Metal complex-substituted polysiloxanes as novel coatings for capillary electrophoresis and capillary electrochromatography

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Abstract

Two novel polysiloxane-based polymers, which contain metal complexes, have been prepared. To prepare the $Co(TACN)_2^{3+}$ (TACN=1,4,7-triazacyclononane) based polymers, an orthoamide derivative of TACN was added to bromobutane-substituted methylpolysiloxane and hydrolyzed with base. Co(II) was then coordinated to the TACN, followed by cobalt oxidation to make polymer **A** or followed by *N*-octyl TACN coordination and cobalt oxidation to make polymer **B**. In both materials, TACN forms thermodynamically and kinetically stable $Co(TACN)_2^{3+}$ complexes in which the six coordination sites of the Co(III) are occupied by nitrogens from the TACN. The polymers were coated on fused-silica capillary columns and spherical silica particles, which were used for capillary electrophoresis and capillary electrochromatography, respectively. The open and packed columns showed strong and pH-independent reversed electroosmotic flow.   © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Capillary electrophoresis (CE) and capillary electrochromatography (CEC) are used to detect and separate compounds. The performance of CE and CEC is often limited by the negatively charged SiO^- groups on the silica surface, which lead to zone broadening and tailing as well as pH dependent electroosmotic flow (EOF). To overcome zone broadening and pH dependent EOF, new silica coatings are being pursued.

Two types of coatings, nonionic and ionic polymers, have been used in CE. For nonionic coatings, covalently bonded or cross-linked polymers are used to end-cap or cover SiO^- groups and form a neutral polymer layer on the capillary wall. Although these coatings can effectively decrease adsorption of analytes, suppressed EOF may not be desired, e.g., in fast separations. In contrast, ionic coatings employ cationic groups (quaternary amines) or strong acidic groups (sulfonic acids) as charged groups on the capillary wall to generate EOF [1]. Since these functional groups cannot be neutralized easily in solution, their concentrations do not change with variation in pH. This affords stable EOF over a relatively wide pH range. However, these newly added charged groups still attract analytes, which results in adsorption. This adsorption behavior can compromise separation efficiency and reproducibility.

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CEC has recently been developed to improve the selectivity and resolution of CE [2], by allowing differential interactions of analytes between mobile and stationary phases. The plug-like flow profile generated in CEC is largely responsible for enhanced separation efficiency. The hybrid nature of CEC provides a unique selectivity that cannot be achieved by either high-performance liquid chromatography (HPLC) or CE alone. However, due to the lack of stationary phases that are suitable for CEC, this technique has not been applied to the separation of a wide range of compounds.

To date, only two types of stationary phases have been used in CEC. Among the most common are commercially available standard HPLC stationary phases [3,4], including silica-based ODS, ion-exchange, and chiral packing materials. These stationary phases are often not ''end capped'' and the exposed SiOH groups on the surface are used to generate EOF and drive the flow of mobile phase and analytes. ''Mixed-mode'' packing materials [5], having both alkyl chains and strong ion-exchange groups ($-SO_3^-$, $-NR_4^+$), have also found application in CEC. The ionic groups carry negative or positive charge over a wide pH range. However, like CE coatings, the issues of pH dependent EOF and analyte adsorption still exist. General methods to deactivate the active charge sites, such as end-capping and polymer coverage, are not desirable because charge sites are required to generate EOF. Herein, we report a novel approach to ''deactivating'' the silanol charged 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 coating in CE and as stationary phase in CEC. Instead of utilizing conventional charged groups, such as SiO^-, $-SO_3^-$ and $-NR_4^+$, we have employed a metal ion (Co^{3+}) coordinated to organic ligands to provide positive charge for the generation of EOF. The novel coatings reported here are based on a polysiloxane backbone, which has been substituted with $Co(TACN)_2^{3+}$ complexes. Polysiloxane phases are known to possess good diffusion properties [6]. Diffusion of solutes in the stationary phase is necessary for optimal performance. The two synthesized polysiloxane-based materials contain small amounts of octyl substituents, which enable them to be cross-linked and immobilized onto a silica surface. This is one of the first examples in which a transition metal ion has been used in a CE coating and CEC stationary phase [7].

The metal–organic ligand complex is designed to remain chemically stable. Positively charged Co^{3+} sites are inaccessible to analytes but remain electrically-accessible to generate EOF, which drives the analytes to the detector. The organic part of the complex, however, is accessible to analytes under CEC conditions and provides separation selectivity. We have chosen to use the $Co(TACN)_2^{3+}$ (TACN= 1,4,7-triazacyclononane) complex because of its kinetic inertness and high charge. Saturated polyazamacrocycles, such as TACN, have long been known for their remarkable binding ability towards transition metal cations [7–10]. The increased stability of TACN complexes is associated with the favorable entropy effects arising from the endodentate conformation of TACN, in which all coordinating atoms are directed towards the center of the macrocycle, minimizing rearrangement upon coordination. When coordinated to the six nitrogens of two TACN molecules, Co(III) is low spin and forms strong metal ligand bonds that are kinetically inert. The two TACN ligands bound to cobalt, enclose the cobalt and do not allow it to coordinate to other molecules or participate in chemical adsorption.

2. Experimental

2.1. Materials and reagents

Reagents and analytical-grade materials were obtained from commercial suppliers and used without further purification except as otherwise noted. Tetrahydrofuran (THF) was distilled from Na/benzophenone, and dichloromethane was dried over CaH_2 prior to use. Dimethylformamide was dried over 4 Å molecular sieves. Deionized water (18.2 Ω) was obtained from a Milli-Q water system (Millipore, Milford, MA, USA). **Caution!** *Although no problems were encountered in this work, perchlorate salts of transition metal complexes are potentially explosive and should therefore be prepared in small quantities and handled with care.*

2.2. Physical measurements

Nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature with a Unity Inova 500 MHz spectrometer (Varian, Palo Alto, CA, USA) using a 5 mm broadband switchable probe operating at 499.919, 125.716, and 99.319 MHz for ^{1}H, ^{13}C, and ^{29}Si NMR spectroscopy, respectively. All chemical shifts are reported relative to tetramethylsilane. For ^{13}C NMR, proton composite pulse decoupling was applied during acquisition. Typical parameters for ^{29}Si NMR spectroscopy included a spectral width of 40 kHz, 104k data points, 1.3 s acquisition time, 22 kHz filter band, 4.0 s d1 delay and 10k scans to obtain sufficient signal-to-noise ratio. Proton composite pulse decoupling was not applied for ^{29}Si NMR. UV absorption spectra were recorded using an HP 8452 UV–Vis spectrophotometer operating with a HP UV–Vis Chemstation. Metal ion analysis was performed using an Optima 2000 DV inductively-coupled plasma optical emission spectrometry (ICP-OES) system (Perkin-Elmer, Norwalk, CT, USA). Electron impact fast atom bombardment (EI/FAB) mass spectra were obtained using a JEOL JMS-SX 102A instrument operating at <20 eV electrospray ionization voltage. Elemental analyses were conducted by M-H-W Labs. (Phoenix, AZ, USA).

2.3. Synthesis of TACN (**2**)

TACN was synthesized by an improved Richman–Atkins method [11]. A solution of 1,4,7-tri(*p*-toluenesulfonyl)-1,4,7-triazacyclononane (**1**) [12] (25 g, 0.042 mol) in concentrated H_2SO_4 (70 ml) was slowly (30 min) heated to 180 °C while being stirred. The resulting dark brown solution was heated at 180 °C for 1 h and cooled to below 80 °C upon standing. The solution was then vigorous stirred and cooled in an ice bath, and ether (200 ml) was slowly added to it. The organic layer was decanted and the black precipitate was washed thoroughly with ether (3×100 ml) and dried under vacuum. Distilled water (25 ml) was added to the black precipitate and the resulting suspension was heated at reflux for 30 min. The hot solution was filtered through a 0.45-μm membrane filter disc. Concentrated HCl (25 ml) was added drop-wise, to the clear hot filtrate and the

volume of the resulting solution was reduced to 15 ml before it was stored in a refrigerator for 24 h. The gray crystals were filtered, washed with cold concentrated HCl (10 ml), ethanol (3×20 ml), ether (3×20 ml) and dried to afford a light gray solid (9 g, 0.038 mol). To the gray solid was added anhydrous benzene (400 ml), crushed NaOH (8.72 g, 0.218 mol) and crushed glass. The suspension was heated at 40 °C for 3 days and filtered through a celite pad. The leftover white solid was washed with benzene (150 ml). The combined filtrates were dried with powdered anhydrous sodium sulfate for 3 h. After removing the sodium sulfate by filtration, the solvent was removed under vacuum to yield a pale yellow viscous oil (4.01 g, 74%), with NMR signals identical to those in the literature [13][1].

2.4. Synthesis of 1,4,7-triazatricyclo[5.2.1.0^{4,10}]decane (**3**)

TACN (4.97 g, 0.038 mol) was warmed to 67 °C (without solvent). Dimethylaminoformaldehyde dimethyl acetal (4.58 g, 0.042 mol) was added in three portions with vigorous stirring while the temperature was held between 67 and 72 °C. The reaction mixture was heated at 73 °C for 18 h after which the low-boiling reagents were evaporated at 73 °C. The remaining yellow residue was distilled to give the TACN orthoamide derivative as a colorless oil (5.35 g, 84%). Its boiling point (160 °C) and NMR chemical shifts are identical to those in the literature [14].

2.5. Synthesis of N-8-octane-1,4,7 triazacyclo[5.2.1.0^{4,10}]decane bromide (**4**)

To a solution of **3** (0.408 g, 2.89 mmol) in dry THF (8 ml) was added 1-bromooctane (0.571 g, 2.96 mmol). The mixture was stirred in the dark at room temperature for 7 days. The solvent was removed under reduced pressure and the white precipitate was washed with ether (25 ml). The solid was dried under vacuum overnight to give a white solid (0.6018 g, 63% yield). ^{1}H NMR (D$_2$O, 500 MHz): δ 5.51 [s, 1H, HC-(N)$_3$], 2.82–3.83 (m, 14H, CH$_2$–N), 1.37–1.28 (m, 12H, CH$_2$), 0.861(t, 3H, CH$_3$).

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[1]This is a modification of the literature preparation.

$C^{13}\{^1H\}$ NMR (D$_2$O, 125 MHz): 116.12, 60.582, 58.07, 53.06, 51.13, 31.10, 28.29, 28.27, 25.82, 24.01, 22.13,13.53; high-resolution electron impact-mass spectrometry (HREI-MS) m/z: (M$^+$) 252.2443 (90.9%); calculated 252.2518.

2.6. Synthesis of N-8-octane-1,4,7-triazacyclononane (**5**)

Compound **4** (600 mg, 1.81 mmol) was dissolved in water (9 ml) and the resulting mixture was refluxed for 9 h. NaOH (140 mg, 3.5 mmol) was added to the solution, and reflux was continued for another 14 h. Toluene (30 ml) was added, water was azeotropically removed, and additional NaOH (0.1 g) was added toward the end of water removal. The toluene solution was filtered and toluene was removed under reduced pressure to give a yellow oil, (430 mg, 98%). ^{1}H NMR (CDCl$_3$, 500 MHz): δ 3.18–2.2 (m, 14H, CH$_2$–N), 1.01 (m, 12H, CH$_2$), 0.62 (t, 3H, CH$_3$). $C^{13}\{^1H\}$ NMR (CDCl$_3$, 125 MHz): δ 58.09, 57.47, 53.27, 49.23, 31.85, 29.54, 29.33 28.03, 27.42, 22.64, 14.14; high-resolution (HR)FAB-MS (thioglycerol+H$^+$ matrix) m/z: 242.2631 (90%), calculated 242.252.

2.7. Synthesis of poly(oxytrimethyldisiloxanediyl) (**6**)

A polysiloxane polymer with molecular mass of about 8000, was synthesized in a similar manner to that reported in the literature [15,16]. Octamethylcyclotetrasiloxane (2.96 g, 10.0 mmol) (Aldrich), 1,3,5,7-tetramethylcyclotetrasiloxane (2.40 g, 1.0 mmol) (UCT), CF$_3$SO$_3$H (25 µl, 9.8·10^{-5} mmol) (Aldrich), and 1,3-di-n-octyltetramethyldisiloxane (0.24 g, 0.67 mmol) (Gelest) were combined and stirred for 24 h at room temperature. Hexamethyldisilazane (16 µl, 9.8·10^{-5} mmol) (UCT) was added to the white slurry to neutralize the CF$_3$SO$_3$H, and the reaction mixture was washed with water (3×20 ml). The oil was dissolved in CH$_2$Cl$_2$ (25 ml) and washed with methanol (25 ml) and water (5 ml). The CH$_2$Cl$_2$ was evaporated under vacuum to give a colorless poly(oxytrimethyldisiloxanediyl) oil (3.8 g,71%). ^{1}H NMR (CDCl$_3$, 500 MHz): δ 4.71 (t, J=8.79 Hz, 1 H, Si–H), 0.21 (s, 10.3 H, Si–CH$_3$). $C^{13}\{^1H\}$ NMR (CDCl$_3$, 125 MHz): δ 33.7, 32.18,

29.6, 29.5, 23.44, 23.36 18.50, 14.34, 1.47, 0.96, 0.28. Ratios of Si–CH$_3$ peaks (0.1 ppm) to Si–H peak (4.71 ppm) indicate approximately 45% hydrogen-substituted polysiloxane.

2.8. Synthesis of bromobutyl poly(oxytrimethyldisiloxanediyl) (**7**)

A 50 ml PTFE centrifuge tube was charged with toluene (25 ml), **6** [265 mg, 2 mmol –O–Si(CH$_3$)$_2$–O–Si(CH$_3$)(H)–O subunit], 4-bromo-1-butene (675 mg, 5 mmol) and 20 µl of platinum(0)-1,3-vinyl-1,1,3,3-tetramethyldisiloxane complex. The reaction mixture was heated to 130 °C for 24 h, cooled to room temperature, and its solvent evaporated under reduced pressure. The resulting brown oil was dissolved in CH$_2$Cl$_2$ (50 ml) and the solution was filtered through a celite pad. Water (3×40 ml) and methanol (3×40 ml) were used to wash the CH$_2$Cl$_2$ solution and the CH$_2$Cl$_2$ was evaporated to give a light gray viscous material (503 mg, 98% yield/subunit). ^{1}H NMR (CDCl$_3$, 500 MHz): δ 3.41 (m, 2 H, CH$_2$–Br), 1.87 (m, 2 H, BrCH$_2$–CH_2–CH$_2$), 1.50 (m, 2 H, CH$_2$–CH_2–CH$_2$Si), 0.528 (m, 2 H, CH$_2$–CH_2–Si), 0.08 (s, 10.3 H, CH$_3$–Si). $C^{13}\{^1H\}$ NMR (CDCl$_3$, 125 MHz): δ 36.03, 33.58, 21.73, 16.61, 1.27, 0.02. ^{1}H NMR shows no remaining peak at 4.37 ppm for Si–H. Bromobutyl is estimated to be 45% substituted in the polysiloxane based on the ratio of Si–CH$_3$ peaks (0.08 ppm) to –CH$_2$– peaks of bromobutane (1.87 ppm).

2.9. Synthesis of 37% N-4-but-1-ane-1,4,7-triazacyclo[5.2.1.0^{4,10}]decane bromide substituted polysiloxane (**8**)

Compound **7** [206 mg, 0.801 mmol –O–Si(CH$_3$)$_2$–O–Si(CH$_3$)(H)–O subunit] was added to **3** (112 mg, 0.81 mmol) in dry THF (9 ml). The mixture was stirred at room temperature under N$_2$ with the exclusion of light. After 3 days, THF was evaporated under reduced pressure and the residue was washed with diethyl ether (3×25 ml). A yellow viscous material (251 mg, 82% yield/subunit) was obtained after drying [17][2]. ^{1}H NMR [(CD$_3$)$_2$CO/D$_2$O, 500 MHz ppm]: δ 5.47 [s, 1 H, CH–(N)$_3$],

[2]Higher yields can be obtained on leaving the THF solution in the dark for an extended period of time.

3.54 (m, 0.35 H, CH_2–Br), 3.71–3.23 (m, 12.35 H, CH_2–N), 1.84 (m, 2 H, NCH_2–CH_2–CH_2), 1.48 (m, 2 H, CH_2–CH_2–CH_2Si), 0.60 (m, 2 H, CH_2–Si), 0.02 (s, 10.6 H, CH_3–Si). $C^{13}\{^1H\}$ NMR (D_2O, 125 MHz): δ 117.95, 60.05, 58.48, 53.93, 51.48, 35.93, 27.82, 21.69, 20.14, 1.2. The ^1H NMR ratio of CH_3–Si peaks (0.02 ppm) to capping TACN methylene peak (5.47 ppm), indicates 37% *N*-4-but-1-ane-1,4,7-triazacyclo[5.2.1.0^{4,10}]decane substitution.

2.10. Synthesis of 37% N-4-but-1-ane-1,4,7-triazacyclononane-substituted polysiloxane (**9**)

Compound **8** [530 mg, 1.47 mmol, –O–Si(CH_3)$_2$–O–Si(CH_3)(H)–O subunit] was dissolved in a solution of water (25 ml), ethanol (15 ml) and THF (7 ml). The resulting mixture was refluxed at 90 °C for 24 h. NaOH (107.5 mg, 2.7 mmol) was added, and reflux was continued for an additional 48 h. The solution was cooled before toluene (150 ml) was added. Water was azeotropically reduced at 130 °C using a Dean–Stark moisture trap to 1–2 ml and more NaOH (120 mg) was added. After the water layer was fully removed, the suspension was filtered and the solvent was removed under vacuum to give a yellow oil (370 mg, 88% yield/subunit). ^1H NMR (CDCl$_3$, 500 Hz): δ 3.51 (m, 0.35 H, CH_2–Br), 2.70–2.44 (m, 12.35 H, CH_2–N), 1.43 (m, 2 H, NCH_2–CH_2–CH_2), 1.29 (m, 2 H, CH_2–CH_2–CH_2Si), 0.47 (m, 2 H, CH_2–Si), 0.02 (s, 10.6 H, CH_3–Si). $C^{13}\{^1H\}$ NMR (CDCl$_3$, 125 MHz): 57.70, 53.22, 52.03, 47.59, 31.48, 30.01, 21.05, 17.23.

2.11. Synthesis of 37% Co(TACN)$_2^{3+}$-substituted polysiloxane (polymer **A**)

The synthesis was performed under nitrogen. A solution of **9** (44 mg, 0.15 mmol subunit) in CH_2Cl_2 (4.2 ml) was degassed by the freeze–thaw method five times. This degassed solution was added drop-wise to the degassed solution of Co(ClO$_4$)$_2$·6H$_2$O (83 mg, 0.22 mmol). The mixture was stirred under nitrogen at room temperature for 2 h, and it turned purple. A solution of **5** (57 mg, 0.24 mmol) in CH_3CN (3.5 ml) was also degassed and added to the purple colored reaction mixture. Stirring was continued under nitrogen at room temperature for an additional 6 h, after which the solution was exposed

to oxygen for 5 h to cause Co^{2+} oxidation. The resulting dark red solution was concentrated under vacuum to give a red solid. After being washed with CH_2Cl_2 (3×10 ml), the red solid (70 mg, 82% yield/subunit) was dried overnight under vacuum. ^1H NMR (CD$_3$CN, 500 MHz): δ 3.54–2.92 (br, 26.3 H, CH_2–N), 1.36 (br, 15.6 H, CH_2), 0.95 (br, 2.8 H, CH_3–CH_2), 0.94 (br, 2 H, CH_2–Si), 0.1 (s, 11.3 H, CH_3–Si); UV–Vis [ϵ/Co(III), 1 mol^{-1}cm^{-1}]: 490 nm (100), 343 nm (80).

2.12. Synthesis of bis(N-4-but-1-ane-1,4,7-triazacyclononane) Co(III) polysiloxane (polymer **B**)

To a degassed solution of **9** (54 mg, 0.18 mmol) in CH_2Cl_2 (3.5 ml) was added a degassed CH_3CN (2.5 ml) solution of Co(ClO$_4$)$_2$·6H$_2$O (32 mg, 0.087 mmol). The reaction mixture was stirred for 2 h at 50 °C under nitrogen. The mixture was cooled and exposed to an oxygen atmosphere for 5 h. The solvent was removed under reduced pressure and the orange–red residue was washed with CH_2Cl_2 (10 ml) and dried under vacuum to yield an orange–red solid (70 mg). ^1H NMR (CD$_3$CN, 500 MHz): δ 3.57–2.79 (m, 11.7 H, CH_2–N), 1.84 (m, 2 H, NCH_2–CH_2–CH_2), 1.53 (m, 2 H, CH_2–CH_2–CH_2), 0.73 (m, 2 H, CH_2–Si), 0.20 (s, 10.3 H, CH_3–Si); UV–Vis [ϵ/Co(III), CH_3CN, 1 mol^{-1}cm^{-1}]: 500 nm (100), 341 nm (80).

2.13. Coating of polymers on capillary columns

Fused-silica capillary tubing (75 μm I.D.×365 μm O.D.) (Polymicro Technologies, Phoenix, AZ, USA) was coated using the dynamic coating procedure. The coating solution was an acetonitrile solution containing polymers **A** or **B** (5%, w/v) and dicumyl peroxide (0.75%, w/v). Trimethylsilyl trifluoromethanesulfonate was also used to deactivate the residual silanol groups on the fused-silica capillary wall. The film thickness of the coated columns was determined by a procedure used in gas chromatography [18]. The concentration of the coating solution and coating speed were selected such that the coated stationary phase had a film thickness of 0.022 μm.

2.14. Coating and crosslinking of polymers on silica particles

A previously reported reaction vessel was used for coating and crosslinking [19]. Dicumyl peroxide was used as free radical initiator for polymer crosslinking. The coating process was carried out in a chromatographic oven. The residual silanol groups on the bare silica were deactivated with trimethylsilyl trifluoromethanesulfonate.

2.15. Packing of capillary columns

The CEC columns were packed and handled as previously described [20]. Fused-silica tubing (75 μm I.D.×365 μm O.D.) was first coated with polymer **A** and then it was packed with silica particles that were also coated with polymer **A**. The coated columns were packed using liquid carbon dioxide with a Lee Scientific Model 600 SFC/GC pump. The packing pressure increased from 60 to 300 atm at 5 atm/min and was maintained at 300 atm for 30 min (1 atm=101325 Pa).

2.16. Capillary electrophoresis and capillary electrochromatography

The instrument used in this work was laboratory-built and has been reported previously [21]. It consists of a Model SL60PN30 high-voltage power supply (Spellman, Haupauge, NY, USA), a scanning UV detector, Model UV3000 (Thermo Separations, Sunol, CA, USA) with a fiber optic assembly for remote detection, a 12-bit Model ADIO 1600 A/D card (Industrial Computer Source, San Diego, CA, USA), two laboratory-built holder assemblies, two solenoid valves (Clippard Instrument Lab., Cincin-

nati, OH, USA), and laboratory-made control circuits. Both CE coated and CEC packed columns were conditioned with 10 mM Tris buffer and mobile phase before use. A 20% (v/v) acetonitrile 10 mM Tris buffer solution was used as the mobile phase. Thiourea and acetone were used as EOF markers.

3. Results and discussion

3.1. Ligand synthesis

A key component of the new polymeric coating is the metal complex. To synthesize the metal complex, we started by making the tridentate ligand 1,4,7-triazacyclononane (TACN, **2**). With its three nitrogens able to coordinate in a facial manner to metal ions, it forms very stable coordination complexes. When two TACN molecules coordinate to a single metal ion, the metal is tightly held and sandwiched between two ligands. To synthesize TACN, we found that good yields were achieved by heating the tosylated derivative of TACN (**1**) in concentrated sulfuric acid at 180 °C for 1 h. Concentrated hydrochloric acid was then added to the oil to obtain pure crystalline TACN·3HCl. A sodium hydroxide–toluene suspension was used to neutralize the acid and extract TACN into toluene. The procedure afforded high yields of TACN (58 to 80%) (Fig. 1).

The TACN was then reacted with neat $(MeO)_2CHNMe_2$ to form the orthoamide derivative 1,4,7-triazatricyclo[5.2.1.0^{4,10}]decane (**3**) [14], which is an excellent reagent to react with haloalkanes to make mono-N-substituted TACN compounds (Fig. 1). The 1,4,7-triazatricyclo[5.2.1.0^{4,10}]decane was then added to bromooctane in THF to give a mono-N-alkylated amidinium salt (**4**). Finally, base hy-
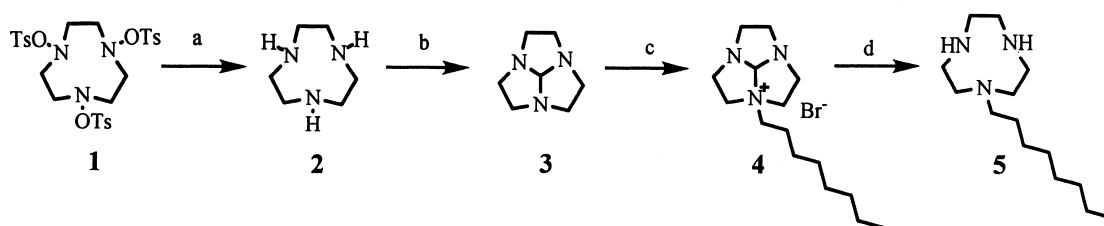

Fig. 1. Synthetic scheme of octyl substituted TACN. Conditions: (a) (i) conc. H_2SO_4, 180 °C, 1 h, (ii) water, conc. HCl, 60 °C, (iii) NaOH; (b) dimethylaminoformaldehyde dimethyl acetal, neat, 70 °C; (c) 1-bromooctane, dry THF, room temperature; (d) NaOH, water, reflux.

drolysis yielded the mono-*N*-substituted TACN derivative, *N*-octyl-1,4,7-triazacyclononane (**5**).

3.2. Polysiloxane

Along with synthesizing ligands for the metal complexes, we synthesized the polymer material to be used as column coating. Stationary phases in gas chromatography are predominantly polysiloxanes due to their stability at high temperature, flexibility at low temperature and resistance to oxygen degradation. We thus chose to use a polysiloxane-based material for the coating material. To synthesize the polysiloxane we used a literature procedure that combined octamethylcyclotetrasiloxane, 1,3,5,7-tetramethylcyclotetrasiloxane, and 1,3-di-*n*-octyltetramethyldisiloxane [16]. Although line broadening in the proton NMR spectrum made characterizing the polymer's microstructure difficult, we were able to resolve different resonances by increasing the temperature and lowering the polymer concentration. The ^{1}H NMR spectrum shows two peaks one at 4.71 ppm (H–Si) and the other at 0.21 ppm (CH$_3$–Si). These are in a ratio of 1 to 10.3 (instead of a 1 to 9) and thus show a 45% Si–H incorporation in the polysiloxane. Heterogeneous broadening was less of a problem in the ^{13}C and ^{29}Si NMR spectra.

There are two main subunits, –O–Si(CH$_3$)$_2$–O– and –O–Si(H)(CH$_3$)–O–, that make up most of the polysiloxane. Each subunit can be arranged next to a subunit like itself or next to another subunit. Silicon NMR is a powerful tool for monitoring the placement of the two subunits and in characterizing the reactions at the H–Si bond. The ^{29}Si NMR spectrum of the synthesized polysiloxane consists of peaks in two regions. It has three peaks at −18.83, −20.21, and −21.57 ppm corresponding to –O–Si(CH$_3$)$_2$–O– subunits and five peaks at −33.85, −34.95, −36.10, −37.38, and −38.44 corresponding to –O–Si(H)(CH$_3$)–O– subunits. Due to neighboring silicon groups, the silicons in the –O–Si(CH$_3$)$_2$–O– subunits have three chemical shifts. These come from a subunit being surrounded by two –O–Si(H)(CH$_3$)–O–, a –O–Si(H)(CH$_3$)–O– and a –O–Si(CH$_3$)$_2$–O–, or two –O–Si(CH$_3$)$_2$–O–. Integration of the three peaks (1:2:1) shows a statistical population of the three environments. The silicons in the –O–Si(H)(CH$_3$)–O– subunits also have three

chemical shifts due to the same three arrangements of groups, however, it has added complexity arising from Si–H coupling ($^1J_{SiH}$=220 Hz). The Si–H coupling results in each peak being split into a doublet, with peaks from two of the doublets overlapping in the middle peak at −36.10 ppm. From the complexity of the spectrum, we conclude that the polymer is not alternating the two subunits as has been proposed for other polysiloxanes [22].

The terminal dimethyloctylsiloxane groups in the polysiloxane, expected to have chemical shifts at around 10 ppm (^{29}Si NMR), were not detected. Therefore, the synthesized polymer has a relatively high average chain length and molecular mass, resulting in a low abundance of terminal groups. All of the ^{29}Si NMR chemical shifts for the synthesized polysiloxanes were negative, common to oxygen-bonded silicon. No signals at −66 or −79 ppm for –Si(–OR)$_3$ groups were detected in any of the polymers, suggesting that the synthesized polymers have chain-like structures [23]. Proton-coupled Si^{29} NMR spectra were recorded instead of broadband hydrogen decoupled spectra because nuclear Overhauser effects (NOEs) on hydrogen-decoupled spectra can reduce intensities of silicon resonances.

3.3. TACN-substituted polysiloxane

After synthesis of the polymer, we then attached the TACN ligand to it, in preparation for metal complex formation. Before attaching TACN, we bonded butyl bromide to the polysiloxane (**6**) by mixing it with 4-bromobutene in the presence of catalyst (Fig. 2). We found that with a 1:1 molar ratio of bromobutene to polymer subunit, less than 50% of the H–Si groups were substituted. However, when bromobutene and H–Si subunit were used in a 2.5:1 molar ratio, the substitution went to completion as shown by the disappearance of the H and Si peaks at 4.2 ppm (^{1}H NMR) and −33.85 to −38.44 ppm (^{29}Si NMR). There was also a new peak in the ^{29}Si NMR spectrum at −22.9 ppm, which we attribute to the new O–Si(CH$_3$)(C$_4$H$_8$Br)–O– subunit. The ^{29}Si signal due to the –O–Si(CH$_3$)$_2$–O– group shifted slightly to −21.8 ppm and became one peak due to all of the silicons in the polysiloxane being bound to two alkyl groups and in similar environments.
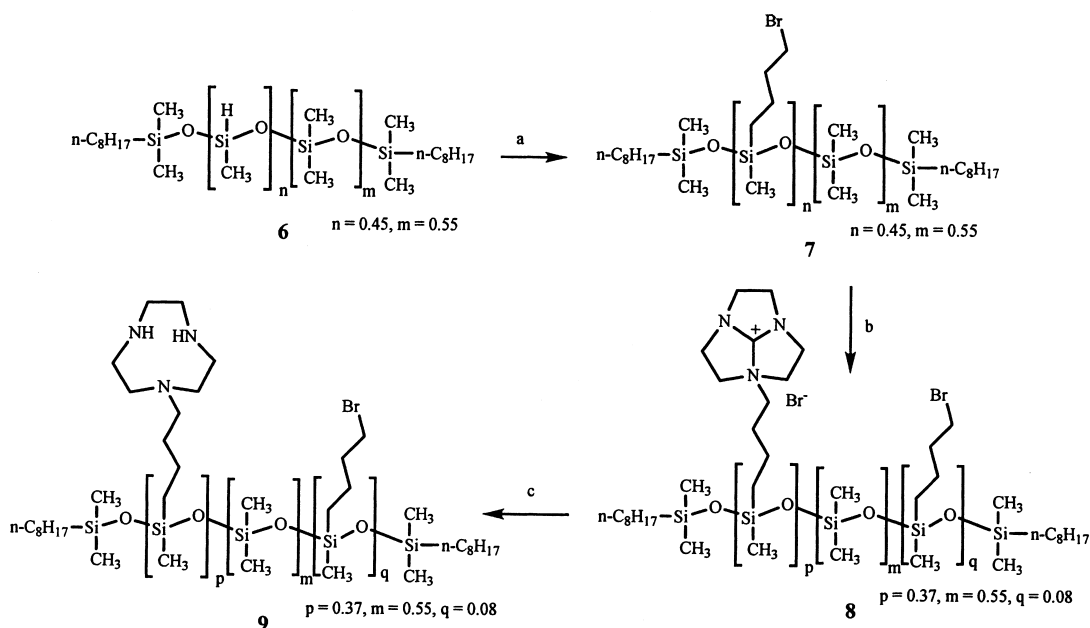
With bromobutyl attached to the polysiloxane,

Fig. 2. Synthesis of TACN substituted polysiloxane. Conditions: (a) 4-bromo-1-butene, platinum(0)-1,3-vinyl-1,1,3,3-tetramethyldisiloxane, toluene, reflux; (b) **3**, THF, room temperature; (c) NaOH, water–ethanol–THF, reflux.

TACN could then be bonded to the butyl chain. The attachment of 1,4,7-triazacyclo[5.2.1.0^{4,10}]decane (**3**), the tricyclic orthoamide derivative of TACN, to the bromobutyl substituted polymer (**7**) was done in dry THF for 1 week (Fig. 2). Removal of the THF yielded a light yellow viscous intermediate (**8**). Hydrolysis of this salt was performed by stirring it in a water–ethanol–THF (5:3:1, v/v) solution for 3 days. The water in the solution was removed azeotropically after adding toluene, and the TACN substituted polysiloxane (**9**) was obtained upon removing the toluene. Attachment of TACN can be monitored by ^{1}H NMR. The intensity of the ^{1}H signal at 3.41 ppm corresponding to $-CH_2Br$ decreases, while a complex set of multiplets for the hydrogens of $N-CH_2-CH_2-N$ from TACN appear at 2.44–2.70 ppm. In an attempt to increase the TACN substitution on the polymer chain, an excess of TACN was used during the substitution reaction. However, even when the amount of TACN was increased to five times that of the alkyl bromide in the polymer chain, some bromobutyl still persisted, probably due to TACN being sterically hindered from attaching. The residual peak at 3.5 ppm and the TACN protons

integrating to less than expected show that some of the bromoalkyl groups remain unattached to TACN. Just over 80% of the bromobutyl groups were accessible, resulting in a polysiloxane with TACN attached to 37% of its subunits. Attachment of the nitrogen rich TACN gives rise to two new ^{29}Si peaks at -19.5 and -20.3 ppm and the spectrum still has the -21.8 ($-O-Si(CH_3)_2-O-$) and -22.9 [$-O-Si(CH_3)(C_4H_8Br)-O-$] ppm peaks.

3.4. Metal complex substituted polysiloxane

With TACN attached to the polymer, we then put charged groups on the polysiloxane by coordinating metal ions to the TACN. We did this in two ways and made two materials. To prepare material **A**, we mixed the appropriate stoichiometric amounts of **9** and $Co(ClO_4)_2 \cdot 6H_2O$ in $CH_2Cl_2-CH_3CN$ (1:1, v/v), followed by addition of a CH_2Cl_2 solution of **5** (Fig. 3). After the reaction suspension was stirred for 6 h, it was exposed to oxygen for 5 h. Over the course of the reaction, the solution changed to deep red in color due to the bonding of Co^{2+} and oxidation of Co^{2+} to Co^{3+}. The Co^{3+} in the strong
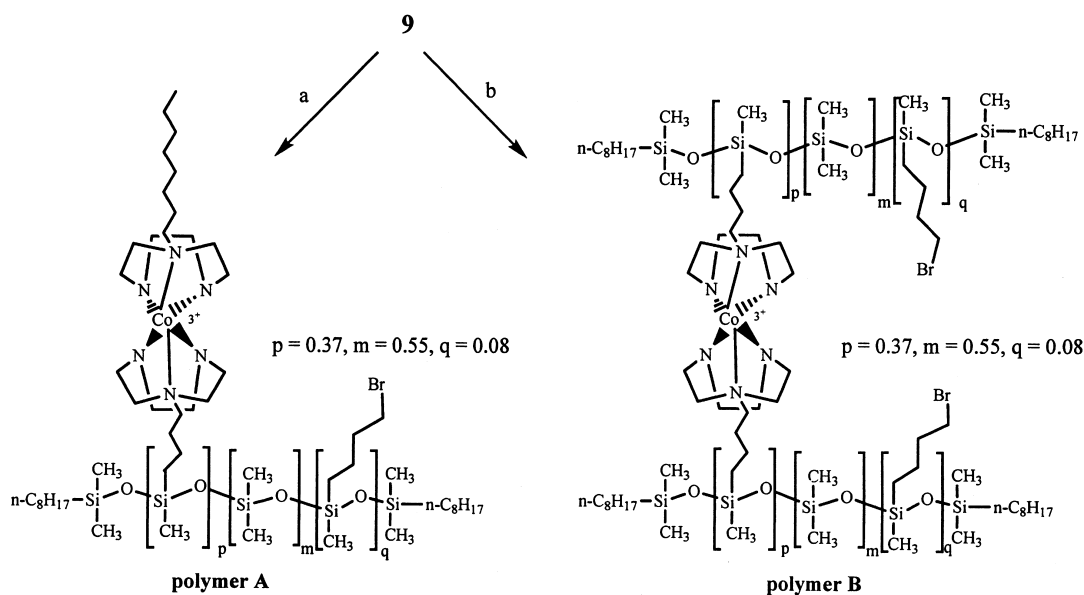
Fig. 3. Synthesis of polymers **A** and **B**. Conditions: (a) (i) **5**, Co(ClO$_4$)$_2$, CH$_2$Cl$_2$–CH$_3$CN, room temperature, (ii) O$_2$; (b) (i) Co(ClO$_4$)$_2$, CH$_2$Cl$_2$–CH$_3$CN, 50 °C, (ii) O$_2$.

field environment of the six nitrogens from the two TACNs is low spin and diamagnetic, therefore, NMR characterization of polymer **A** is possible. The ^{1}H NMR spectrum of polymer **A** has five sets of peaks: 0.1, 0.97. 1.36, 2.92, and 3.56 ppm. The polysiloxane ^{29}Si NMR resonances shift downfield upon cobalt coordination to -13.0, -14.2, and -16.0 ppm. We have tentatively assigned these peaks to the –O–Si(CH$_3$)$_2$–O–, –O–Si(CH$_3$)(C$_4$H$_8$Br)–O–, and –O–Si(CH$_3$)(C$_4$H$_8$TACN–Co)–O– groups, respectively.

Polymer **B** was synthesized in a similar manner to polymer **A**. TACN-substituted polysiloxane **9** in CH$_2$Cl$_2$ was added to Co(ClO$_4$)$_2$·6H$_2$O in CH$_3$CN, after which the reaction mixture was stirred for 2 h under nitrogen (Fig. 3). The solution was then exposed to oxygen for 5 h and the solvent removed. The orange–red polymer **B** was washed with CH$_2$Cl$_2$ and dried. Its ^{1}H and ^{29}Si NMR resonances were very similar to those of material **A** and in agreement with the structures of these diamagnetic compounds.

The UV–Vis absorption spectra of polymers **A** and **B** were as expected for compounds containing Co^{3+} with d^6 electronic configurations and in an octahedral N$_6$ environment. Both polymers containing Co(TACN)$_2^{3+}$ complexes show absorption maxima at

343 nm ($\epsilon = 80$) and 500 nm ($\epsilon = 100$), which absorptions arise from d–d transitions. These absorptions corresponded nicely to those of the simple [Co(TACN)$_2$]$^{3+}$ which has absorptions at 333 and 458 nm [8]. The Co(TACN)$_2^{3+}$ complexes attached to the polysiloxane have alkyl substituted TACN molecules, which could account for the red shift of the absorptions.

3.5. Chromatographic characterization of polymer coatings by CE and CEC

Preliminary investigation of the polymer coatings was conducted. Since the nature of the chemical group bound to the silica support surface, the degree of surface deactivation and the content of polymer coating determine the dependence of EOF on pH, EOF was used to measure the effectiveness of the polymer coatings and bonding procedures. The mobility of the EOF in both CE and CEC, μ_{eof}, is given by:

$$\mu_{eof} = \epsilon \zeta E / 4 \pi \eta \qquad (1)$$

where ϵ and η are the dielectric constant and

viscosity of the liquid, respectively, E is the electric field strength, and ζ is the potential across the diffuse double layer. Among these parameters, ϵ and η depend on properties of the mobile phase; ζ is the only factor that is affected by the nature of the silica support, and is proportional to the charge density on the surface of the capillary wall or stationary phase. In this study, the positively charged Co^{3+} contained in the polymers provided a positively charged surface on the silica support, which, in turn, counteracted the effect of the residual silanol groups residing on the bare silica support. From Eq. (1), it is anticipated that the magnitude and direction of EOF in the coated columns would be determined by the relative contributions of the positively charged Co^{3+} moieties anchored on the silica support and by the negative surface charge due to the non-capped residual silanol groups. The resultant EOF would be in the direction of the greater of these two opposing forces.

Capillary columns coated with polymers **A** and **B** have a strong EOF in the reverse direction. The EOF values were $-7.4 \cdot 10^{-4}$ and $-4.3 \cdot 10^{-4}$ cm^2/V s for polymer **A** and **B** coated capillaries, respectively. The migration time of thiourea in the polymer **B** coated capillary was approximately two times that in the polymer **A** coated capillary, suggesting that with the same mass concentrations of coating solution, the EOF generated in the polymer **A** coated capillary is two times stronger than the EOF in a capillary coated with polymer **B**. The cathodic EOF exhibited by the uncoated open column was found to be $6.7 \cdot 10^{-4}$ cm^2/V s with thiourea as an EOF marker in an 80% acetonitrile aqueous electrolyte with 10 mM Tris–HCl buffer at pH 7.8.

Once EOF was observed, its dependence on pH was studied over the pH range of 2–11[3]. The effect of pH on the EOF was studied using hydroorganic eluents composed of 10 mM Tris aqueous buffer–acetonitrile (20:80, v/v). The pH of the buffer was adjusted by mixing the 10 mM Tris acid or base solution with 0.1 M HCl or 0.1 M NaOH [24][4]. After each EOF measurement at a certain pH, the coated capillary was rinsed with acetonitrile, dried with helium, and then conditioned with running electrolyte for 20 min before the next measurement. Fig. 4 shows the effect of pH on EOF for coated and bare capillaries. The coated column generated a very stable reversed EOF over the pH range of 2 to 10, while the bare capillary showed a strong dependence on pH. As described by Eq. (1), under otherwise identical conditions, surface charge density determines the EOF. In the bare capillary, the surface charge density is controlled by the dissociation constant of the free hydroxyl groups on the silica surface. The charge density (σ) can be expressed as:
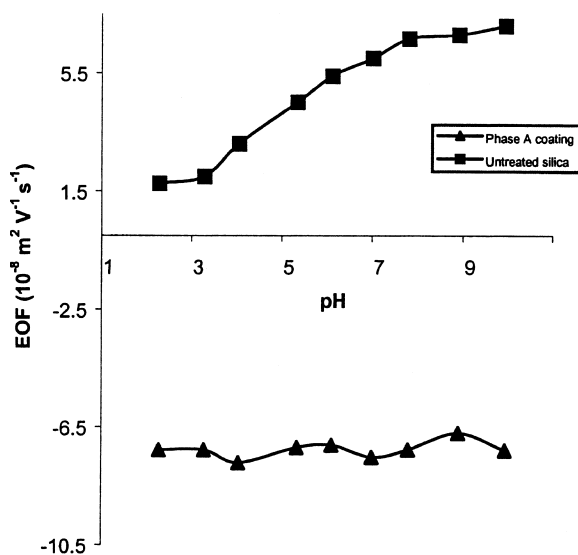

Fig. 4. Comparison of the EOF mobility at different pH values obtained on an untreated fused-silica capillary and polymer **A** coated silica support. Conditions: 2 kV for 2 s injection, -15.1 kV, thiourea as EOF marker, 80% (v/v) acetonitrile in 10 mM Tris buffer as running electrolyte.

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[3]Measurements were also carried out at pH 1 and 12; however, the higher electric current due to an increased transport of OH$^-$ and H$^+$ generated a higher amount of Joule heat, causing a decrease in viscosity. This resulted in very high experimental EOF values.

[4]Throughout the text, the stated electrolyte pH refers to the pH of the aqueous solution on which it is based, and not the ''apparent'' pH of the mixed solvent system, which was not measured due to the difficulty in measuring accurately an organo–aqueous solution. It is generally assumed that the incorporation of acetonitrile in the electrolyte has little effect on either pH or ionic strength.

$$\sigma = \gamma/(1 + [H^+]/K) \qquad (2)$$

where γ is the number density of surface SiOH groups, K is the dissociation constant $(4.2 \cdot 10^{-10})$ for surface hydroxyl groups on the fused-silica and $[H^+]$ is the bulk buffer hydrogen ion concentration. Since the pK_a of the surface hydroxyl group is large, its ionization varies dramatically at different pH values, leading to strong pH dependence of EOF.

For the coated column, due to the stability of the positively charged $Co(TACN)_2^{3+}$ complex, the charge on polymer **A** remains constant when pH is changed. Proton NMR spectra and UV–Vis absorption spectra of polymer **A** in CD_3CN–water (1:1, v/v) solution show no change even in highly acidic (pH 1) or basic solution (pH 11.5). The stability of the $Co(TACN)^{3+}$ complex, which leads to pH-independent EOF, can be attributed to the chelate effect of TACN and the strong ligand field created by the six nitrogens from the two TACNs. The EOF for the polymer **A** coated column at pH 2 to 10 is $-7.3 \cdot 10^{-4}$ cm^2/V s, which is even higher than the highest EOF value ($7.1 \cdot 10^{-4}$ cm^2/V s at pH 8.9) for the fused-silica capillary. For a coated column, this EOF is high [5]. The high and constant EOF value over a wide pH range should allow fast separations at both high and low pH values with reproducible retention times. However, further studies are needed to observe if resolution is reduced due to the strong EOF.

Polymer **A**, which contains octyl groups attached to the metal complexes that can interact with analytes, was used as a coating for CEC as well. We coated polymer **A** on the silica particles and on the inside surface of the fused-silica columns. Again, as with the CE columns, we observed reverse EOF with the CEC columns. Under the same experimental conditions, the EOF mobility in the packed columns was very high ($-8.2 \cdot 10^{-4}$ cm^2/V s), which is even higher than was found in CE. This is one of the highest EOF values reported for CEC [5b,25].

Along with production of EOF, polymer **A** coated CEC columns also separate compounds. Polymer **A** coated capillary columns were packed with polymer **A** coated silica particles and separation of benzene and alkylbenzenes was attempted. Fig. 5 shows a representative separation of benzene and alkylben-
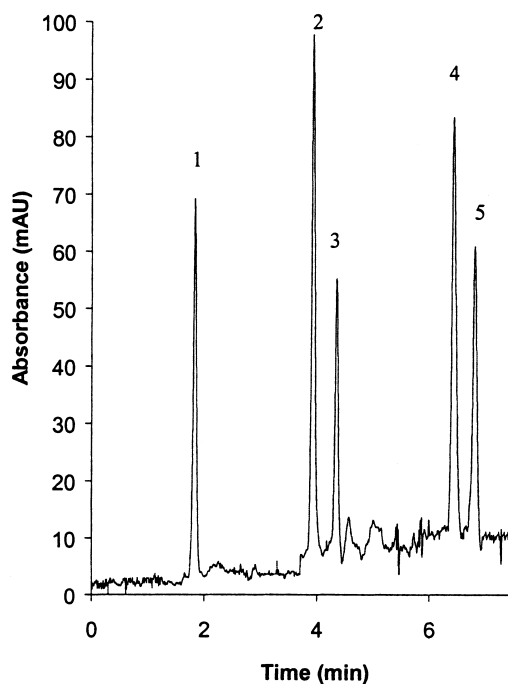

Fig. 5. CEC separation of a mixture of alkylbenzenes using a column packed with polymer **A** coated silica particles. Peaks: (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, (5) butylbenzene. Conditions: 29.7 cm (20.4 cm effective length)×75 μm I.D. column; 3 μm silica packing (20% polymer loading); mobile phase, 80% (v/v) acetonitrile in 10 m*M* Tris buffer, pH 7.8; −15.1 kV applied voltage; UV detection at 214 nm.

zenes obtained on the packed column using an 80% acetonitrile aqueous mobile phase with 10 m*M* Tris buffer at pH 7.8. Five compounds were baseline separated according to carbon number with symmetrical peak shapes in 7 min. The electrochromatogram shows that even though polymer **A** is highly positively charged, it facilitates separation of such compounds.

Another positive feature of the polymer coatings is their excellent long term stability. In 7 days continuous use, the EOF only decreased 7.5% for a polymer **A** capillary coating, 10% for a polymer **B** capillary coating, and 10% for polymer **A** coated silica particles. The life time of these material coatings was extremely long, approximately 2 months. Several factors contributed to the strong chemical stability of these coatings: the stability of the two polymers, strong interactions between the polymers and silica

supports, and small contamination from samples due to the shielding of the charge sites.

4. Conclusions

Successful synthesis of two novel polysiloxane coatings for CE and CEC separations with $Co(TACN)_2^{3+}$ complexes attached to them, was achieved. ^1H, ^{13}C, and ^{29}Si NMR spectroscopy provided structural information including chemical composition, branching and microstructure parameters. Strong EOF, good chromatographic performance, and high stability of coatings bode well for both polymers as promising coatings for electrokinetic separations. Applications of these cationic polymers in CE and CEC for a wide range of compounds are currently under investigation.

Acknowledgements

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