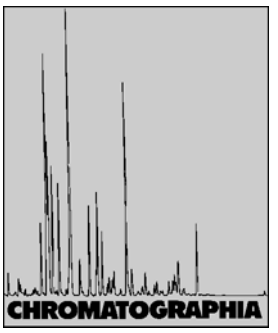
Biopolymer-coated Fused Silica Capillaries for High Magnitude Cathodic or Anodic Electro-osmotic Flows in Capillary Electrophoresis



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Key Words

Capillary electrophoresis
Electroosmotic flow
Coating
Biopolymers

Summary

The manipulation of electroosmotic flow in capillary electrophoresis was achieved by coating the inner wall of a fused silica capillary with the biopolymers α-chymotrypsinogen A and dextran sulfate. Simple coating procedures were based on flushing the fused silica capillary with α-chymotrypsinogen A solution to obtain a α-chymotrypsinogen A coating, or to additionally coat with dextran sulfate solution to obtain a α-chymotrypsinogen A -dextran sulfate coating. The biopolymers α-chymotrypsinogen A coated capillary exhibited strong reversed (anodic) electroosmotic flow values as high as $-81.7 \times 10^{-9}\ m^2 V^{-1} s^{-1}$ at pH 2.0. The α-chymotrypsinogen A -dextran sulfate coated capillary exhibited a cathodic EOF of $62.2 \times 10^{-9} m^2 V^{-1} s^{-1}$ which remained virtually unaltered over the pH range 2 – 9. Both coatings showed high stability, as demonstrated by electroosmotic flow reproducibility of 1.0% and 0.7% RSD (n = 50), respectively. The α-chymotrypsinogen A coating was found to be tolerant to 0.1 M HCl, whilst the α-chymotrypsinogen A-dextran sulfate coating was tolerant to 1 M NaOH, CH_3OH and CH_3CN. The coating-to-coating repeatability for the two coatings, as determined by the RSD of the resultant electroosmotic flow values, were 2.25% and 1.85% (n = 4), respectively. Four anions and five cations were used as test substances to examine the separation performance of the α-chymotrypsinogen A and α-chymotrypsinogen A -dextran sulfate coatings and high efficiencies (80,000 to 200,000 theoretical plates) and rapid separations were obtained. The separation of isomers of chloroaniline was carried out using the α-chymotrypsinogen A-dextran sulfate coating and a pH 2.5 electrolyte in about one third of the time needed when using a FS capillary. A α-chymotrypsinogen A coated capillary was used for ultra-rapid separation of nitrate and nitrite at acidic pH using a co-electroosmotic flow mode. The separation was completed in less than 10 s with a migration time reproducibility of 0.3% RSD (n = 10) and sub-μM detection limits.

Introduction

Capillary electrophoresis (CE) has developed into a powerful analytical technique with the outstanding features of high separation efficiency and rapid analysis. Control of the electro-osmotic flow (EOF) is critical for achieving reproducible separations. EOF is strongly dependent on the pH of the background electrolyte (BGE) and approaches zero at low pH and increases rapidly in the pH range 5 – 10. This leads to separation reproducibility problems when there are even slight changes in the pH of the BGE and although buffered electrolytes are often applied in practice [1, 2], the availability of a capillary with a pH-independent EOF is desirable. On the other hand, it is often necessary in CE to manipulate the EOF in order to maximise the resolution between sample components, or to enable the separation to be achieved in the fastest possible time. Since the EOF in the fused silica capillary originates from the native negative charges on the inner wall due to ionisation of surface silanol groups, manipulation of EOF is achieved by modifying the capillary wall to change its charge. Many such modifications simultaneously reduce or eliminate analyte-wall interactions, such as adsorption of proteins.

Modification procedures for fused silica (FS) capillaries can be generally categorised into two main groups [3, 4]. The first involves formation of covalent bonds, either by direct bonding of a coating agent to the FS surface, or through bonding with cross-linking polymers previously adsorbed to the capillary wall. Ex-

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amples include covalent coatings with polymers covalently attached to the FS capillary surface using a reaction with γ-methacryloxy propyl trimethoxy silane [5], where the polymer can be neutral (e.g. poly(acrylamide) [5, 6]) or charged (e.g. a copolymer of vinylpyrrolidone and vinylimidazole [7] or homopolymer of poly (2-aminoethylmethacrylate hydrochloride) (PALM) [8]). A further example of covalently bonded coatings is cross-linking of a hydrophilic polymer layer, such as poly(vinylmethylsiloxanediol) [9].

The second main group of FS capillary modifications is based on non-covalent modifications by adsorption of a coating agent(s). This can be accomplished either in a dynamic fashion, when the coating agent is usually added into the electrolyte and it is in a dynamic equilibrium with its adsorbed portion at the FS capillary wall based. This is the case for most surfactants which coat the FS wall by micellar surface aggregation [10], and examples of coating surfactants include cetyltrimethylammonium bromide (CTAB) [11], tetradecyltrimethylammonium bromide (TTAB) [12] or didodecyldimethylammonium bromide (DDAB) [3].

Alternatively, a semi-permanent coating can be achieved by rinsing the capillary with a coating solution of a compound that will be strongly enough adsorbed onto the capillary wall to form a non-covalently semi-permanently adsorbed monolayer, so that the coating agent is not present in the electrolyte. Such coating agents are usually positively charged polymers, such as polyamines [13, 14], poly(dimethyldiallylammonium chloride) (PDMAC) [15–18], poly(ethyleneimine)(PEI) [19, 20], polybrene (PB) [21, 22], poly(arginine) (PA) [23], aromatic polyaniline [24], double-strand polyaniline [25], polymetal complex-substituted polysiloxanes [26], as well as some neutral polymers, such as poly(vinylpyrrolidone) (PVP) [27], or poly(vinyl alcohol) (PVA) [28]. Double-chained cationic surfactant didodecyldimethylammonium bromide (DDAB) [29], as well as double-chained zwitterionic surfactant 1,2-dilauroyl-sn-phosphatidylcholine (DLPC) [30] were also investigated for their use to constitute a semi-permanent monolayer coating. On the other hand, semi-permanent coatings can also be established by successive coatings with ionic polymers resulting in multiple layer coatings. Katayama et al. [31, 32] first reported this type of coating procedure by successively rinsing the capillary with PB and dextran sulfate (DS). Similar procedure was taken by Bendahl et al. [33] to set up a polymer polybrene (PE)-poly(vinylsulfonate) (PVS) bi-layer coating. Some semi-permanent coating approaches combine features of some of the other coating methods, such as when Rodriguez-Delgado et al. [34] reported a high molecular weight PEI coated capillary that provided strong cathodic EOF at pH as low as 1 when SDS was added to the electrolyte. It is also worthwhile to point out that some coating agents could be categorised as both dynamic and semi-permanent – those which do not adsorb strongly enough to form a stable semi-permanent coating but do adsorb strongly enough to allow the coating to be renewed after/before each run with a short flush, so that they do not have to be always present in the electrolyte.

The several coating approaches have their advantages and disadvantages. Although covalent modification results in "permanent" coatings which exhibit longer operational lifetimes and require less maintenance than other coatings, the coating procedure can be complicated and time-consuming and reproducibility between capillaries can be poor. Dynamically coated capillaries offer the advantages of good reproducibility and simplicity of preparation, but they are generally not suitable for protein analyses because of denaturation of proteins by surfactants. Severe problems are also created when mass spectrometry (MS) is combined with CE because the presence of the coating agent in the BGE may deteriorate the ionization of the analytes [35]. Non-covalently adsorbed semi-permanent coatings offer a promising alternative to both covalent and non-covalent dynamic coatings and can be obtained through adsorption of a polymer monolayer or polymer multilayer onto the FS wall. However, in the past the coating agents have been mainly confined to a few synthetic polymers. Moreover, the goals of the coating were generally to manipulate the EOF and to suppress the adsorption of macromolecular analytes, but in some cases a fast and stable EOF is much more desirable. With this incentive in mind we have investigated two examples of natural biopolymers (proteins and polysaccharides) for use as polyelectrolytes for semi-permanent coatings in the present work. Proteins in aqueous solutions can be positively or negatively charged or neutral, depending on the pH of the solution. It is also well known that proteins adsorb easily onto the FS capillary surface, so it appear natural for proteins to be used as capillary coatings. Although proteins have been widely used as chiral selectors in chiral CE [36], it is surprising that to our knowledge there is no reports regarding semi-permanent protein coating for the purpose of controlling EOF and the few existing reports rather focused on control of protein adsorption. In one example, protein fibrinogen was adsorbed onto FS and then stabilised by thermal treatment [37]. Another related example [38] is the use of proteins, such as albumin or haemoglobin, in a weakly acidic buffer to form a polycation, as an initiator for the capillary surface and to saturate the sites of adsorption at the capillary wall to prevent adsorption of cationic species. In subsequent electrophoresis separation, a polyanion was added to the electrolyte as a dynamic coating additive to dynamically modify the capillary wall and to provide a stable and pH-independent EOF. As proteins will be charged when adsorbed on the FS wall, they can be expected to be able to adsorb secondary layers of another charged biopolymer through electrostatic interactions. In this way, the adsorbed protein can be used as an intermediate layer for further modification of the capillary wall to form a bi-layer coating.

The goal of this work was to develop a new coating strategy for manipulation of EOF in FS capillaries using biopolymers as coating agents. The biopolymers chosen in this work were a protein, α-chymotrypsinogen A (ChA), to form a single-coated capillary, and dextran sulfate (DS) to adsorb in a secondary layer onto the ChA in order to achieve a pH-independent EOF.

Experimental

Reagents

Coating reagents, α-chymotrypsinogen A (ChA) (Type II, from bovine pancreas, lot 16H7075) and dextran sulfate (DS) (from dextran with an average MW of 500,000, lot 128H1185) were from Sigma (St. Louis, MO). All test solutes were from Aldrich (Milwaukee, WI, USA). All other chemicals were analytical grade. Mesityl oxide (MSO) was used as an EOF marker. All solutions were prepared with deionized water from a Milli-Q unit (Milli-

pore, Bedford, MA, USA), and were degassed using vacuum sonication and filtered though a 0.45 μm syringe filter (Activon, Thornleigh, Australia) before use.

Instrumentation

Separations were performed with a HP[3D] Capillary Electrophoresis instrument (Agilent Technologies, Waldbronn, Germany). Uncoated FS capillaries were obtained from Polymicro (Phoenix, AZ, USA). Sample injections were performed by pressure (20 mbar for 3 s). In order to realise ultra-rapid separation of nitrate and nitrite (see Results and Discussion), a small change to the commercial capillary cassette was made such that the body of the cartridge permitted the capillary to take shortest possible path from the injection outlet through the detector interface and to the detection side outlet, with the total capillary length being 18.5 cm (originally 21.5 cm).

Coating Procedures

Coating of an FS capillary was performed using the following steps. A new capillary was preconditioned by rinsing with 1 M NaOH for 20 min, and then with water for 20 min, after which the preconditioned capillary was flushed with 1% ChA prepared in phosphate buffer at pH 3.0 for 30 min, and then allowed to stand for 60 min. The ChA coated capillary was ready to be tested after excess ChA was rinsed away. The bi-layer coated capillary was formed by flushing; the ChA coated capillary with 1.5% DS solution for 30 min, and then allowing the capillary to stand for 60 min. After excess DS was removed by flushing the capillary, the ChA-DS coated capillary was ready for use. Before each electrophoresis run, the coatings were flushed with the electrolytes for 2 min All rinsing steps were performed by using the flushing function of the Agilent CE instrument at 25 °C.

Result and Discussion

EOF *vs.* pH Profiles

The EOF *vs.* pH profiles of an uncoated FS capillary, a ChA coated capillary and a ChA-DS coated capillary are shown in Figure 1. As will be discussed in more detail below, the reproducibility of EOF is already evident by the fact that each point in Figure 1 is made of an average of 5 replicate analyses showing RSD values of less than 0.81%.

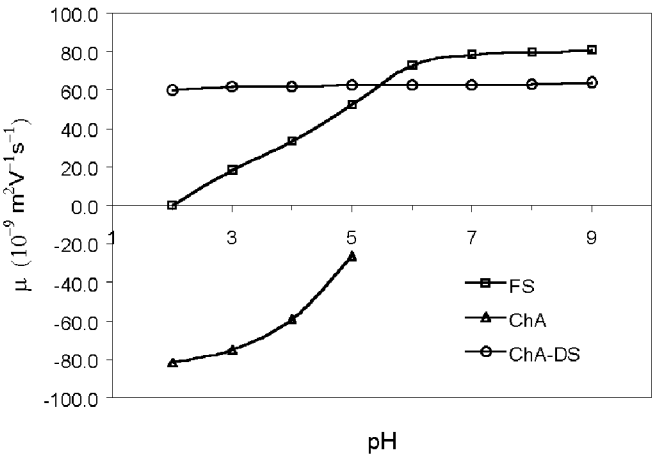

Figure 1. EOF *vs.* pH profiles for an uncoated FS, ChA coated and ChA-DS coated capillaries. Conditions: electrolyte, 10 mM sodium phosphate; capillary, 50 μm i.d. × 55 cm (46.5 cm to detector); applied voltage, +30 kV for uncoated FS, +25 kV for ChA-DS, and −30 kV for ChA coated capillary; detection, 200 nm; number of measurements, n = 5.

Table I. Magnitudes of EOF for various capillary surfaces.

Coating	pH or pH range	Ionic Strength (mM)	EOF ($\times 10^{-9}$ $m^2V^{-1}s^{-1}$)	Reference
ChA[a]	2.0	10	-81.7 ± 0.14 (Mean ± SD, n = 5)[a]	this work
ChA-DS[a]	2.0–9.0	10–30	62.2 ± 1.1 (n = 8)[b]	this work
DDAB[c]	7.2	20	−46	[3]
CTAB[c]	7.2	20	−34	[3]
PDMAC	4.0–8.0	500	−34.5 (n ≥ 2)	[16]
PEI[c]	3.0	25	−43	[19]
PB	3.0	50	−37.7	[31]
PB-DS	3.0	50	37.5	[31]
PB-DS-PB	3.0	50	−34.5	[32]
PB-PVS	2–10	30	49.07 ± 0.1 (n = 9)	[33]

[a] Conditions as in Figure 1. [b] Statistic results were from the determined values at 8 pH values from pH 2 to pH 9. [c] EOF was estimated from the graphs given in the references.

ChA is a basic protein with an isoelectric point (pI) of 8.8 [20]. The ChA coated capillary gave a reversed EOF (i.e. direction of flow from cathode to anode), and the magnitude of the EOF was found to increase with decreasing pH. At pH 2.0, a very high EOF of $-81.7 \pm 0.16 \times 10^{-9}$ $m^2 V^{-1} s^{-1}$ was obtained, which is a much higher anodic EOF than for any previous report using coated capillaries [3, 16, 19, 31]. A comparison between the various values reported in the literature and the values obtained in this work is given in Table I. These remarkable and significant differences can be attributed to the surface structure of the coating. It has been well known that ChA, as a protein, is easily adsorbed onto the capillary wall due to intensive Coulombic and/or hydrophobic interaction between them [31]. In acidic pH, large amounts of absorbed ChA will result in high positive charge density. Although some studies of the surface structure such as by atomic force microscopy [3] were used to explain some properties of capillary coatings, detailed studies of the surface structure would be needed to correlate the surface structure parameters with the magnitude of EOF. The EOF became progressively smaller when the pH approached the neutral region, as expected from the pI of 8.8 and also effects of increased ionization of surface silanol groups which neutralise the positive charges of the ChA.

Dextran sulfate is a heparin-like polysaccharide biopolymer containing up to three sulfate groups per glucose molecule, which gives the DS a relatively high density of negative charge, and because of the low pK_a values of sulfate groups, the charge will be constant across a wide operational pH range. When the DS was coated as a second layer onto the ChA, the direction of the EOF was towards the cathode. Importantly, the magnitude of

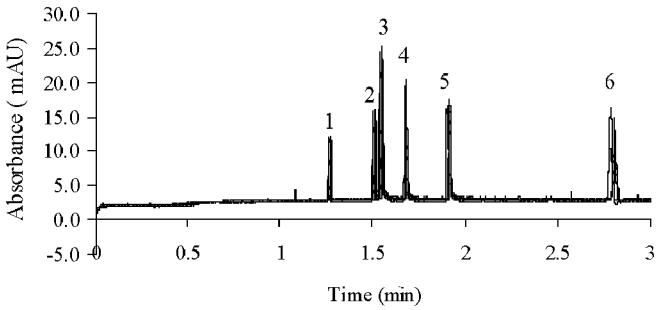

Figure 2. Overlaid electropherograms for the 1st, 10th, 20th, 30th, 40th and 50th run. Conditions: coating, ChA-DS; electrolyte, 10 mM phosphate at pH 3.0, 1. Peaks: **1** = Imidazole, **2** = $C_6H_5CH_2NH_2$, **3** = $C_6H_5CH_2N(CH_3)_3^+$, **4** = $C_6H_5CH_2N(C_2H_5)_3^+$, **5** = $C_6H_5CH_2N[(CH_2)_3CH_3]_3^+$, **6** = EOF; other conditions as Figure 1.

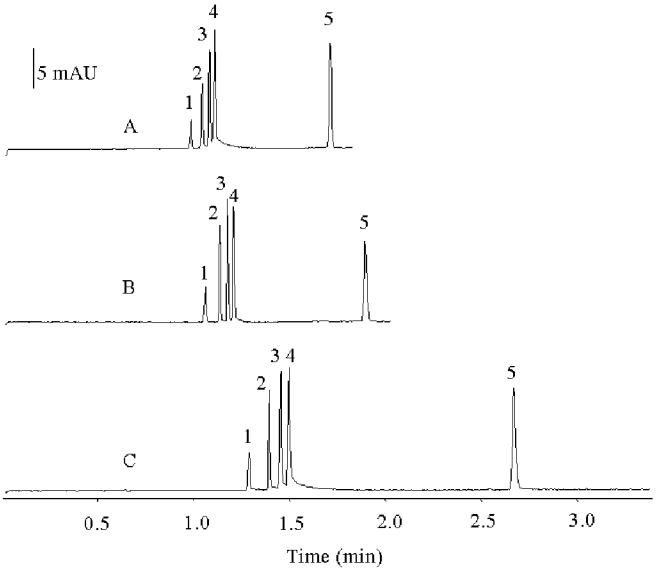

Figure 3. Electropherograms of periodate, benzylsulfonate, 4-methylbenzenesulfate and 4-ethylbenzenesulfate on a ChA coated capillary under co-EOF conditions at pH 2.0 **(A)**, 3.0 **(B)** and 4.0 **(C)**. Conditions as Figure 1. Peaks: **1** = IO_3^-, **2** = $C_6H_5SO_3^-$, **3** = p-$CH_3C_6H_4SO_3^-$, **4** = p-$C_2H_5C_6H_4SO_3^-$, **5** = EOF.

Table II. Comparison of the baseline noise (mAU) at various wavelengths between coated and uncoated capillaries (values shown are the average of three measurements)*.

Capillary	200 nm	214 nm	254 nm
Uncoated	0.049	0.030	0.027
ChA	0.065	0.030	0.028
ChA-DS	0.079	0.037	0.035

* Conditions: electrolyte, 10 mM phosphate, pH 3.0; other conditions as in Figure 1.

Table III. Relative standard deviation (RSD) (%, n = 5) for the EOF of the coating, and the separation efficiencies.

pH	EOF RSD (%, n = 5)[a]			Efficiency (10^3)[b]		
	Uncoated	ChA	ChA-DS	Uncoated[c]	ChA[d]	ChA-DS[c]
2.0		0.16	0.46		80.6	134
3.0	2.88	0.17	0.50	14.0	95.5	151
4.0	1.40	0.81	0.11	39.0	128	149
5.0	1.44	0.64	0.74	54.3	86.0	155
6.0	1.41	n.d.[e]	0.36	57.3	n.d.	120
7.0	0.50	n.d.	0.44	63.7	n.d.	170
8.0	0.84	n.d.	0.29	65.0	n.d.	201
9.0	0.71	n.d.	0.75	60.2	n.d.	168

[a] Conditions as in Figure 1. [b] Calculated using the test substance and formula $N = 5.54\,(t_m/W_{1/2})^2$. [c] Test substance: $C_6H_5CH_2[N(CH_2)_3CH_3]_3^+$. [d] Test substance: p-$C_2H_5C_6H_4SO_3^-$. [e] Not determined.

the EOF was almost constant for the pH range 2–9. Because the magnitude of the anodic EOF exhibited by the ChA coated capillary was high, indicating a high density of positive charge, it can be anticipated that significant amounts of the negatively charged DS would be adsorbed, leading to high values of cathodic EOF. Table I shows a comparison of the cathodic EOF values obtained in this work and those for other reports on coated capillaries [31–33] and indicates that the EOF for the ChA-DS capillary was higher than obtained previously and almost reached the maximum value attainable with bare FS at high pH.

Detection Performance due to Background Absorbance of the Coatings

Proteins are known to absorb light in the low-UV region and therefore the effect of the ChA coating on the detection performance should be determined. In order to investigate this effect, the baseline noise for a ChA coated and a FS uncoated capillary were measured under typical CE operational conditions at 200 nm, 214 nm and 254 nm, respectively, and the results are summarized in Table II. Somewhat higher noise was observed at 200 nm but this poses no significant detection problem, whilst the differences in noise at 214 nm and 254 nm were insignificant. It can be assumed that the adsorbed layer of ChA is very thin and therefore the resulting background absorbance is very small and consequently does not significantly affect the detection parameters.

Coating Stability

The long-term stability of the coatings was examined at pH 3.0 by successively performing 50 replicate analyses of the test substances and the EOF marker. Some of the resulting electropherograms of the 50 replicate separations with ChA-DS capillary (including the first and last runs) are shown overlaid in Figure 2. No significant differences within the 50 replicate values of EOF and efficiency were observed with the RSDs of EOF for ChA coated and ChA-DS coated capillaries being 1.0% and 0.7%, respectively. By comparison, a synthetic polymer PB coating could only endure 25 runs [20], and for a PB-PVS bi-layer coating the systematic

Table IV. Tolerance of the coating to some BGE additives (n = 3)[a].

Solvent	ChA			ChA-DS	
	EOF_1[b] (10^{-9} $m^2V^{-1}s^{-1}$)	EOF_2[c] (10^{-9} $m^2V^{-1}s^{-1}$)	Degradation (%)[d] (10^{-9} $m^2V^{-1}s^{-1}$)	EOF_1[b] (10^{-9} $m^2V^{-1}s^{-1}$)	EOF_2[c]	Degradation (%)
0.1 M HCl	−72.9	−72.5	0.6	61.5	N.D.[e]	–[f]
1 M NaOH	−72.5	−67.8	6.4	61.2	60.3	1.5
100% CH_3CN	−67.8	−58.6	14	60.3	59.9	0.7
100% CH_3OH	−58.6	−32.1	45	59.9	59.6	0.4
0.1 M SDS	−70.7	−31.7	55	59.6	54.2	9.1

[a] Conditions: electrolyte, 10 mM phosphate at pH 3.0; applied voltage, −30 kV for ChA and 25 kV for ChA-DS; others as in Figure 1. [b] Measured before rinsing with additive. [c] Measured after rinsing with additive. [d] Defined as $(EOF_1-EOF_2)/EOF_1 \times 100\%$. [e] EOF marker not detected within 60 min. [f] Degradation ratio could not be calculated.

migration time increased by 6–12% between the first and fifty fourth run if PVS was omitted in the electrolyte [33] The stability of the coatings at various pH values was investigated by measuring EOF and the resultant RSD values are summarized together with separation efficiencies in Table III. For both the ChA coated capillary at pH 2.0 to 3.0 and for the ChA-DS coated capillary throughout the pH range 2–9 the stability of EOF was excellent.

The tolerance of the coatings to some common electrolyte additives such as organic solvents, acids, bases and surfactants was investigated to evaluate the stability, robustness and applicability of the coated capillaries under practical conditions. The assessment procedure described by Katayama [31] based on comparing the EOF values measured before and after exposure to a certain concentration of the additive was adopted. Briefly, the EOF of a newly prepared coating was measured, then the coated capillary was rinsed with the electrolyte containing the investigated additive for 20 min, then with water for 2 min, and the running electrolyte for 2 min. The EOF was then measured again. The tolerance to the additive was evaluated by the degradation ratio of EOF after the rinsing. It is evident from the results shown in Table IV that the ChA coated capillary showed limited tolerance towards the investigated additives, but the ChA-DS coated capillary exhibited very high tolerance towards a wide range of additives, with the exception of SDS.

Repeatability of the Coating

Capillary-to-capillary coating repeatability is essential from the practical requirement to prepare coated capillaries which exhibit little variation in performance. The results for four ChA and four ChA-DS coated capillaries were evaluated based on RSD values of EOF. For the ChA coated capillary a RSD of 2.25% was obtained, whilst for the ChA-DS coated capillary, the RSD was 1.85%. These RSD values are larger than those reported for synthetic polymer coatings (0.5% of RSD of the EOF) [31]. However, taking into account the commonly encountered difficulties in achieving repeatable EOF values for FS capillaries, the RSD values for the coated capillaries of around 2% can be viewed as satisfactory.

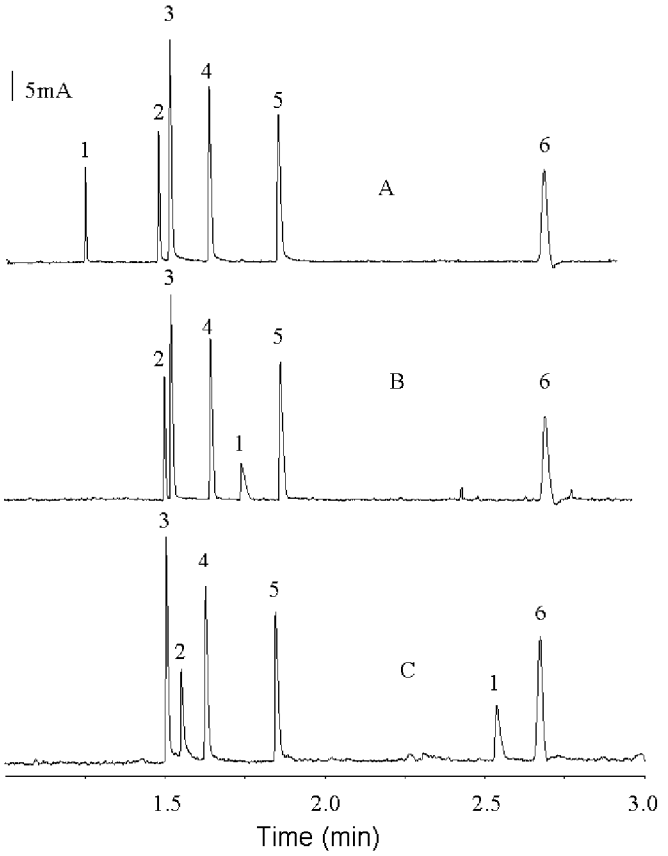

Figure 4. Electropherograms of five organic cations on a ChA-DS coated capillary at pH 3.0 **(A)**, 7.0 **(B)** and 9.0 **(C)**. Conditions as Figure 1. Peaks: **1** = Imidazole, **2** = $C_6H_5CH_2NH_2$, **3** = $C_6H_5CH_2N(CH_3)_3^+$, **4** = $C_6H_5CH_2N(C_2H_5)_3^+$, **5** = $C_6H_5CH_2N[(CH_2)_3CH_3]_3^+$, **6** = EOF.

Separation Performance

Practical separation performance of the coated capillaries was investigated using four test analyte anions for the ChA coating and five test analyte cations for the ChA-DS coating, together with the EOF marker. Representative electropherograms are given in Figures 3 and 4 and the separation efficiencies at various pH values are included in Table III. A very stable baseline, symmetrical peaks and high separation efficiencies were achieved with the coated capillaries. The coated capillaries exhibited much higher separation

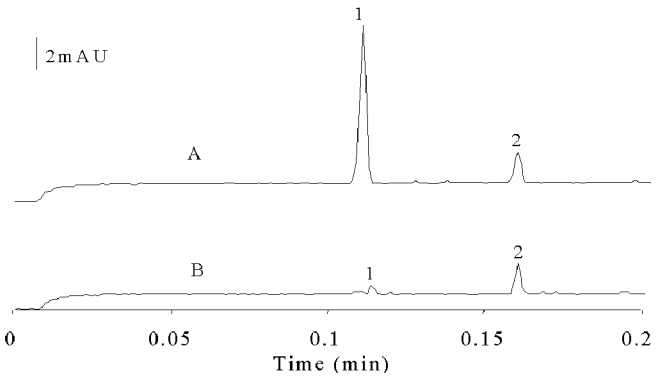

Figure 5. Ultra-fast separation of nitrate and nitrite in 50 µM standard mixture **(A)** and tap water spiked with 50 µM nitrite **(B)**. Conditions: 10 mM phosphate at pH 3.0; ChA coated capillary, 50 µm i.d. × 27 cm, 8.5 cm effective length; applied voltage, 30 kV; detection, 200 nm. Peaks: **1** = NO_3^-, **2** = NO_2^-.

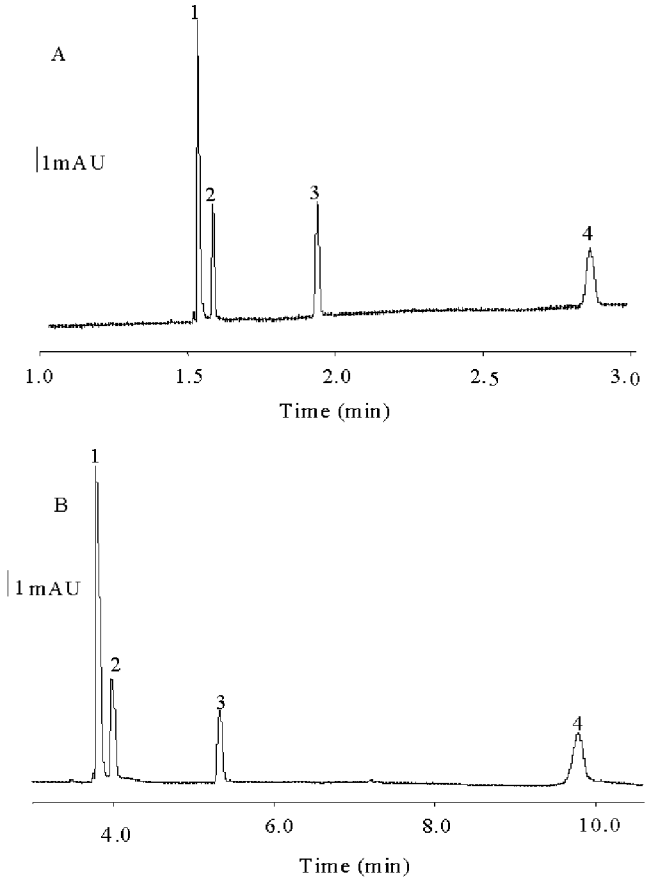

Figure 6. Comparison of separation of chloroaniline isomers on a ChA-DS coated capillary **(A)** and an uncoated FS capillary **(B)**. Conditions: electrolyte, 10 mM phosphate at pH 2.5, other conditions as in Figure 1. Peaks: **1** = 4-chloroaniline, **2** = 3-chloroaniline, **3** = 2-chloroaniline, **4** = EOF.

efficiencies than the uncoated FS capillary which may be partly due to a smaller amount of longitudinal diffusion with shorter analysis time.

Use of ChA-coated Capillary for Ultra-rapid Determination of Nitrate and Nitrite in Water

The separation of nitrate and nitrite is often needed in areas such as environmental monitoring or clinical toxicological examinations, but two problems are encountered when applying conventional methods. First, the mobilities of nitrate (μ_0 = $-68.3 \times 10^{-9}\ m^2 V^{-1} s^{-1}$) and nitrite ($\mu_0$ = $-68.8 \times 10^{-9}\ m^2 V^{-1} s^{1}$) [39] are very similar under neutral and alkaline conditions, which will typically result in poor resolution. Second, if an acidic BGE is used the low EOF will cause long separation times in an uncoated FS capillary using either positive or negative separation voltages. These problems can be overcome by a strategy of using a low pH electrolyte in a co-EOF mode, provided the EOF is sufficiently high. Nitrite has a pK_a of 3.15, and the use of a low pH electrolyte can therefore selectively reduce its effective mobility. At this low pH the selectivity between nitrate and nitrite is so large that the separation can be conducted in a short capillary. By using the short-end injection technique [40], minimising the total capillary length (using a modified capillary cartridge, see Experimental) and performing a co-EOF separation with a high-magnitude reversed EOF at acidic pH, a very rapid separation should be possible. The very high anodic EOF in an acidic electrolyte offered by the ChA coated capillary is ideal for this purpose. An 8.5 cm capillary (measured from the detector to the outlet vial) was used for the separation, with the sample being injected at the outlet end of the capillary and the separation being performed with the cathode at the outlet vial.

With these parameters adopted, nitrate and nitrite were separated in less than 10 s, as shown in Figure 5. Compared with the results of Melanson and Lucy [29] conducted using a similar setup and conditions, the separation time was shortened by 20%, no pre-rinse procedure was needed, and the resolution was greatly increased to 10 compared to only about 1.6 in the previous study. This indicates that an even shorter capillary length could be adopted, for instance on a chip. The RSD of the migration times for nitrate and nitrite from 10 consecutive runs were 0.28 and 0.32%, respectively. The detection limits were 400 nM for nitrate and 2 µM for nitrite using electrokinetic injection (1 kV for 2 s) at S/N of 3.

Use of ChA-DS-coated Capillary for Fast Separation of Chloroaniline Isomers

Modified capillaries having pH-independent-EOF offer significant advantages for both counter-EOF separations of anions and co-EOF separations of cations at acidic pH [31]. In this work, the separa-

tion of chloroaniline isomers as protonated bases in an acidic BGE was evaluated using the ChA-DS coated capillary. Figure 6 shows the separation of 3 chloroaniline isomers (2-chloroaniline, 3-chloroaniline and 4-chloroaniline) having pK_a values of 2.65, 3.46 and 4.15, respectively. At pH 2.5, differences in the degree of protonation between the analytes gives a separation selectivity which allows a baseline separation to be completed in less than 2 min (Figure 6A). Although it is also possible to separate these analytes using an uncoated FS capillary, the separation time was about 3 times as long as that for the ChA-DS coated capillary (Figure 6B).

Conclusions

The highest reported EOF values for coated capillaries have been achieved for both a α-Chymotrypsinogen A coated positively charged capillary (at pH 2.3) and a α-Chymotrypsinogen A-dextran sulfate double-coated negatively charged capillary (over the pH range 2–9). These coatings showed highly reproducible EOF values and repeatabilities of the coatings, and in the case of the double-coated capillary the EOF was independent of pH over the range examined. Moreover, the coated capillaries exhibited much greater separation efficiencies than uncoated FS, showing that wall interactions were minimal in the coated capillaries. Biopolymers therefore offer a promising new alternative to conventional synthesized polymers as semi-permanent coatings for the control of EOF and wall interactions in CE.

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