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Coated versus fused-silica capillaries for the separation of inorganic and organic cations by capillary zone electrophoresis

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Abstract

The performance of a hydrophilic-coated capillary supplied by Dionex has been compared to an unbonded fused-silica capillary for the separation of various inorganic and organic cations. The coated capillary yielded both improved run-to-run migration time reproducibility and, due to reduced electro-osmotic flow, enabled the separation of analytes which coelute using the fused-silica capillary. The coating also showed increased stability to acidic samples and allowed the migration of analytes which adsorb onto a fused-silica surface. Migration time reproducibility and resolution were significantly improved for a mixture of alkali, alkaline earth and transition metal standards. The separation of a series of organic amines, unsatisfactory with a fused-silica capillary, was successfully obtained in the coated capillary.

Keywords: Capillary columns; Inorganic cations; Organic cations; Alkali metals; Alkaline earth metals; Transition metals

1. Introduction

The hydrophilic capillary coating used in this work was developed primarily for basic protein analysis. The analysis of proteins is a problem because of their non-specific binding to silanol groups and hydrophobic sites, and the hydrophilic capillary coating is one of many designed to alleviate this problem [1–8].

This work highlights another advantage of using a coated capillary, for applications outside the protein field.

Fused-silica capillaries used in capillary zone electrophoresis (CZE) contain surface silanol groups, which may become ionised in the presence of the electrophoretic medium or electrolyte. Ionisation of the silanol groups increasingly occurs over the approximate pH range 3 to 9, with complete ionisation at pH 9 [1]. Electro-osmotic flow is a consequence of the silanol ionisation. The negative charges on the capillary wall cause the formation of an electrical double layer, a diffuse layer of mobile cations in the electrolyte. The potential at the surface of shear between the charged surface and the electrolyte solution is termed the zeta potential.

When an electric field is applied, all charged species within the electrolyte will migrate towards the appropriate electrode. The cations of the double layer migrate towards the cathode, causing the whole electrolyte to move with 'plug flow' in the same direction. The velocity of this electro-osmotic flow is directly proportional to the zeta potential. Since the zeta potential relies on the charge density of the

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silica surface, both it and the electro-osmotic flow are pH-dependent.

In the presence of electro-osmotic flow,

$$t = \frac{L^2}{(\mu + \mu_{eo})V} \quad (1)$$

where t = migration time, L = capillary length, μ = mobility of solute (velocity in unit electric field), μ_{eo} = coefficient of electro-osmotic flow and V = applied voltage [9].

Thus the rate of migration of any solute is the sum of its electrophoretic mobility and the electro-osmotic flow, but separation is obtained on the basis of differing solute mobilities. Electro-osmotic flow sweeps the solutes through the capillary so that for cations, the presence of electro-osmotic flow gives fast separations with very sharp peaks whilst allowing little time for good resolution to be obtained. Any reduction in this electro-osmotic flow would increase run times for cations but provide the possibility of improved separations with greater resolution.

If the silanol groups are masked, either by protonation [10] or modification of the silica surface as is the case with coated capillaries, then the electro-osmotic flow can be reduced, eliminated or even reversed. In so doing, band broadening and efficiency limitation caused by solute interaction with the charged silica wall can also be reduced, and it is for this reason that capillary modification has generated so much interest.

Much of the work in capillary coatings for CZE has centred around the separation of proteins, due to their inherent tendency to adsorb onto the silica capillary walls which has made their analysis difficult. Protonation of the silanol groups by adjusting the electrolyte pH would be one solution to the problem; however, running at pH 2 may not be viable in terms of solute stability and migration properties.

Several materials have been used to modify the internal silica surface in order to reduce adsorption and electro-osmotic flow, including the following: poly(ethylene glycol) (PEG) [2,3], poly(acrylamide) via siloxane bonds [4,5] and via Si–C bonds [6], poly(ethylene imine) [1], C_1, C_{18} hydrocarbons and Carbowax-20M [7], dextran and methylcellulose [2,8].

The inorganic cation separation studied here is particularly pH-sensitive in that both electro-osmotic flow and the transition metal separation mechanism [complexation with α-hydroxyisobutyric acid (HIBA)] are pH-dependent. This work shows that a proprietary coating giving reduced electro-osmotic flow yields improved migration time stability and resolution for the separation of alkali, alkaline earth and transition metals.

The coated capillary was designed to enable the separation of proteins by preventing their adsorption onto the capillary wall. The quaternary amine studied here was also prone to adsorb onto fused silica, and in this paper it is shown that the separation of organic amines is also greatly improved by using the coated capillary.

2. Experimental

2.1. Chemicals

Imidazole, α-hydroxyisobutyric acid (HIBA) and 18-crown-6 were obtained from Fluka (Buchs, Switzerland). Glacial acetic acid (HPLC grade) was obtained from BDH (Poole, UK), as were the Spectrosol cation standards used as samples. Diallyldimethylammonium chloride (DADMAC) was obtained from Aldrich (Gillingham, UK) and the other amines (ADMA, mono-, di- and tri-methylamine hydrochlorides) from Sigma (Poole, UK). The fused-silica capillary tubing was obtained from Composite Metal Services (The Chase, Hallow, UK) The coated capillary was kindly donated by Dionex (Camberley, UK).

2.2. Apparatus and electrophoresis

A Dionex capillary electrophoresis system (CES) was used. Fused-silica capillary tubing with an outer coating of polyamide, 375 μm O.D., 75 μm I.D. was cut to 60 cm total length (55 cm to detector). The window for the on-column detector was made in the capillary by burning off a small (ca. 0.5 cm) section of the polyamide coating with a match or butane lighter, then wiping off the residue with methanol. Standard rinse procedures were used between each run, i.e. 6 s each for source and destination reservoirs

and 120 s for the capillary. Samples were injected hydrostatically by raising the sample vial containing the inlet end of the capillary to a height of 100 mm above the detector end for 30 or 60 s. The supply voltage was positive to give cathodic flow. Indirect UV detection at 215 nm or 220 nm was used, with negative signal polarity giving 'positive' peaks. Electropherograms were obtained and data processing achieved by a computer running Dionex AI450 software, via a Dionex ACI interface. All running buffers were prepared using degassed distilled deionised water, and the pH of each was adjusted with glacial acetic acid.

3. Results and discussion

3.1. Analysis of metal ions

3.1.1. Retention time stability

Mixed Spectrosol standards were run, each containing the following nine cations: ammonium, potassium, sodium, calcium, magnesium, manganese, cobalt, nickel and zinc, each 5 mg/l.

Twenty repeat runs were performed. The voltage was set (Fig. 1) so that the zinc peak eluted at approximately the same time on all runs, i.e. 15.5 min.

The recorded retention times for the nine-cation standard were plotted and are displayed in Fig. 1. The relative standard deviations of the zinc peak retention times for each set of data are 0.265% for the silica capillary and 0.083% for the coated capillary ($n=5$).

It can be seen from the graphs (Fig. 1) and statistical data that the coated capillary gave an obvious improvement in the run-to-run retention time reproducibility, exhibiting much more stable and predictable behaviour than the silica capillary. Although the retention times gradually increase, the smaller run-to-run discrepancies should greatly improve automatic peak detection.

3.1.2. Resolution

To compare the resolution obtained with the different capillaries, mixed standards were run under optimum conditions for a fused-silica capillary [11], each containing the following fourteen cations: ammonium, caesium, potassium, calcium, sodium, magnesium, manganese, strontium, cobalt, chromium, nickel, barium and zinc, cadmium, all 5 mg/l. Buffers and voltages were exactly as for the retention time studies. In addition an attempt was made to further optimise the separation for the coated capillary, by slightly adjusting the pH and the HIBA concentration.

The cobalt–chromium and the barium–zinc pairs coeluted in the fused-silica capillary, even using the best possible separation conditions. The coated capillary partially resolved both pairs using the same conditions, and a slight adjustment of the buffer, from 10 m*M* to 8 m*M* HIBA and from pH 3.5 to 3.8, improved the separation further, giving peaks which were close to being baseline resolved. This same adjustment had no effect on the separation in the fused-silica capillary.

3.1.3. Acid strength of samples

Real samples of low-level inorganic cations are often stabilised in acid. An additional study compared the migration times of a 5 mg/l zinc standard in varying concentrations of nitric acid. Zinc standards of 5 mg/l were prepared at acid concentrations varying from 0.0025 *M* to 0.1 *M*, and each sample was run three times, the average migration time being plotted against acid concentration (Fig. 2). Similar results were seen with other metal standards.

The results shown in Fig. 2 clearly indicate that the retention times of individual standards are significantly affected by the acid strength of the sample matrix for fused silica. The pH stability is much higher with a coated capillary, such that there is almost no change in the zinc migration time over a 400-fold increase in acid concentration.

The Spectrosol metal standards used in this experiment, like many real samples, are acid-stabilised, being supplied as 1000 mg/l metal in 0.5 *M* HNO_3. Thus a mixed standard of nine metals will contain nine times the acid of a single standard. Results indicated that there was 5–10% difference in retention time between single and mixed standards in a fused silica, but that this difference was reduced to 0.5–3% in the coated capillary.

In a fused-silica capillary the silanol groups are in a delicate equilibrium at pH 3.5, such that any change in pH is capable of dramatically changing the

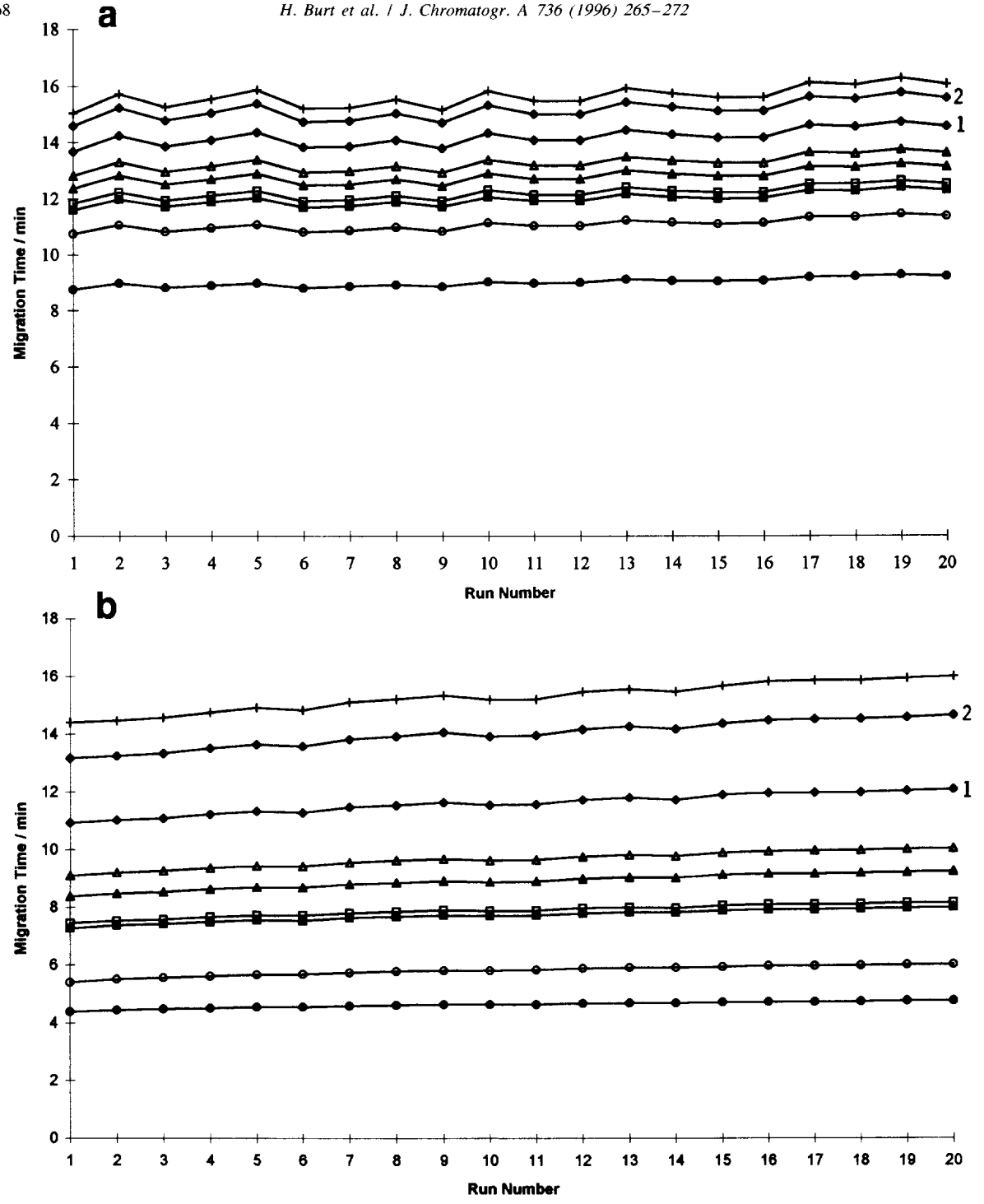

Fig. 1. Retention time stability for metal-ion standards. Migration time against run number for a capillary zone electrophoretic analysis of a standard solution of nine alkali, alkaline earth and transition metals. (a) Fused-silica capillary: 375 μm O.D., 75 μm I.D., 60 cm length (55 cm to detector); buffer: 12.5 m*M* imidazole, 10 m*M* HIBA, 3 m*M* 18-crown-6, pH 3.5; control: 9.75 kV, detector cathodic; injection: gravity, 100 mm for 30 s; detection: indirect UV, 215 nm. (b) Coated capillary: experimental conditions as for (a) except, control: 25 kV. Symbols: ● = Ammonium, ○ = potassium, ■ = calcium, □ = sodium, ▲ = magnesium, △ = manganese, ◆1 = cobalt, ◆2 = nickel, + = zinc.

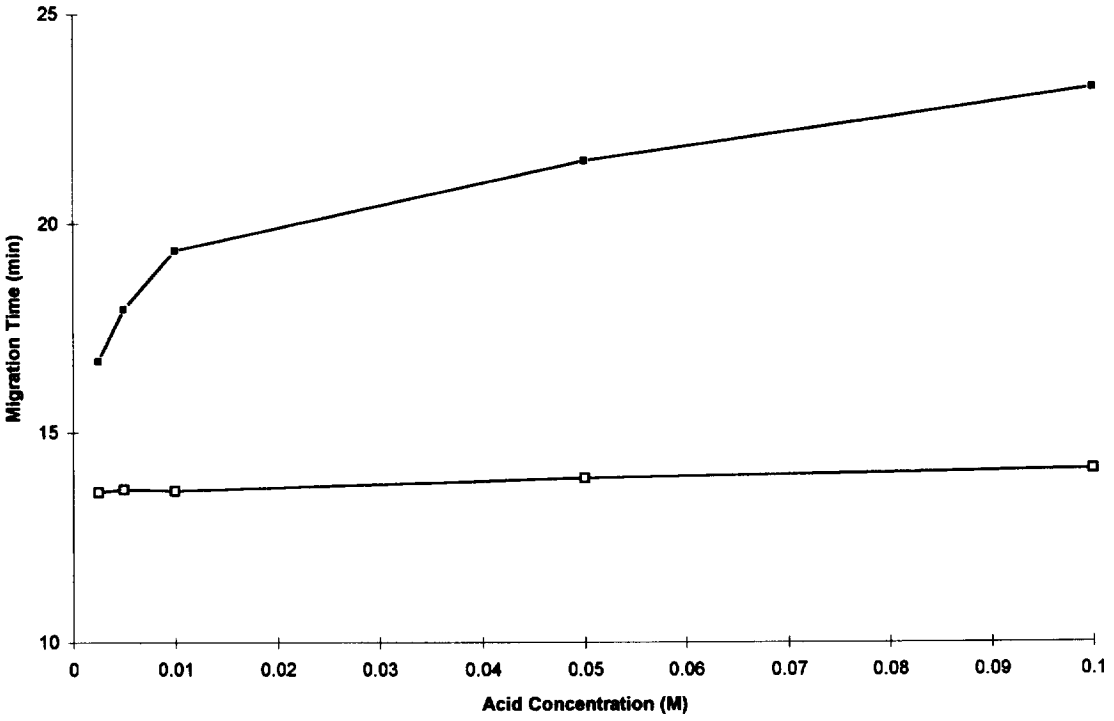

Fig. 2. Migration times of acidic samples. Graph of migration time against acid concentration of sample for an acidified 5 mg/l zinc sample, for fused-silica and coated capillaries. Capillaries: fused silica and coated, both 375 μm O.D., 75 μm I.D., 60 cm length (55 cm to detector); buffer: 12.5 m*M* imidazole, 8 m*M* HIBA, 3 m*M* 18-crown-6, pH 3.8; control: 9.75 kV (fused-silica capillary) or 20 kV (coated capillary), detector cathodic; injection: gravity, 100 mm for 30 s; detection: indirect UV, 215 nm; samples: 5 mg/l zinc in HNO_3 at 0.0025 *M*, 0.005 *M*, 0.01 *M*, 0.05 *M*, 0.1 *M*. Symbols: ■= Fused-silica capillary; □=coated capillary.

degree of ionisation of the silanol groups. This in turn will affect the zeta potential and the electro-osmotic flow. Injections of acidic samples such as Spectrosol cation standards or other acid-stabilised samples can have such an effect, which can be seen in the retention time instability.

The coating on the capillary wall alters the zeta potential by effectively masking silanol groups, giving a reduced electro-osmotic flow and hence lowering the overall pH-sensitivity of the separation. Thus the retention times are more stable, and the reduced electro-osmotic flow gives better resolution than is available from the conventional fused-silica capillary. However, the pH is still an important parameter since it governs the extent to which the transition metals form complexes with the HIBA. Care must therefore be taken to ensure that it, and other conditions, remain constant from one buffer batch to the next. The discrepancies between retention times of single and mixed standards shown by the fused-silica capillary suggest that the use of external standards for peak identification may be unreliable. This view is reinforced by the fact that the retention time instability may often cause automatic peak-detection software to mis-assign peaks, particularly among the transition metals. This is much less likely to be a problem with the coated capillary.

3.2. Capillary spectra

When the coated capillary was used for cation analysis at around 215 nm, it gave a four-fold lower response than its uncoated equivalent. Using the capillary electrophoresis system to measure the absorbance of the capillaries at different wavelengths, ultraviolet spectra were obtained (Fig. 3).

These spectra can only be directly compared if it

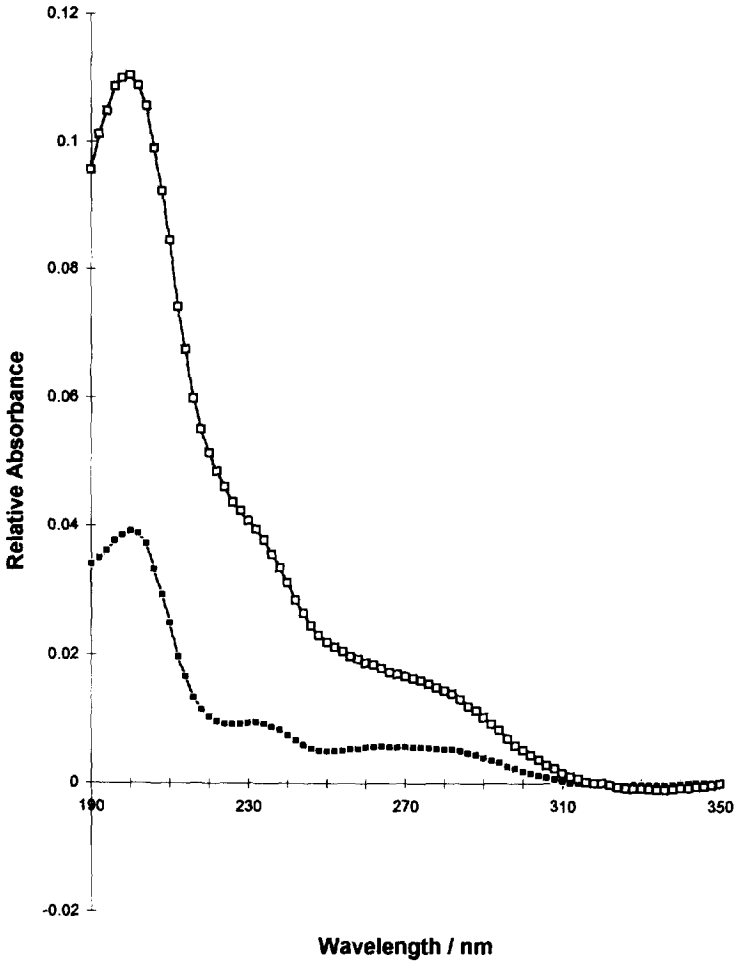

Fig. 3. Plot of capillary spectra. Graph of relative absorbance against wavelength for coated (□) and fused-silica (■) capillaries, both water-filled.

is assumed that both the capillaries have negligible absorbance at 350 nm, but in any case it is obvious that the coated capillary has a greater ultraviolet absorbance than an uncoated one. The noisier baselines which result may cause problems when working at extremely low concentrations with the coated capillary, although the levels used in this study (ca. 5 mg/l) were easily detectable.

3.3. Analysis of amines

The separation of a mixed amine standard was used to compare the performance of fused-silica and coated capillaries. The standard consisted of: methylamine hydrochloride, dimethylamine hydrochloride, trimethylamine hydrochloride, allyldimethylamine (ADMA) and diallyldimethylammonium chloride (DADMAC), each 10 mg/l.

It was possible to obtain a separation of the first four amines (i.e. no DADMAC present) on fused silica under the following conditions: buffer 5 m*M* imidazole pH 3.5, injection 100 mm, 60 s, detection indirect UV at 220 nm, control 20 kV.

When a mixed standard containing all five amines was repeatedly run under the same conditions, only the first four amines eluted (not DADMAC), and the retention times rapidly increased, until by the fourth run no peaks at all were seen within 60 min. The

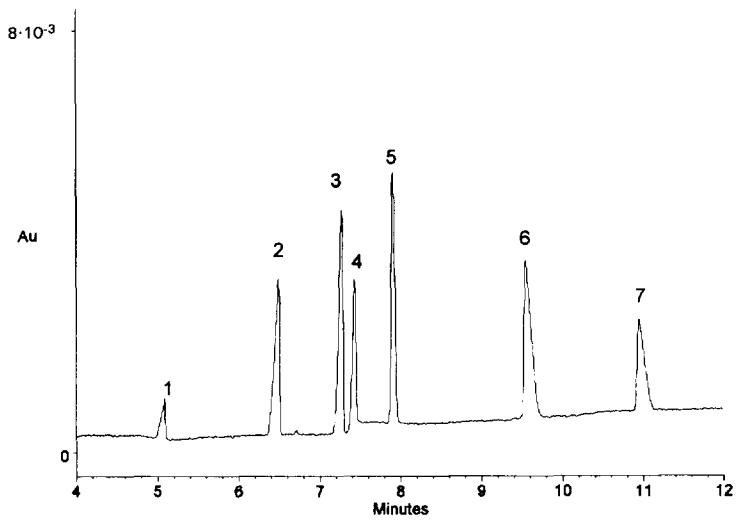

Fig. 4. Organic amine separation, coated capillary. Capillary zone electrophoretic separation of organic amines. Capillary: coated, 375 μm O.D., 75 μm I.D., 60 cm length (55 cm to detector); buffer: 5 m*M* imidazole, pH 3.5; control: 20 kV, detector cathodic; injection: gravity, 100 mm for 60 s; detection: indirect UV, 220 nm. Peaks: 1 = unknown, 2 = methylamine hydrochloride (10 mg/l), 3 = dimethylamine hydrochloride (10 mg/l), 4 = unknown, 5 = trimethylamine hydrochloride (10 mg/l), 6 = allyldimethylamine (ADMA) (10 mg/l), 7 = diallyldimethylammonium chloride (DADMAC) (10 mg/l).

baseline also showed a downward drift. Both of these factors are indicative of the strong adsorption of the quaternary amine onto the capillary wall. Regeneration of the capillary surface with 1 *M* NaOH and rinsing regained the separation for a single run, but the baseline was far more noisy than before.

When similar runs were performed on the coated capillary, complete, reproducible separation of all five amines was obtained. It was necessary to increase the voltage across the capillary to compensate for the reduced migration rates seen as a result of the reduced electro-osmotic flow within the capillary, but all other experimental conditions were identical. The typical separation achieved is shown in Fig. 4. The fronting and tailing of the peaks in this figure is believed to be due to the amine mobilities differing from that of imidazole, rather than as a consequence of any significant adsorption.

4. Conclusions

The coated capillary used in these studies showed a marked improvement over a conventional fused-silica capillary for retention time stability of alkali, alkaline earth and transition metal cations. It also improved the agreement between the retention times of samples of varying acid strength, and enabled the hitherto unsatisfactory separation of organic amines to be achieved. The lower signal obtained from the coated capillary gave comparatively noisy baselines, which may adversely affect the lower limit of detection. Thus the overall characteristics of the coated capillary, as highlighted in this study, result in far greater confidence in peak identification of cationic components in real samples than that possible with conventional fused-silica capillaries.

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