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Capillary electrophoresis of anions in an untreated polypropylene hollow fiber

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Abstract

Capillary electrophoresis has been performed primarily in small-bore fused-silica capillaries. The walls of these capillaries are acidic, generating a large electroosmotic flow (EOF) at elevated pH. In order to reverse the high EOF in these capillaries to allow for early detection of the fast, inorganic anions, electroosmotic flow modifiers (OFMs) are added to the electrolyte or the walls of the capillary are covalently altered. Surface-modified, hollow polypropylene fibers have been employed recently for the separation of large biomolecules where sample adsorption to the walls of silica capillaries is a problem. The nonpolar inner surface of the polypropylene capillaries is an ideal site for graft copolymerization of polymeric coatings to aid in these separations. We have successfully used these polypropylene capillaries without wall modification, or addition of an OFM, to separate small, inorganic anions. Detection limits and resolution are comparable to those achieved using fused-silica capillaries and separations at elevated pH values are possible with this system.

Keywords: Capillary columns; Inorganic anions

1. Introduction

Since its inception, capillary zone electrophoresis (CZE) has most commonly been performed using fused-silica capillaries [1,2]. However, detection of fast-moving inorganic anions in these capillaries, at the anode, is difficult due to the high electroosmotic flow (EOF) toward the cathode. Quaternary ammonium salts have been used by some researchers as electroosmotic flow modifiers (OFMs) in order to slow or reverse this high EOF [3–5]. In order to reduce sample adsorption of large biomolecules, several groups have employed covalently or dynamically modified fused-silica capillaries [6–12]. Tindall and Perry [13] used covalently modified capillaries to study the separation of small, organic molecules. Several other polymeric and/or buffer

additives have been used for these same purposes [14–18]. Jones and Jandik [19] previously used an OFM, which reverses EOF, for the early detection of inorganic anions using a fused-silica capillary.

Other types of capillaries have been characterized and their qualities compared with those of fused-silica capillaries. Lukacs and Jorgenson [20] used capillaries made of borosilicate glass, fused-silica or Pyrex to study the physical parameters affecting separations in these capillaries. Schützner and Kennedler [21] studied the separation capabilities of polyfluorocarbon (PFC), polyethylene (PE), and poly(vinyl chloride) (PVC) capillaries. Nielen [22] studied the use of polypropylene capillaries in capillary zone electrophoresis for the detection of large, organic molecules and small cations. These capillaries were pre-equilibrated with a surfactant solution prior to performing separations. Lee and coworkers [23–25] recently studied the use of covalently and

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dynamically coated polypropylene capillaries for the separation of large biomolecules.

A pure polypropylene polymer would have no net charge and therefore no EOF. In practice, however, there is a slight negative charge on the inner surface of the capillary which produces a small EOF. This EOF is smaller than that of fused-silica capillaries and is also pH dependent. This low EOF allows for the early detection of inorganic anions without an OFM. These polypropylene capillaries demonstrate good stability at high electrolyte pH, are low in cost, and are transparent to visible and near-UV light [22]. Resolution is comparable to that achieved with fused-silica capillaries and detection limits are nearly equivalent to those reported for fused-silica capillary systems.

2. Experimental

2.1. Instrumentation

Analyses were performed on a Dionex capillary electrophoresis system (CES) (Dionex, Sunnyvale, CA, USA). Indirect UV detection was carried out at 254 nm. Polypropylene capillaries were provided by Polymers (Middlebury, VT, USA). Dimensions of the polypropylene capillaries were 85 μm I.D. and 60 cm total length. Standard fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) 75 μm I.D. and 60 cm in length were used in the EOF experiment presented in this report. The effective length of the capillary from source to detector was 55 cm in both capillaries. Data acquisition was carried out using a Dionex Advanced Computer Interface (ACI) connected to a personal computer running Dionex AI-450 chromatography software.

2.2. Operation

The CES allows three methods of sample injection: gravity, electromigration, and pressure injection modes. For the EOF experiment in this study, the gravity injection method was employed to allow the EOF marker (water) to enter the capillary end. The signal resulting from the dilution of the electrolyte with a gravity-injected sample of water was used to measure EOF. Electromigration injection was

used for all anion separations performed in this report. All analyses were performed with voltage maintained at 15 kV.

2.3. Reagents

All chemicals used in making electrolyte solutions and anion standards were reagent grade. Milli-Q reagent grade water deionized to 18 M Ω resistivity (Millipore, Bedford, MA, USA) was used to prepare electrolyte solutions and anion standards. A 100 mM chromate stock solution was prepared from anhydrous Na₂CrO₄ (Mallinckrodt). The 5 mM chromate electrolyte was prepared from subsequent dilutions of this 100 mM solution. Stock solutions of 100 mM NaOH (Mallinckrodt) and 100 mM H₂SO₄ (Mallinckrodt) were prepared and then added to the 5 mM chromate solution to adjust pH. Before use, all electrolyte solutions were filtered through a 200-ml, 0.45- μm cellulose acetate membrane filter (Corning, NY, USA) and degassed by vacuum sonication. For the separation and detection of anions in fused-silica capillaries, 0.5 mM tetradecyltrimethylammonium bromide (TTAB) (Aldrich, Milwaukee, WI, USA) was added to the electrolyte to serve as an OFM. This effectively switched direction of the EOF from the cathode to the anode and allowed for the early detection of inorganic anions. All standard anion solutions were prepared from sodium salts (Mallinckrodt). The stock solutions were stored in poly-methylpentene containers (Nalgene, Rochester, NY, USA).

2.4. Determination of electroosmotic flow

Electroosmotic flow was determined by analysis of a gravity-injected sample of deionized water. EOF is positive toward the cathode. The migration velocity (v_{EOF}) of the neutral marker (in cm/s) was measured using the migration time of the peak at its maximum. When this value was divided by the field strength (E) (in V/cm), the value for EOF (in cm²/V·s) was determined by the equation

$$\mu_{\text{EOF}} = \frac{v_{\text{EOF}}}{E} \quad (1)$$

with μ_{EOF} being the electrophoretic mobility of the

electrolyte, v_{EOF} the velocity of the electrolyte, and E the electric field.

3. Results and discussion

Although the polypropylene polymer itself generates no charged surface upon exposure to the electrolyte solution, impurities in the polymer give a slight charge to these capillaries and, consequently, a small EOF. These impurities are due to the polymerization process and arise from several sources: 1) polymer bound sulfate and sulfonate initiator groups can be generated by a metal persulfate anion radical initiation; 2) oxidation can result in carboxylic acid formation in the polymer; and, 3) some surfactants used in emulsion or suspension polymerization, such as alkyl phosphates or dialkyl sulfosuccinates, can contribute to a slight negative charge within the walls of the polymer [26–28]. Like the deprotonated silicate groups on the walls of a fused-silica capillary, these acidic impurities form a net negative charge on the walls of the polypropylene capillaries. This gives rise to an EOF toward the cathode, i.e., the same direction for both fused-silica and polypropylene capillaries. However, because these impurities are in trace amounts, the charge on the walls of the polypropylene capillaries is much smaller at a given pH than that of fused-silica capillaries. When the EOF is low, fast anions can be detected at the anode without an OFM being added to the electrolyte. With no OFM present, the first anion of a nine-anion standard was detected in just over 6 min (Fig. 1).

The EOF was determined by gravity injecting a sample of deionized water, then applying a 15 kV potential with the detector located 5 cm from the cathode. Each separation was repeated three times at each pH, within the range of 2 to 12, increasing by one pH unit increments. The EOF of the polypropylene capillaries is pH dependent as shown in Fig. 2. The pH dependence and direction of the EOF is evidence of acid sites on the polypropylene capillary walls, similar to fused-silica capillaries. Comparison to the EOF in standard fused-silica capillaries is also made in Fig. 2. Polypropylene capillaries display a considerably lower EOF which varies less with electrolyte pH than that obtained

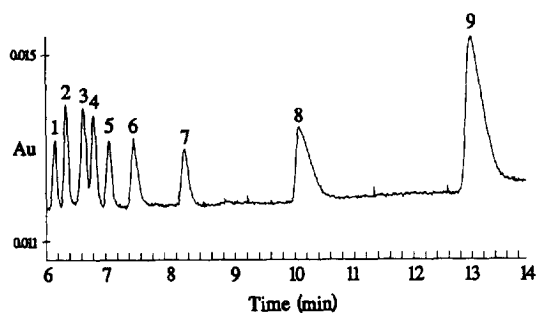


Fig. 1. Electropherogram of nine-anion standard by polypropylene capillary, electrolyte pH=8.4. Electromigration injection at 1 kV for 15 s. Peak identities: 1=bromide (4 ppm); 2=chloride (2 ppm); 3=sulfate (4 ppm); 4=nitrite (4 ppm); 5=nitrate (4 ppm); 6=molybdate (10 ppm); 7=chlorate (4 ppm); 8=fluoride (1 ppm); 9=acetate (4 ppm). Electrolyte is 5.0 mM sodium chromate. Detection near anode. Separation voltage, 15 kV. Indirect UV detection, 254 nm. Detection mode inverted on CE instrument to give positive peak signals on electropherogram.

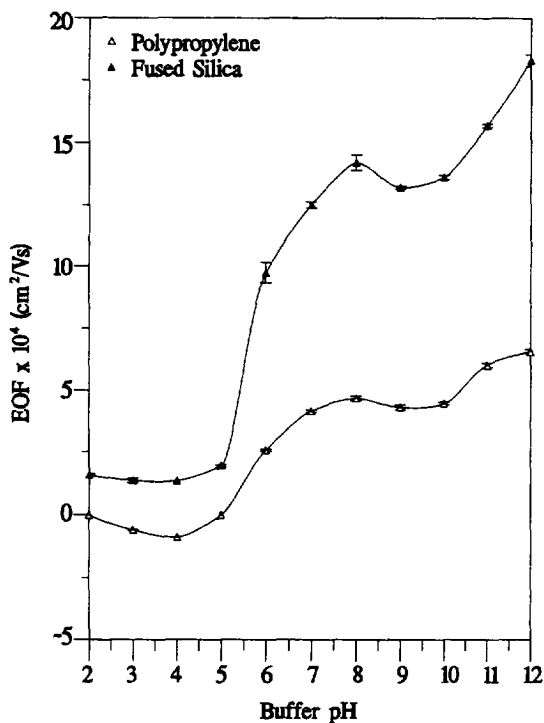


Fig. 2. Electroosmotic flow profile with varying pH for polypropylene and fused-silica capillaries. Gravimetric injection, 100 nm, 30 s. Electrolyte is 5.0 mM sodium chromate, increments of 1 pH unit. Indirect UV detection, 254 nm. Detection near cathode. Separation voltage, 15 kV.

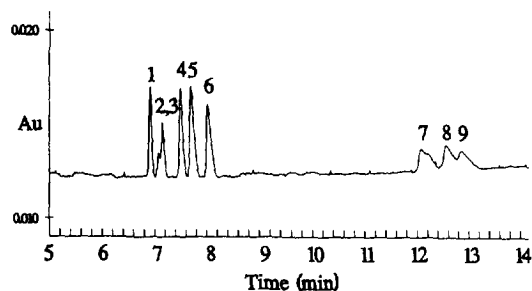


Fig. 3. Electropherogram of nine-anion standard by polypropylene capillary, electrolyte pH=11.5. Electromigration injection at 1 kV for 15 s. Peak identities: 1=bromide (4 ppm); 2=chloride (2 ppm); 3=sulfate (4 ppm); 4=nitrite (4 ppm); 5=nitrate (4 ppm); 6=molybdate (10 ppm); 7=chlorate (4 ppm); 8=fluoride (1 ppm); 9=acetate (4 ppm). Electrolyte is 5.0 mM sodium chromate. Detection near anode. Separation voltage, 15 kV. Indirect UV detection, 254 nm. Detection mode inverted on CE instrument to give positive peak signals on electropherogram.

with fused-silica capillaries. Unlike the fused-silica capillary, which suffers dissolution to form monosilicic acid and silicate ions at elevated pH, the polypropylene capillary remains intact over a wide pH range. Fused-silica capillaries exhibit a hysteresis effect when pH is varied [29], but no such effect was observed with the polypropylene capillaries, i.e. the EOF was reproducible at a given pH even after the pH had been raised and lowered. These polypropylene capillaries maintained their structural integrity and continued to provide good separations up to a pH of 11.5. Fig. 3 shows a representative electropherogram at pH 11.5. This separation com-

pares very well with that obtained at a much lower pH of 8.4, the pH of a 5.0 mM sodium chromate solution (Fig. 1).

Due to the low EOF in the polypropylene capillary at electrolyte pH 8, separation of small, fast-moving anions in a 5 mM sodium chromate electrolyte without an OFM was possible with detection at the anode. The separation compares favorably with those attainable using fused-silica capillaries with an OFM in the electrolyte. Although peak efficiency is lower with polypropylene, as indicated by the number of theoretical plates, resolution is nearly equivalent for all peaks in the polypropylene and fused-silica capillaries (Table 1). Furthermore, reproducible electropherograms were obtained in polypropylene capillaries after only four injections (Fig. 4). By comparison, manufacturers recommend that fused-silica capillaries be presoaked with dilute sodium hydroxide and rinsed with electrolyte a much larger number of times before use to ensure reproducibility.

These polypropylene capillaries were more difficult to install and align in the detector path in our CE instrument than were fused-silica capillaries. Great care was also needed to cut the capillary properly. It was found that a new razor blade was required each time a new capillary was cut to avoid damaging the tip of the capillary with a dull blade. Initially, deviation in detection sensitivity from capillary to capillary was high. However, when the capillaries were cut with a new blade and carefully aligned with the detector, we noticed a significant drop in deviation. Most likely this was caused by an

Table 1

Comparison of separation efficiencies and peak resolution of inorganic anions on fused-silica capillaries (with OFM) and untreated polypropylene capillaries (without OFM)

Anion	Fused-silica capillary			Polypropylene capillary		
	Migration time (min)	Theoretical plates	Resolution ^a	Migration time (min)	Theoretical plates	Resolution ^a
Br ⁻	3.38	73 000	0.91	6.13	14 000	0.88
Cl ⁻	3.43	52 000	1.40	6.32	13 000	1.36
NO ₂ ⁻	3.50	123 000	0.80	6.62	14 000	0.74
NO ₃ ⁻	3.54	56 000	1.08	6.78	17 000	1.16
SO ₄ ²⁻	3.61	43 000	-	7.03	16 000	1.25
MoO ₄ ⁻	-	-	-	7.43	5 000	-

Injection: electromigration, 1 kV for 15 s. Electrolyte: 5.0 mM sodium chromate, pH 8.4. Detection: near anode, indirect UV at 254 nm. Control voltage: 15 kV.

^aBetween listed sequential peaks.

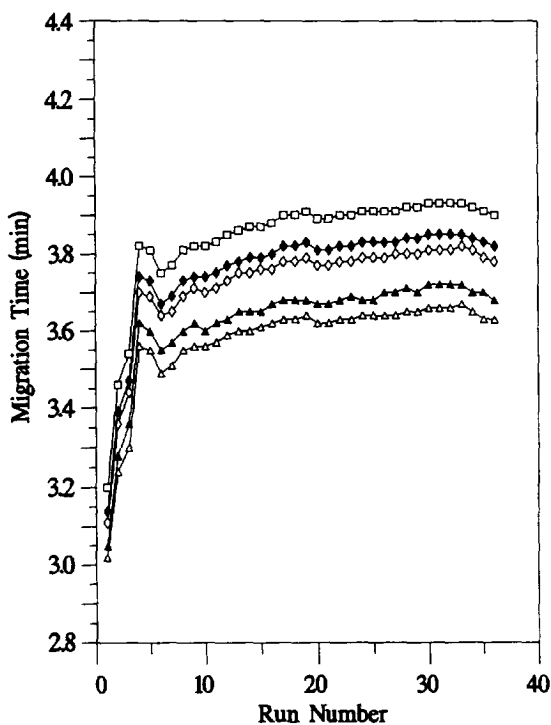


Fig. 4. Migration times for each of the first five anions of a nine-anion standard plotted against run number. Electrolyte is 5.0 mM sodium chromate, pH=8.4. Detection near anode. Separation voltage, 15 kV. Indirect UV detection, 254 nm. Symbols: Δ =bromide, \blacktriangle =chloride, \diamond =sulfate, \blacklozenge =nitrite, \square =nitrate.

uneven cut on the capillary which allowed sample to drip from the end between sample injection and separation.

Polypropylene and fused-silica capillaries exhibit nearly equivalent detector response to a given concentration of sample injected. Absorbance is directly proportional to path length, which is roughly equiva-

lent to the inner diameter (I.D.) of the capillary. Because the I.D. of the polypropylene capillaries is 13.3% larger than that of the fused-silica capillaries, total absorbance ought to be larger by the same ratio. This can be seen by comparing peak areas detected using the two capillaries (Table 2). Steps were taken to ensure that equal volumes of sample were gravity injected into the two different capillaries by varying the time of injection. This was determined according to the equation [30]

$$V = k H t \frac{D^4}{L} \quad (2)$$

which includes the following parameters: volume injected V (in nl), time t (in s) at height H (in mm) of injection, the capillary inner diameter D (in μm), and constant $k = 2.84 \cdot 10^{-8}$ nl cm/(s mm μm^4). Injection height was held at 100 mm and capillary length L was 60 cm for both capillaries. Injection times calculated and used were: 30 s for fused-silica and 18 s for polypropylene. Inner diameters were given earlier. These injection times give volumes of 44.93 and 44.47 nl for the fused-silica and polypropylene capillaries, respectively.

Because of the larger peak height and efficiency for fused-silica capillaries, one would expect to realize lower detection limits for these over polypropylene capillaries. However, Jones and Jandik [19] reported detection limits in fused-silica capillaries using a chromate/OFM system below the ppb threshold. We determined our system detection limits for the chloride ion using the 5.0 mM chromate electrolyte and a polypropylene capillary to be 1 ppb at three times baseline noise peak to peak. Data were obtained for chloride concentrations within the range of 100 ppt to 100 ppm. Each concentration was

Table 2
Relative absorbances between fused-silica and polypropylene capillaries

Anion	Polypropylene peak area (arbitrary units)	Fused-silica peak area (arbitrary units)	Peak area ratio
Bromide	102 200	88 800	1.15
Chloride	72 700	67 000	1.09
Nitrite	198 900	176 500	1.13
Nitrate	88 700	78 700	1.13
Sulfate	165 400	147 600	1.12

Electrolyte: polypropylene (85 μm I.D.): 5.0 mM sodium chromate, pH=8.4; fused-silica (75 μm I.D.): 5.0 mM sodium chromate, 0.5 mM TTAB, pH=8.4. Detection: near anode, indirect UV at 254 nm. Control voltage: 15 kV. Gravity injection.

analyzed three consecutive times and the peak areas averaged. When this average was then plotted against concentration, the linear detection range was determined to be from 1 to 500 ppb with the linear regression coefficient of 0.9974. Standard deviations for migration time using polypropylene capillaries were $6 \pm 0.5\%$, while standard deviations for fused-silica capillaries were $3 \pm 0.5\%$ on our instrument.

4. Conclusion

Anion separations by capillary electrophoresis have been carried out successfully using untreated polypropylene capillaries. The electroosmotic flow is much lower than that of fused-silica capillaries at a given pH, which makes it possible to analyze for fast-moving anions without capillary-surface or electrolyte modification. Migration times at high pH are low enough to make these capillaries useful for practical separations under these conditions. In addition, baseline noise is minimal even at elevated pH levels, making it possible to determine trace amounts of substance at high electrolyte pH. Resolution of species is comparable to fused-silica capillaries. Peak efficiency is lower and migration times are longer. Resolution and standard deviation of migration times are comparable to those of fused-silica capillaries. In addition, we observed no hysteresis effect on migration times upon variation of the electrolyte pH. These capillaries provide a simple and easy means of separating fast-moving inorganic anions without pretreatment and are available at low cost.

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References

- [1] J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298–1302.
- [2] C.A. Monnig and R.T. Kennedy, *Anal. Chem.*, 66 (1994) 280R–314R.
- [3] T.W. Garner and E.S. Yeung, *J. Chromatogr.*, 640 (1993) 397–402.
- [4] T. Tsuda, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 622–624.
- [5] N.J. Benz and J.S. Fritz, *J. Chromatogr. A*, 671 (1994) 437–443.
- [6] Z. Zhao, A. Malik and M.L. Lee, *Anal. Chem.*, 65 (1993) 2747–2752.
- [7] D. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho and B.L. Karger, *J. Chromatogr. A*, 652 (1993) 149–159.
- [8] J.K. Towns, J. Bao and F.E. Regnier, *J. Chromatogr.*, 599 (1992) 227–237.
- [9] S. Hjertén and K. Kubo, *Electrophoresis*, 14 (1993) 390–395.
- [10] J.T. Smith and Z. El Rassi, *Electrophoresis*, 14 (1993) 396–406.
- [11] M. Strega and A. Lagu, *Anal. Chem.*, 63 (1991) 1233–1236.
- [12] R.M. McCormick, *Anal. Chem.*, 60 (1988) 2322–2328.
- [13] G.W. Tindall and R.L. Perry, *J. Chromatogr. A*, 696 (1995) 349–352.
- [14] M. Giles, M.H. Kleemis and G. Schomburg, *Anal. Chem.*, 66 (1994) 2038–2046.
- [15] D.W. Armstrong, Y. Tang and T. Ward, *Anal. Chem.*, 65 (1993) 1114–1117.
- [16] J.R. Mazzeo and I.S. Krull, *Anal. Chem.*, 63 (1991) 2852–2857.
- [17] C. Schwer and E. Kenndler, *Anal. Chem.*, 63 (1991) 1801–1807.
- [18] C. Stathakis and R.M. Cassidy, *Anal. Chem.*, 66 (1994) 2110–2115.
- [19] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445–458.
- [20] K.D. Lukacs and J.W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 407–411.
- [21] W. Schützner and E. Kenndler, *Anal. Chem.*, 64 (1992) 1991–1995.
- [22] M.W.F. Nielsen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 16 (1993) 62–64.
- [23] M. Huang, G. Yi, J.S. Bradshaw and M.L. Lee, *J. Microcol. Separ.*, 5 (1993) 199.
- [24] P.Z. Liu, A. Malik, M.C.J. Kuchar, W.P. Vorkink and M.L. Lee, *J. Microcol. Separ.*, 5 (1993) 245.
- [25] P.Z. Liu, A. Malik, M.C.J. Kuchar and M.L. Lee, *J. Microcol. Separ.*, 6 (1994) 581.
- [26] K.F. O'Driscoll and P. Ghosh, *Structure and Mechanism in Vinyl Polymerization*, Marcel Dekker, New York, 1969.
- [27] H.H.G. Jellinek, *Degradation and Stabilization of Polymers*, Elsevier, New York, 1983.
- [28] B.M.E. Van der Hoff, *Solvent Properties of Surfactant Solutions*, Marcel Dekker, New York, 1983.
- [29] D.N. Heiger, *High Performance Capillary Electrophoresis: An Introduction*, Hewlett-Packard, France, 2nd ed., 1992, p. 20, 21.
- [30] Dionex Capillary Electrophoresis System I Operator's Manual: Appendix F, Dionex Corp., 1990, p. 1201.