported previously [18].

Metal ion analysis was performed using an inductively-coupled plasma-optical emission spectrometer (ICP-OES), Model Optima 2000 DV (Perkin-Elmer,

Norwalk, CT, USA). Elemental analyses were conducted by M-H-W Laboratories (Phoenix, AZ). A JEOL JSN 840A (10 kV accelerating potential) scanning electron microscope was used to analyze the particles after sputtering them with a 200 Å gold coating.

2.2. Preparation of coated capillary columns

Before coating, the capillary was cleaned to produce a uniform surface capable of binding the polymer. The fused-silica capillaries were treated in sequence with 1.0 M NaOH for 1 h, distilled water for 45 min, 0.1 M HCl for 1 h and water for 45 min. Residual water was evaporated by connecting the capillary to a gas chromatographic oven (HP-5670, Hewlett-Packard, Avondale, PA, USA) at 100 °C under helium (400 kPa) for 12 h.

A stable reverse EOF was achieved by a two-step coating procedure. The capillaries were first dynamically coated using acetonitrile solution containing metal complex polymer A or B (5% w/v) and dicumyl peroxide (0.75%, w/v). The coating solution was forced through the capillary at room temperature with a syringe pump for 50 min and the

capillary was allowed to stand for 40 min. The coating solution was then forced out of the capillary with helium at 400 kPa for 1 h. With helium purging, the coated capillary was heated from 40 to 200 °C at 5 °C/min, and held at the final temperature for 5 h in a chromatographic oven. The capillary was then rinsed with CH₃CN for 40 min and dried with helium (400 kPa).

A deactivating solution consisting of 5% (w/w) of trimethylsilyltrifluoromethanesulfonate and 7.23% (w/w) of 3,5-lutidine in dry CH₂Cl₂, prepared by mixing both reagents under nitrogen, was stored in a desiccator with drying agent prior to use. The deactivating solution was forced through the capillary with a syringe pump for 5 h. The capillary was then rinsed with dry CH₂Cl₂ for 1 h and CH₃CN for 1 h. The coated capillary was finally dried under a helium purge at 400 kPa for 2 h. Before the coated capillary was used, it was conditioned with buffer solution for 10 min.

2.3. Coating and deactivation of silica particles for CEC

A previously reported [19] reaction vessel was used for coating and cross-linking of silica particles with the polymer. Polymer A (5 to 11 mg) and dicumyl peroxide (1.97 mg, 7.3×10^{-3} mmol) were dissolved in 10 ml of acetonitrile. The solution was transferred to the reaction vessel containing porous bare silica (55 mg). Helium was bubbled (200–500 ml/min) through the mixture at room temperature for 4 h. In order to accelerate solvent evaporation, the reaction vessel was placed in an ultrasonic bath (Branson Ultrasonics, Danbury, CT, USA). This process facilitated uniform coating of the polymer on the silica surface as observed by SEM pictures and filling of the pores in the silica particles with the polymer. After the acetonitrile was evaporated, the vessel was placed in a chromatographic oven under helium purge (150 ml/min) and warmed from 40 °C to the reaction temperature of 200 °C at 5 °C/min. The vessel was kept at 200 °C for 5 h to carry out cross-linking and dehydrocondensation. Upon cooling, the solid was washed with acetonitrile and dried under vacuum to yield brown particles. Helium was further bubbled through the particles for 2 h at 500 ml/min. To the dried particles was added 7.5 ml of a

dry CH₂Cl₂ solution of 5.0% (w/w) trimethylsilyltrifluoromethanesulfonate and 7.2% (w/w) 3,5-lutidine. The suspension was stirred for 24 h in a desiccator with drying agent at room temperature. (The deactivation reaction must be carried out under a dry atmosphere.) The suspension was filtered and washed with dry CH₂Cl₂ (3×10 ml), CH₃CN (2×8 ml), and CH₂Cl₂ (2×5 ml). The brown particles were further dried by purging with helium (500 ml/min) for 4 h.

2.4. Preparation of CEC columns

The CEC columns were packed using supercritical carbon dioxide with a Lee Scientific Model 600 SFC/GC pump. Capillary columns were first coated with polymer before being packed with particles coated with the same polymer. A stainless steel frit or screen (Valco, Houston, TX, USA) was used to retain the packing material in the column during the packing process. Details of the packing procedure have been described elsewhere [20]. Inlet and outlet frits were prepared using a resistive heating coil (InnovaTech, UK) while water was pumped through the column at 40,000 kPa. Since the frit formation process creates some small gaps around the frit inside the packed column, an outlet frit was made while water was pumped in the reversed direction. Extra packing material outside the outlet frit was washed away while pumping mobile phase through the column (4000 p.s.i.). A detection window was made immediately past the outlet frit using a resistive heating coil, or just prior to the frit by dripping hot concentrated sulfuric acid on the polyimide coating.

Before the packed columns were installed in the CEC instrument, they were rinsed and equilibrated with mobile phase using an Isco model μ LC-500 pump (Isco, Lincoln, NE, USA). A typical conditioning cycle employed an initial treatment with 80:20 acetonitrile–water solution or mobile phase until no bubbles eluted from the outlet frit (4000 p.s.i. for 12 h or longer, sometimes 2 to 3 days). Column conditioning was then continued until current through the packed bed could be observed (6 h or overnight at 2 kV) and then 5 kV for 30 min and 10 kV for 10 min were applied.

2.5. CE and CEC instrumentation

The instrument used in this study was a laboratory-built apparatus, which has been described in the literature [20,21]. The apparatus consisted of a computer controlled high-voltage power supply (0–40 kV), two pressurized electrolyte reservoirs and a fiber optic assembly for UV detection. Injections of samples were made electrokinetically for 1 s at 2 kV, which corresponds to an injection volume of 1.5 nL.

3. Results and discussion

3.1. Coatings

We have functionalized polysiloxanes with cationic metal complexes to obtain stable pH-independent EOF and minimize adsorption of solutes on the capillary or packing material surface. The metal complexes contain the tridentate ligand 1,4,7-triazacyclononane (TACN), which has a high affinity for metal ions due to the cooperative binding by its three nitrogen atoms (Fig. 1). Two TACN ligands coordinated to one transition metal ion can occupy all of the available metal coordination sites, and thus make a coordinately saturated metal complex. When two TACN molecules bind to a Co(III) ion, a low-spin and kinetically stable complex is formed. This $\text{Co}(\text{TACN})_2^{3+}$ complex is stable over a wide range of pH values and ion concentrations. As seen by proton NMR and UV–Vis absorption spectra, the synthesized polymers do not change in highly acidic (pH 1) or basic (pH 11.5) solutions [18]. Furthermore, highly charged Co(III) ions are trapped in the bis-macrocycle frame and are shielded from interacting with solutes. By means of ^1H and ^{29}Si NMR spectroscopy, a straight chain polysiloxane backbone structure was confirmed. The NMR spectra also demonstrated that 35% of the silicon hydride groups on the polymethylsiloxane chain were substituted by $\text{Co}(\text{TACN})_2^{3+}$ groups. The stability of the synthesized materials led to pH-independent EOF in polymer-coated columns.

Due to the fixed charges, both polymers A and B can be used as CE coatings to influence EOF. However, only polymer A is suitable for CEC due to its dual functionality of charged sites and alkyl

chains. In polymer A, the C_8 chains bonded to the TACN groups provide enough free volume into which analytes can partition. In typical CEC stationary phases, alkyl chains are linked directly to the silica support through Si–C bonds, which reduces the number of charged Si–O[−] sites [22]. With these partially end-capped phases, it is difficult to simultaneously obtain a high density of charged sites needed for strong EOF and a large number of alkyl chains needed for partitioning of analytes. In polymer A, octyl chains were covalently linked to the TACN rings instead of being directly linked to the charged silanol sites, therefore, making it possible to obtain strong electroosmotic pumping and substantial partitioning.

Another important feature of the polymers is their high molecular masses (greater than 20,000 g/mol, NMR analysis). One advantage of high molecular mass polymers is their increase of the double layer viscosity at the silica support, which reduces the variance of electroosmotic flow caused by SiO[−] groups and minimizes zone broadening [23]. In practical terms, this permits the use of extreme conditions including high ionic strength, very high or low pH and different organic modifiers.

3.2. Coating of polymer bonded phases

To achieve a permanent coating, the polymethylsiloxanes were immobilized onto the silica support via direct covalent bonding and cross-linking. Coating of an open tubular column with the polymers involves filling a fused-silica capillary with a coating solution and keeping the solution in the capillary for a short time (e.g. 1.5 h) to allow for physical adsorption of the polymers to the capillary inner wall. The coated capillary is then thermally treated to promote chemical bonding of the polymer to the column and rinsed with a series of solvents. Next the coatings are cross-linked via free radicals formed from the terminal groups ($\text{C}_8\text{H}_{17}\text{–Si}(\text{CH}_3)_2\text{–}$) of the polysiloxane chain. Simultaneously, some remaining silylhydride groups (HSi–) on the polysiloxane backbone react with silanol groups (HOSi–) on the silica surface [24]. Such a stable cross-linked and chemically bonded layer is not only resistant to washing with water and mobile phase, but also covers the silica surface, including some unmodified

silanol groups. In this study, cross-linking and coating were conducted at 200 °C [19,25]. Temperatures between 200 and 350 °C were investigated, and no apparent improvement in deactivation was observed by the higher temperatures.

In order to maintain a strong anodic EOF, we attempted to cover most of the silanol groups. After coating, most residual SiO^- groups were shielded by the positively charged polymer coating and did not contribute to the EOF. However, the EOF was weak ($< -0.2 \times 10^{-5} \text{ cm}^2/\text{V s}$) due to the cathodic EOF of some silanol groups counteracting the anodic EOF of the metal complexes. To eliminate the adverse effects of these silanol groups, trimethylsilyltrifluoromethanesulfonate was used to form TMS ethers with silanol groups and 3,5-lutidine was used to scavenge protons during the end-capping process. Although an increase of carbon content in the materials was hardly noticeable after treatment with the silylating reagents, the electrical effects were appreciable as shown by the anodic EOF generated after end-capping. After trimethylsilyltrifluoromethanesulfonate treatment for 5 h, very strong anodic EOF in both open tubular CE and packed CEC columns was observed. End-capping was thus done on all columns and stationary phases coated with polymer.

Uniformity of the polymer coating and the number of $\text{Co}(\text{TACN})_2^{3+}$ moieties are the two major concerns in coating silica particles with polymer A for CEC. Uniformity of the coating is important for separation efficiency, while strong anodic EOF requires high $\text{Co}(\text{TACN})_2^{3+}$ content. A main challenge to prepare a uniform polymer coating is that the particles tend to agglomerate when high concentrations of polymers are used. However, a porous silica substrate helps eliminate this and uniformly coated particles can be obtained for a polymer loading as high as 25% (w/w) [26]. Different polymer loadings, 10% and 20% w/w, were investigated with porous silica particles in an attempt to obtain fast and efficient separations.

Scanning electron micrographs (SEM) show the difference between the surfaces of untreated bare silica, 10% loading of polymer A and 20% loading of polymer A. The particles coated with a 20% polymer solution have a smoother surface compared to the particles that were not coated or those coated

with a 10% polymer solution. It is expected that most of the pores are coated by polymer chains as the polymer forms a “mat” layer on the particles [27]. Fusing was not observed among the polymer-coated particles with a loading of up to 20%.

3.3. Measurement of electroosmotic flow (EOF)

Due to the difficulty in characterizing the coated silica surface by chemical or physical methods, the magnitude and direction of the EOF was used to indicate the completeness of the coating and changes on the surface. The velocity of the EOF in both CE and CEC is proportional to the charge density on the surface of the capillary wall or stationary phase [28]. The $\text{Co}(\text{TACN})_2^{3+}$ groups provided a positively charged surface on the silica support, which counteracts the effect of the residual anionic silanol groups residing on the bare silica. The resultant EOF of the column would be in the direction of the greater of these two opposing forces.

Polymers A and B were both tested as capillary coatings in CE by measuring the EOF [29]. Polymer A- and B-coated capillaries exhibited EOF values of $-7.4 \times 10^{-4} \text{ cm}^2/\text{V s}$ and $-4.3 \times 10^{-4} \text{ cm}^2/\text{V s}$, respectively. The EOF is in the opposite direction from that of bare fused-silica, which had an EOF of $6.7 \times 10^{-4} \text{ cm}^2/\text{V s}$ under the same conditions. Comparing bare fused-silica to polymer A- and B-coated capillaries, the magnitude of the EOF was reduced by ~36% for the polymer B-coated capillaries, while it was raised by ~11% for the polymer A-coated capillaries. This is attributed to the different $\text{Co}(\text{TACN})_2^{3+}$ contents in the two polymers.

In CEC, it has been reported that for reversed EOF, packed columns with positively charge-coated walls exhibited higher EOF than columns with uncoated walls [15]. Therefore, the polymer A-coated stationary phase was packed into a capillary coated with the same polymer. (Both stationary phase and capillary had been end-capped.) The EOF results obtained for the three packing materials agreed with our SEM observations. Silica with 20% polymer A loading generated much higher anodic EOF ($-8.2 \times 10^{-4} \text{ cm}^2/\text{V s}$) than the 10% loading material ($-3.0 \times 10^{-5} \text{ cm}^2/\text{V s}$). These were in contrast to the EOF generated by bare silica ($+2.0 \times 10^{-4} \text{ cm}^2/\text{V s}$). As described later, the 20% polymer

A loaded silica packing provided more efficient separation than the 10% loaded silica particles due to greater pore blockage with the 20% polymer A. The packed column generated very strong EOF, higher than that found in CE under the same experimental conditions. The strong EOF was in contrast to the suggestion that a lower EOF should result in packed columns on account of the tortuous nature of the packed beds and the increased available cross-sectional area in an open tube [30]. Unexpectedly stronger EOF in packed columns was also observed in studies of SCX-coated and packed columns [31]. Moreover, the EOF value for the polymer A-coated packed columns was even higher than that measured for untreated capillaries. To the best of our knowledge, this is the highest reported EOF value for CEC.

3.4. Reproducibility and long-term stability of coatings

The stabilities of polymers A- and B-coated silica were evaluated by measuring the EOF over several months. Both coatings in CE and CEC showed long-term stabilities (Fig. 2) when exposed to 5–15 kV continuously for 7 days at an apparent pH of 7.8. After 7 days of continual use, the EOF only decreased 7.5% for the polymer A coating, 10% for the polymer B coating, and 10% for the polymer A-

coated silica packing material. Strong EOF was still observed after 2 months of daily usage, though it had been reduced. To regenerate the EOF, the capillary could be recoated with the positively charged polymers again. The regeneration process was found to efficiently recover the original EOF. The long lifetimes indicate that there is minimal analyte adsorption on the charged sites of the coatings. To help maintain EOF during usage over a long time, and to obtain higher reproducibility in day-to-day runs, a “fresh surface” was established every morning by rinsing the capillary with buffer electrolyte for ~30 min. At the end of the day, the capillary was rinsed with 1 ml of acetonitrile to remove all remaining analytes before it was dried by purging with helium gas for 30 min.

As a test for polymer degradation or “bleeding” during application at high voltage, the running electrolyte was analyzed for cobalt. Cobalt was not detected in the outlet vial solutions of either CE or CEC experiments (2 weeks of solutions were collected and analyzed using ICP). If a significant amount of the cobalt had leached from the polymer, it would have been within the detection limits. The strong chemical stabilities of these coatings not only results from the chemical stability of the polymers, but also from the strong interactions between the polymers and silica support, which involved covalent bonding and electrostatic affinity.

Coating reproducibilities between runs, days, and columns were also investigated. EOF reproducibilities between runs and days in the CE columns were high (2.6% RSD, Table 1). The EOF reproducibility for five CE columns was also high (5% RSD). The reproducibility of CEC packed columns was lower than for open tubular columns coated with the same material (7.7% RSD between days and runs and 21.6% RSD between columns). Thus, column packing, frit making, and column conditioning add additional variables to the packed columns. The packed bed was readily disturbed by sample injection and, therefore, required stringent sample clean-up requirements. During column preparation, beds and frits initially appeared uniform, and then voids eventually formed inside the packed bed. The voids or bubbles are probably due to the high density of charged groups on the silica support [32]. We found that exposure of the beds to voltage over time is helpful

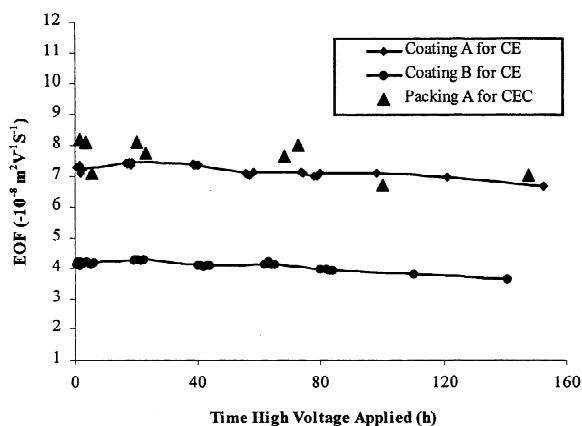


Fig. 2. EOF change with time. As voltage is applied, EOF slightly decreases in polymer A- and B-coated CE columns and polymer A-coated CEC columns. Conditions: injections of 2 kV for 2 s, run at -15 kV, acetone as EOF marker, 80% acetonitrile in 10 mM Tris-HCl buffer (pH 7.8) as background electrolyte.

Table 1

Reproducibility of polymer A- and B-coated silica CE and CEC columns. Experimental conditions are given in Fig. 2

Column	Run to run % RSD (no. of runs)	Day to day % RSD (no. of days)	Column to column % RSD (no. of columns)
Polymer A coated CE	1.46 (16)	1.98 (20)	5.75 (5)
Polymer B coated CE	0.30 (16)	2.64 (20)	4.47 (5)
Polymer A coated and packed CEC	7.72 (5)	7.42 (20)	21.6 (3)

to stabilize the current and EOF due to rearrangement and consolidation of the bed under the applied voltage. However, the effects of inadequate conditioning were difficult to ascertain and, thus, some poorly conditioned columns showed inconsistent elution times and efficiencies, and even inconsistent day-to-day reproducibilities.

3.5. Effect of acetonitrile content on EOF

Solvent, as well as coating, influences EOF, because EOF depends on the dielectric constant and viscosity of the solvent. Polymer A-coated capillaries were used to evaluate how acetonitrile concentration affects EOF. A set of mobile phases containing varying percentages of acetonitrile (0–80%) and 10 mM aqueous Tris–HCl was utilized. The EOF within the untreated capillary decreased by 22% as the acetonitrile content in the buffer solution increased to 80% (Fig. 3) [33]. This is explained by both the dielectric constant and viscosity continuously decreasing as the acetonitrile content increases [34]. In contrast, the magnitude of the EOF for the coated capillary is shifted toward higher negative values as the percentage of organic modifier in the background electrolyte is increased (Fig. 3). This behavior has also been observed by others [35,36] and can be attributed to the accessibility of surface charge. Since polymer A has good solubility in acetonitrile, an increase in the content of acetonitrile leads to a more extended polymer conformation and, thus, higher apparent charge density (the charge density that actually contributes to the EOF).

3.6. Separation of acids by CE

A mixture of aromatic acids when eluted through a column coated with polymer A was separated into individual components (Fig. 4). Even though the

voltage applied was only 15 kV and the column length was more than 45 cm, it was possible to accomplish the separation within 2 min. One reason for the fast separation is that the EOF and electrophoretic mobility were both in the same direction, toward the anode. This is unlike anionic columns where EOF and electrophoretic mobility oppose each other, the EOF in the direction of the cathode and electrophoretic mobility of anions toward the anode. High separation reproducibility (caption to Fig. 4, eight runs) was achieved as a result of the pH-independent EOF and the chemical stability of the polymer A coating. This finding indicates that adsorption of the negatively charged acidic analytes to the positively charged coating is negligible. The peak symmetries of the acid peaks were good and no obvious peak tailing was observed during the experiment, even though the separation conditions were not optimized. The separation efficiency most likely

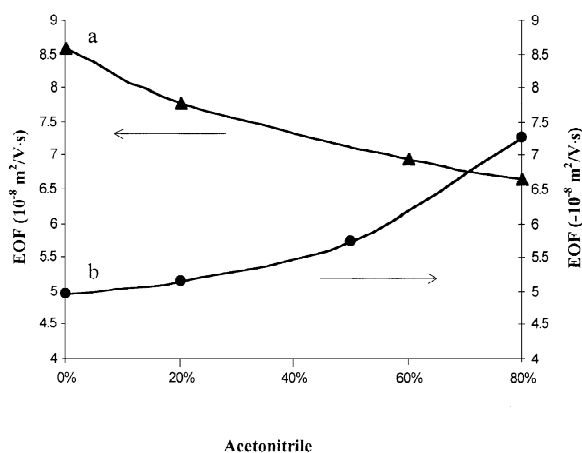


Fig. 3. EOF change with percentage of acetonitrile. The EOF decreases in an untreated fused-silica capillary (a) and increases in a polymer A-coated silica capillary (b) as the percentage of acetonitrile in the mobile phase increases. Conditions: injection of 2 kV for 2 s, run at -15 kV, thiourea as EOF marker, acetonitrile in 10 mM Tris–HCl buffer (pH 7.8) as background electrolyte.

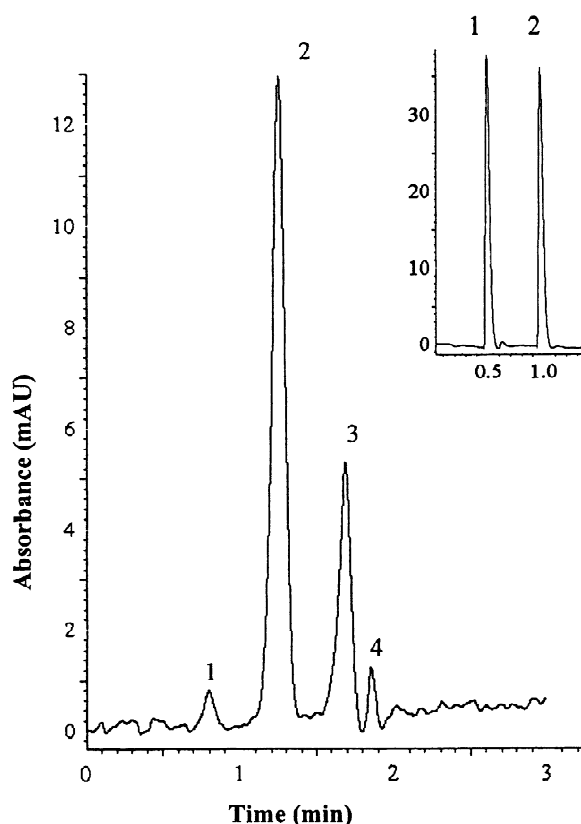


Fig. 4. Separation of aromatic acids using a polymer A-coated capillary and polymer B-coated capillary (inset). Peak identities for polymer A column: (1) 3,5-dinitrobenzoic acid (0.77 min, 2.62% RSD); (2) benzoic acid (1.24 min, 0.77% RSD); (3) pentylbenzoic acid (1.68 min, 1.03% RSD); (4) dodecyloxybenzoic acid (1.83 min, 1.87% RSD). Peak identities for polymer B column: (1) benzoic acid; (2) pentylbenzoic acid. Conditions: 49 cm (40 cm effective length) column for polymer A column, 33 cm (25.5 cm effective length) for polymer B column, 75 μ m I.D. fused-silica capillaries, run at -15 kV, 80% acetonitrile in 10 mM Tris-HCl buffer (pH 7.8) as background electrolyte, UV detection at 200 nm.

could be improved by changing the ionic strength of the mobile phase. Polymer B-coated capillaries can also be used to separate acids (Fig. 4).

Aromatic naphthalene and benzene sulphonates and their hydroxyl and amino derivatives are widely used in industrial processes, particularly as intermediates in the production of dyes and pharmaceutical compounds [37,38]. HPLC and CE are well suited for the separation of these anionic compounds [39–41]. At present, most CE analyses of these acids

use untreated fused-silica capillaries [42,43]. In order to minimize the analysis time and Joule heating from high voltage, reversed EOF has also been utilized to separate aromatic sulphonics acids [44].

We found that satisfactory separation of seven aromatic sulphonics acids could be achieved by using a polymer A-coated capillary (Fig. 5). The migration order of the sulfonates is proposed by considering their mass to charge ratios [45]. Compounds with two sulfonate groups (two anionic charges) eluted first followed by compounds with only one sulfonate group. The naphthol-containing compound is predicted to elute first due to the partial deprotonation of its alcoholic group ($pK_a=9.1$).

3.7. Separation of aromatic and basic compounds by CEC

The separation performance of polymer A-coated particles was evaluated with a test mixture of alkylbenzenes and compared to the performance of

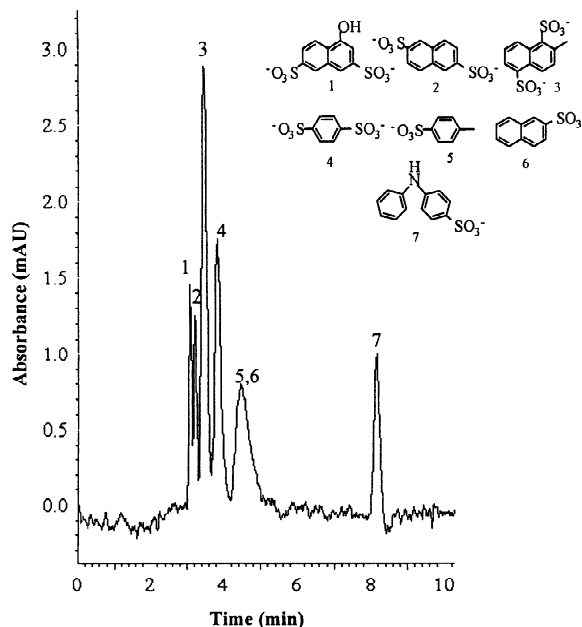


Fig. 5. Separation of aromatic sulphonics acids using a polymer A-coated capillary. Peak identities: (1) 1-naphthol-3,6-disulfonate; (2) naphthalene-2,6-disulfonate; (3) 2-amino-1,5-naphthalene-disulfonate; (4) benzene-1,4-disulfonate; (5) toluene-4-sulfonate; (6) naphthalene-2-sulfonate; (7) diphenylamine-4-sulfonate. Conditions are given in Fig. 4.

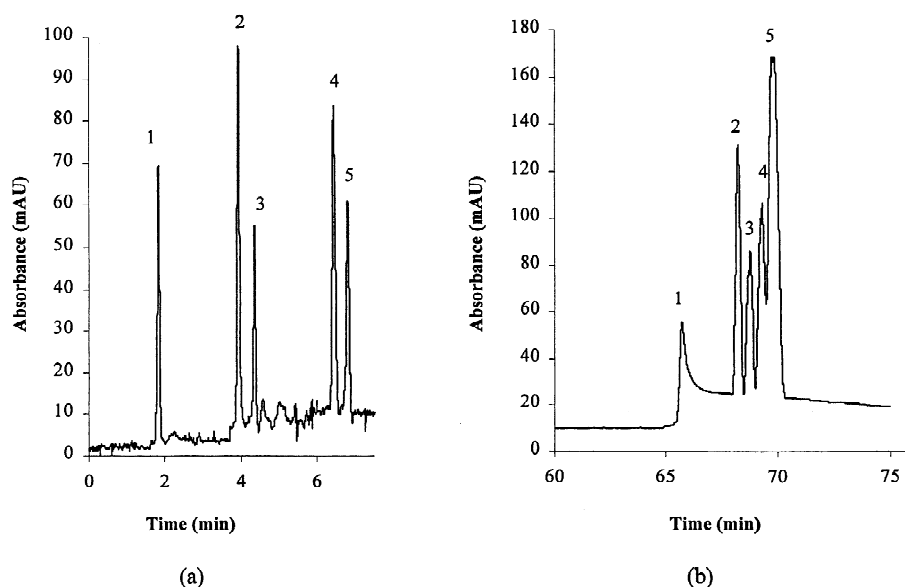


Fig. 6. CEC separation of a mixture of alkylbenzenes using 20% (a) and 10% (b) polymer A loaded silica. Peak identities in (a): (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene. Conditions: 29.7 cm (20.4 cm effective length) column, 75 mm I.D. fused-silica capillary, run at -15 kV, 80% acetonitrile in 10 mM Tris-HCl buffer (pH 7.8) as background electrolyte, UV detection at 214 nm.

octadecyl-silica (ODS) stationary phases which are used in reversed-phase liquid chromatography. For comparison, two columns of the same diameter and length were packed with 10% and 20% (w/w) polymer A-coated silica particles. The 20% polymer A-coated column generated a high EOF and was capable of providing good resolution for the alkylbenzenes (symmetrical peak shapes in 7 min, Fig. 6). In contrast, the alkylbenzenes took 60 min to elute on the 10% polymer A loaded column and then they eluted with peak overlap (Fig. 6). We assume the slow elution is due to the low density of charge sites.

The symmetrical peak shapes of the alkylbenzenes as well as the high separation efficiency (110,000 to 260,000 plates/m) suggest that the $\text{Co}(\text{TACN})_2^{3+}$ moieties on the particles do not detrimentally affect

the separation of these compounds. Since the analytes are neutral, the separation was not due to differences in electrophoretic mobilities. The elution order is, as predicted, based on their carbon numbers or their hydrophobicities [46]. Therefore, the polymer A-coated stationary phase exhibited reversed-phase chromatographic behavior due to the cationic nature and octyl chains on the $\text{Co}(\text{TACN})_2^{3+}$ complexes.

The retention factors, k , for the alkylbenzenes on the polymer A-coated stationary phase are listed in Table 2 and are compared to values obtained from ODS stationary phases [47]. The ODS stationary phase was intentionally designed to have a relatively low octadecyl ligand density (ca. $2.1 \mu\text{mol}/\text{m}^2$) to allow a relatively high EOF velocity. The relatively

Table 2

Retention factors (k) and EOF values for ODS and polymer A-coated CEC columns. Experimental conditions are given in Fig. 6

Column	Retention factor k					EOF $\text{cm}^2/\text{V s}$
	Benzene	Toluene	Ethylbenzene	Propylbenzene	Butylbenzene	
ODS	0.71	1.00	1.36	1.96	2.80	1.15×10^{-4}
Polymer A coated	1.16	3.64	4.14	6.61	7.04	-8.20×10^{-4}

low ligand density corresponds to reacting 25% of the silanol groups to form bonded octadecyl ligands while leaving 75% of the silanols intact. The ODS column was packed at 4000 p.s.i., as was the polymer A column, and similar mobile phase systems were used for both columns. The data in Table 2 suggest that the polymer A-coated silica column had much stronger retention (1.63 to 3.64 times) for alkylbenzenes than the ODS column. This is surprising because the surface of the polymer A-coated silica had a permanently charged sublayer, which should lower the overall hydrophobicity of the column and decrease the retention.

The stronger retention of alkylbenzenes on the polymer A column can be attributed to its higher hydrophobicity as a result of having a higher density of octyl groups than the density of octadecyl groups in ODS. In addition, the EOF value of the polymer A-coated column is 7.1 times greater than the ODS

column. This high velocity explains in part the short analysis time of 7 min for the alkylbenzene homologs on the polymer A column as opposed to 20 min on the ODS column. Thus, polymer A-coated silica has advantages over conventional ODS particles in generating stronger EOF and providing sufficient selectivity for analytes.

Due to peak tailing, basic compounds are difficult to separate efficiently by CEC. Polymer A-coated CEC columns performed well in separating 10 basic compounds (Fig. 7). At the pH (4.5) of the buffer solution, all of the compounds were cationic and, hence, moved efficiently through the column. They were mainly separated on the basis of reversed-phase partitioning. The theoretical plate numbers for the peaks ranged between 120,000 and 210,000 plates/m. The high efficiency obtained by the polymer A packed column is comparable to that obtained in CE [48].

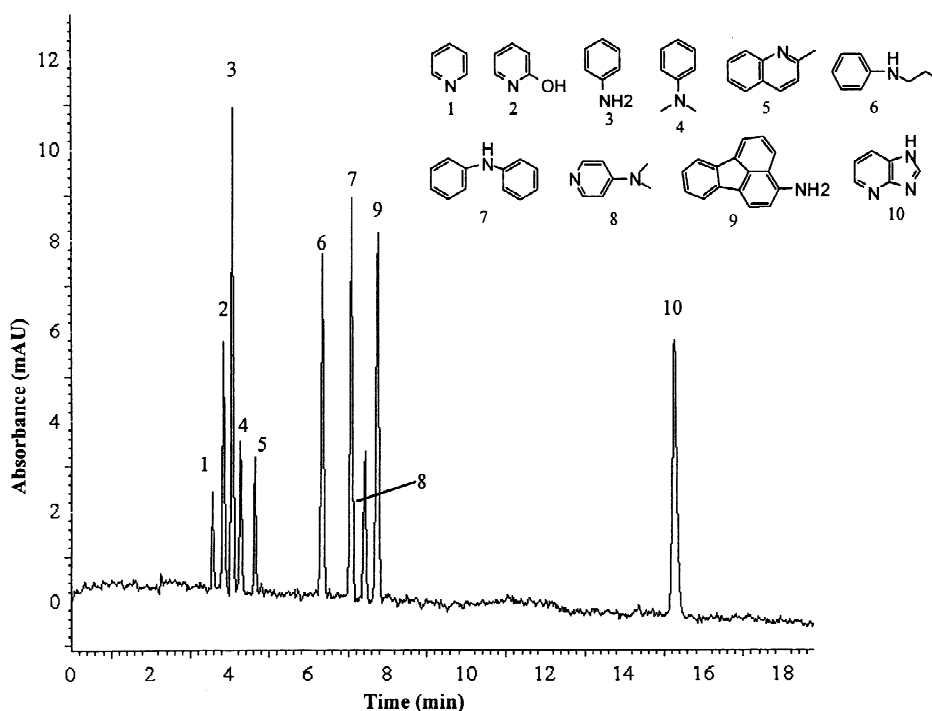


Fig. 7. CEC separation of basic compounds using 20% polymer A loaded silica. Peak identities: (1) pyridine; (2) 2-hydroxypyridine; (3) aniline; (4) *N,N*-dimethylbenzeneamine; (5) quinaldine; (6) *N*-propylaniline; (7) diphenylamine; (8) 4-dimethylaminopyridine; (9) 3-aminofluorene; (10) 4-azabenzimidazole. Conditions: 41.2 cm (30.5 cm packed length), 75 μ m I.D. column; 3.5 μ m silica packing (20% polymer loading); 80% (v/v) acetonitrile in 10 mM Tris buffer (pH 4.5) mobile phase; -15.1 kV applied voltage; UV detection at 215 nm.

4. Conclusions

New organic–inorganic polymer bonded materials have been prepared for use as coatings in CE or as stationary phases in CEC to overcome pH-dependent EOF separations. The cationic polymers were strongly bonded to silica via covalent bonding and cross-linking. The positively charged $\text{Co}(\text{TACN})_2^{3+}$ moieties of the polymers provided strong anodic pH-independent EOF. The incorporation of a deactivation reagent into the coating process proved to be important to generate enhanced reversed EOF. Due to the stable structure of the $\text{Co}(\text{TACN})_2^{3+}$ complex, the lifetimes of these polymer-coated silica materials were longer than those of conventional coatings. Fast, reproducible separations of acids were achieved using polymer A-coated capillaries. The separation of alkylbenzenes and basic compounds with polymer A-coated packed columns demonstrated fast and selective CEC performance.

The novel polymer phases described herein represent a fruitful intersection of materials research and separation science. Research in this direction should lead to the design of new coatings and in turn extend the scope of the highly promising CEC technique.

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