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Journal of Chromatography A, 768 (1997) 3–8

JOURNAL OF CHROMATOGRAPHY A

Electroosmotic flow mobilities in open and packed capillaries with spermine and other amine flow modifiers

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Abstract

The effect of some cationic amines (spermine, spermidine, hexamethonium bromide and tetramethylammonium bromide) on the mobility of electroosmotic flow (EOF) in open fused-silica capillaries was studied. The EOF was found to be significantly reduced with spermine and spermidine and to a lesser extent with hexamethonium bromide. Tetramethylammonium bromide was found to be ineffective as a flow modifier. Spermine (10–20 μM) gave a stable reduced EOF at pH 2.50, while 400–500 μM was required at pH 7.0. In a silica-packed capillary, higher concentrations of spermine were required to significantly change the EOF (up to 1 mM). The very low absorption coefficients (M^{-1} cm^{-1}) of spermine [$\epsilon_{200}=250$, $\epsilon_{210}=35$] and spermidine [$\epsilon_{200}=118$, $\epsilon_{210}=3$] at low UV wavelengths makes them useful as surface-charge modification agents when using UV detection, and their low conductivity makes them suitable for use with conductivity detection.

Keywords: Electroosmotic flow; Buffer composition; Inorganic ion analysis; Spermine; Amines

1. Introduction

Capillary electrophoresis (CE) is most often carried out using fused-silica capillaries that have weakly acidic surface silanol groups [1]. Under most separation conditions, the inner surface of the capillary is negatively charged, resulting in counter-ion condensation towards the wall and the formation of an electric double layer [2–4]. When an electric field is applied, a bulk movement of the solvated counter-ions generates the electroosmotic flow (EOF) [2–4]. The EOF, described by the electroosmotic flow mobility (μ_{EOF}), is given by Eq. (1).

$$\mu_{EOF} = (\epsilon_o \epsilon \zeta / \eta) \quad (1)$$

where ϵ_o is the permittivity of a vacuum, ϵ is the dielectric constant of the background electrolyte (BGE) solution, ζ is the zeta potential of the electrical double layer and η is the viscosity of the BGE.

Since the EOF moves from anode to cathode, the apparent electrophoretic mobility (μ) of cationic species is greater than their effective mobilities (μ_{eff}) as they move with the EOF. In contrast, for anionic species, $\mu < \mu_{eff}$, as they migrate against the EOF. Fast migrating anionic species (e.g. Cl^-) can have very low μ and therefore they require long analysis times in the absence of EOF modification [5]. Control of the EOF is also useful in capillary electrochromatography (CEC) analysis, where the eluent is driven along a packed capillary column by

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PII S0021-9673(96)00835-7

electroosmosis. In CEC, the EOF is determined by the zeta potential at the packing surface, which will be determined by the physico-chemical properties of the bonded phase [6,7].

The EOF in fused-silica capillaries can be controlled by manipulating the pH [8] or, to a lesser extent, the ionic strength [9]. However, these parameters are also likely to affect the mobility of the analyte. Covalently coating the inner surface of the capillary wall with an uncharged polymer can permanently eliminate EOF [10]. The application of external radial electric potential gradients across the capillary wall allows direct control of the zeta potential [11–13]. Alternatively, the EOF can be altered by means of a dynamic surface coating with BGE additives such as surfactants [14–16], amines [17] or cationic polymers [18]. In the case of cationic surfactants, increasing their concentration can result in a reversal of the EOF [14–16].

One problem that we encountered with cationic surfactants, such as cetyltrimethylammonium bromide, was in the analysis of proteinaceous biofluids, when the surfactant strongly interacts with proteins, causing their precipitation and blockage of the capillary. Furthermore, their tendency to form micelles can complicate the separation by adding an extra selection process. In order to avoid these difficulties, different kinds of cationic amine EOF modifiers with less hydrophobicity and less tendency to form micelles should be investigated.

In this study, we report the use of spermine, spermidine, hexamethonium bromide and tetramethylammonium bromide as possible EOF modifiers in open silica capillaries. Dynamic coating of spermine in a silica packed capillary was also studied, in comparison to the open capillary.

2. Experimental

2.1. Chemicals

Sodium dihydrogenphosphate, disodium hydrogenphosphate and acetone were purchased from Fisher Scientific (Montreal, Canada). Phosphoric acid (85%) was purchased from Anachemia (Montreal, Canada). Spermine (N,N′-bis[3-aminopropyl]-1,4-butanediamine), spermidine (N-[3-aminopropyl]-1,4-butanediamine), hexamethonium bromide (hexane-1,6-bis[trimethylammonium] bromide, HMBr), CHES (2-[N-cyclohexylamino]ethanesulfonic acid), lithium hydroxide and Dowex SBR (hydroxide form) were purchased from Sigma (St. Louis, MO, USA). Tetramethylammonium bromide (TMBr) was purchased from Aldrich (Milwaukee, WI, USA).

Phosphate buffer, pH 7.0 (I=26 mM), was prepared by titrating a 12.0 mM solution of disodium hydrogen phosphate with a 12.0 mM solution of sodium dihydrogen phosphate, whereas phosphate buffer, pH 2.50 (I=26 mM), was prepared from 20 mM solutions of sodium dihydrogen phosphate and orthophosphoric acid. For packed-capillary electrochromatography, a pH 7 (I=4.3 mM) buffer was prepared by titrating 2.0 mM solutions of monosodium and disodium phosphate. Buffer solutions were prepared from distilled and doubly deionised water (Milli-Q50 unit, Millipore, Montreal, Canada). The buffer solutions were degassed by sonication and passed through a 0.45-μm membrane filter before use (Millipore). All of the reagents used were of analytical grade.

2.2. Instrumentation

A CE unit from Applied Biosystems (Foster City, CA, USA), Model 270 A-HT, was used for open tubular CE. Packed capillary measurements were performed using an ABI 270A CE instrument. Open fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 45 cm total length×365 μm O.D.×50 μm I.D. were used. The polymer coating was burned off 22 cm from the cathodic end of the capillary to form a detection window. The oven was thermostatted at 30°C and UV absorbance detection was carried out at 240 nm, both for the open and packed capillaries. A Crystal CE system with Concap (45 cm length×365 mm O.D.×50 μm I.D.) and Contip (sensor) from ATI Unicam (Boston, MA, USA) was used for separation of anions with conductivity detection. A Unicam 5625, UV–visible spectrometer (Cambridge, UK) was used to measure the absorption coefficients of the additives.

2.3. Capillary packing

A fused-silica capillary (50 μm I.D., 365 μm

O.D.) was packed using the procedure described previously by Li and Lloyd [6] with slight modification. A suspension of Excil silica (3 μm particles, 8 nm pore size, obtained from CSC, St. Laurent, Canada) in 5 m*M* sodium phosphate buffer (pH 7.0) was packed into the column after an inlet frit was generated by sintering 5 μm silica. After packing, a second frit was made in the column, at a distance of 23 cm from the inlet. The second frit was made by heating the column while the column outlet was under a pressure of 5000 p.s.i. (1 p.s.i. = 6894.76 Pa). Using this procedure for producing the second frit under pressure minimized the disturbance of the adjacent silica particles. A narrow window (~2 mm) for detection was created ~5 mm after the second frit. The column was then purged with the mobile phase by pressurizing the column inlet to 3000 p.s.i for ~1 h to flush out the extraneous silica particles between the frit and the outlet of the column.

2.4. Pretreatment of the capillary

At the beginning of the experiments, open fused-silica capillaries were washed with 0.5 *M* sodium hydroxide for 30 min and then they were washed with deionised water for 10 min. Then the capillaries were equilibrated with BGE for 10 min. In the case of silica packed capillaries, the column was equilibrated only with BGE, which was electrically driven through the capillary by applying an electric field of 223 V cm^{-1} for 30 min. On changing the concentration of the flow modifier in the BGE, the open capillary was re-equilibrated by pressure-flushing with the new solution for 5 min. In the packed capillary, re-equilibration was performed by electroosmotically changing the BGE at a field strength of 223 V cm^{-1} for 10 min.

2.5. Measurement of EOF mobility

Acetone (2% aqueous solution) was used as a neutral marker to monitor the EOF. In the case of the open capillary, the marker was injected hydrodynamically by applying a vacuum at 17 kPa for 5 s. The migration time of acetone was monitored under an applied electric field of 556 V cm^{-1} (pH 7, $i = 21$ μA; pH 2.5, $i = 40$ μA). With a silica packed capillary, marker was injected electrokinetically by applying a voltage of 5 kV for 5 s, and the EOF mobility was observed in the 2 m*M* phosphate buffer with an electric field of 223 V cm^{-1}, which generates ~2 μA of current. The EOF mobility was calculated using Eq. (2).

$$\mu_{EOF} = lL/tV \quad (2)$$

where L is the total length of the capillary, l is the effective length of the capillary to the detector, t is the migration time and V is the applied voltage.

2.6. Absorption coefficients

The UV absorption spectra of spermine, spermidine and HMBr solutions in 12 m*M* phosphate buffer (pH 7, $I = 26$ m*M*) were recorded between 200 and 300 nm, and the absorption coefficients were calculated.

2.7. Analysis of a mixture of anions

Separation of a standard mixture of anions, i.e., chloride, nitrite, nitrate, sulfate, phosphate and carbonate (4 ppm each), was carried out with a conductivity detector in 50 m*M* CHES and 20 m*M* LiOH (pH 9.3) with 25 μ*M* spermine or 1.0 m*M* HMOH (HMBr was converted to the hydroxide form by passing it through Dowex SBR resin, to avoid any interference from bromide in the analysis). The sample was injected by applying a positive pressure, 20 mbar for 0.2 min (~7 nl), and separation was carried out with an applied electric field of −445 V cm^{-1} at 25°C.

3. Results and discussion

EOF is the product of the negative charges at the capillary surface and the use of cationic amine additives, which are adsorbed to the surface, is an effective method of neutralizing these charges and in turn modifying the EOF [14–18]. Table 1 shows the effect of four different additives on the EOF mobility. When 1 m*M* spermine is included as an additive in the pH 7 BGE, the EOF is reduced to 10% of its value in BGE alone. With 1 m*M* spermidine, the EOF dropped to 20% of its value in the absence of

Table 1
Electroosmotic flow (EOF) mobilities with some cationic amines in an open fused-silica capillary[a]

Concentration (m*M*)	Mobilities of EOF ($cm^2\ V^{-1}\ s^{-1}$)[b]			
	Spermine	Spermidine	HMBr[c]	TMBr
0.0	$5.58\cdot10^{-4}$	$6.79\cdot10^{-4}$	$5.61\cdot10^{-4}$	$6.70\cdot10^{-4}$
0.1	$1.20\cdot10^{-4}$	$2.81\cdot10^{-4}$	$5.00\cdot10^{-4}$	$6.57\cdot10^{-4}$
1.0	$3.90\cdot10^{-5}$	$1.34\cdot10^{-4}$	$3.62\cdot10^{-4}$	$6.24\cdot10^{-4}$

[a] Mobilities were measured at pH 7 ($I=26$ m*M*) using 12 m*M* phosphate buffer in an open fused-silica capillary. Acetone (2% in water) was used as a marker to monitor the EOF.
[b] Mobility was calculated from triplicate runs.
[c] Similar changes in mobility were observed for the HMOH form (for 0, 0.1 and 1.0 m*M* HMOH in the BGE, the observed EOF mobilities were $5.88\cdot10^{-4}$, $5.28\cdot10^{-4}$ and $3.87\cdot10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$, respectively).

modifier. A 1 m*M* concentration of HMBr only caused ~ a 30% reduction in the EOF, and there was not much change in the EOF with TMBr up to a concentration of 1 m*M*. It is clear from these data that on a per mole basis, spermine and spermidine are more effective at reducing the EOF.

Changes in the EOF mobility as a function of spermine concentration in an open capillary are shown in Fig. 1A–B (at pH values of 7 and 2.5, respectively), and in a silica packed capillary at pH 7 in Fig. 1C. At pH 7, stable reduced EOF mobility was obtained with 400 to 500 μ*M* spermine in an open capillary. In contrast, at pH 2.5, stable reduced EOF was achieved with ~10–20 μ*M* spermine. At both pH values, the limiting value of EOF achieved was in the range $4–6\cdot10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$. With spermine, much of the change in EOF occurs after the addition of the first ~100 μ*M* concentration of additive, with the change thereafter being much slower.

In a packed capillary, the mobility was found to be ~$1.94\cdot10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ at pH 7, which is about ~30–40% of the value measured in the open capillary. Generation of electroosmosis in packed capillaries occurs at the packing surface rather than at the capillary walls. In the present case, the underivatized silica packing used is expected to have a similar ζ potential to that of the capillary walls. Nevertheless, the magnitude of the EOF is expected to be decreased due to non-alignment of the flow channels in the packed bed with the capillary axis, and by lack of

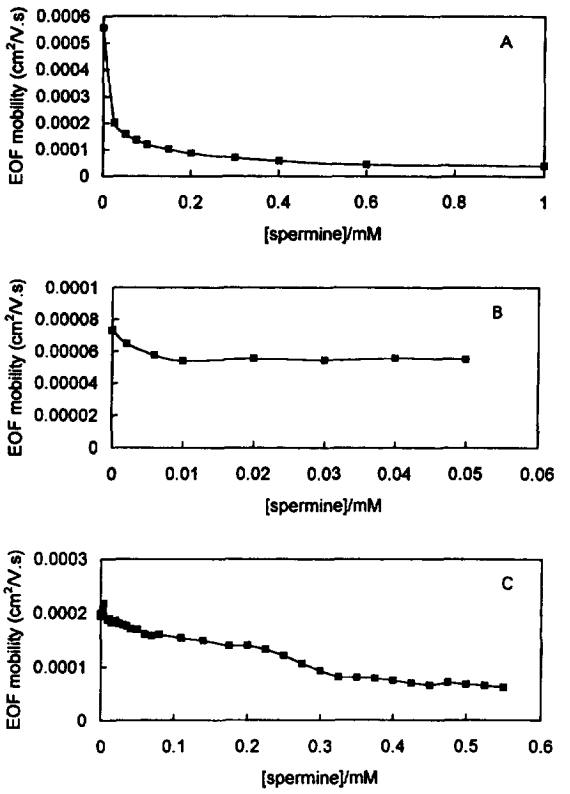

Fig. 1. Changes in EOF mobilities with spermine as a flow modifier. Conditions: fused-silica open capillaries, total length, 45 cm×365 μm O.D.×50 μm I.D.; field strength, 556 V cm^{-1}, (A) BGE: sodium phosphate, pH 7.0 ($I=26$ m*M*) $i=21$ μA. (B) BGE: sodium phosphate, pH 2.5 ($I=26$ mM) $i=40$ μA. (C) Silica packed capillary; field strength, 223 V cm^{-1}, $i=2$ μA; BGE, sodium phosphate, pH 7.0 ($I=4.3$ m*M*).

electro-drive within the particle pores [20]. Unlike the case in the open-tubular system, in the packed capillary almost 0.5 m*M* spermine was required to reduce the EOF mobility to ~30% of its value in BGE. The relative lack of effectiveness of spermine as a flow modifier in packed capillaries is somewhat surprising, since one would expect adsorption of spermine to silica particles to be similar to its adsorption at the capillary wall. The problem does not seem to be one of inadequate time for equilibration, since the results presented in Fig. 1C are an average of triplicate injections, and no systematic drift in the EOF was seen. Alteration of the ζ potential at derivatized packing surfaces can also account for an alteration in EOF mobility when using

bonded phases [6] and the adsorption of spermine to the silica particles used here may not reflect its action with other stationary phases.

Flow modification using spermine may be useful in a variety of applications. In inorganic anion analysis, it is common to reduce or reverse the EOF to speed up the separation and HMOH has been used in this application (HMBr may lead to interferences and vacancy peaks) [19]. Spermine and HMOH were used as flow modifiers to separate some common inorganic anions such as chloride, nitrite, nitrate, sulfate, phosphate and carbonate in a short period of time and with good resolution. The results, using conductivity detection, are shown in Fig. 2. The signal-to-noise ratio was found to be better with spermine than with HMOH. The lower baseline noise with spermine is as a result of its smaller contribution to the BGE conductivity (a background conductivity of 12.4 μS was observed for the BGE with spermine and 18.4 μS was observed for the BGE with HMOH). This is due mainly to the low concentration of spermine (25 μ*M*) needed, compared to HMOH (1 m*M*). Furthermore, spermine and spermidine have low UV absorption coefficients (Table 2). This makes them more suitable for analyses with direct UV detection (e.g. nitrite and nitrate ions, $\lambda_{max}=214$ nm).

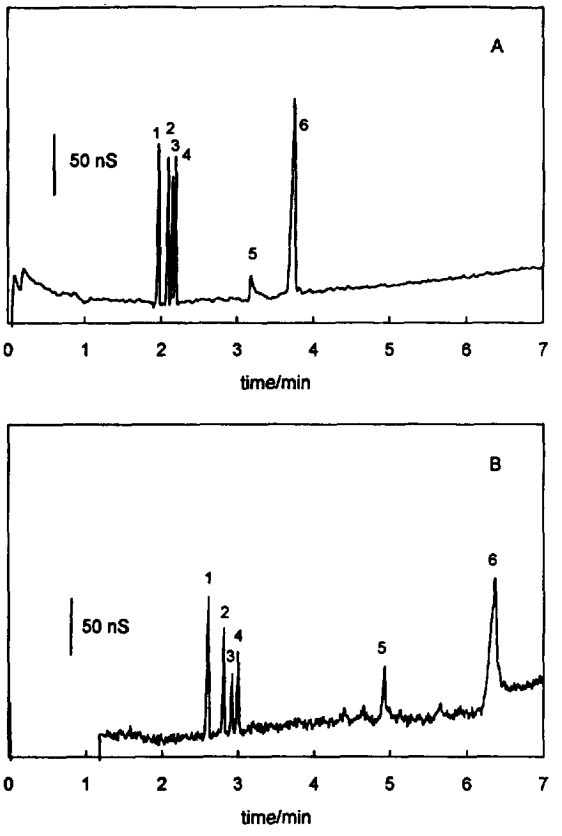

Fig. 2. Separation of inorganic anions by capillary electrophoresis. Conditions: fused-silica Concap, total length of 45 cm×365 μm O.D.×50 μm I.D.; field strength, 445 V cm^{-1}; BGE, 50 m*M* CHES, 20 m*M* lithium hydroxide and (A) 25 μ*M* spermine or (B) 1.0 m*M* HMOH, pH 9.30. Anions (4 ppm each): (1) chloride, (2) nitrite, (3) nitrate, (4) sulfate, (5) phosphate and (6) carbonate.

Table 2
Absorption coefficients of the flow modifiers[a]

Modifier	Absorption coefficients (ϵ/M^{-1} cm^{-1})		
	200 nm	210 nm	220 nm
Spermine	250	35	0.0
Spermidine	118	3	0.0
HMBr	13670	3230	220

[a] Spectra were measured in 12 m*M* phosphate buffer, pH 7.0 ($I=26$ m*M*)

Acknowledgments

This work was funded in part by the Natural Sciences and Engineering Research Council of Canada and by the Respiratory Health Network of Centres of Excellence. D.K.L. is a recipient of a chercheur-boursier award from the Fonds de la Recherche en Santé du Québec.

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