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# Resin coating for capillaries giving a net positive charge and great potential for customised modification of surface properties

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## Abstract

The use of capillary coatings in capillary electrophoresis to manipulate electroosmotic flow (EOF) and chemical interactions is an expanding field which has generated much interest. In this study the use of a reactive polyamide resin is explored, which is straightforwardly applied to give a positively charged coating on the capillary wall. A permanent positive coating offers distinct advantages, particularly in the analysis of low-molecular-mass anions. Analysis of such highly mobile anions has usually been achieved by the addition of cationic surfactant (EOF modifier) to the electrolyte, to provide a temporary positive coating on the wall, reversing the direction of EOF within the capillary and, under appropriate conditions, forcing the anions towards the detector. The more permanent positive charge achieved here eliminates the need for such electrolyte additives. Additionally, the coating chemistry is such that there is potential for further modification to provide great flexibility in manipulation of surface properties. This offers the possibility of various coatings to suit a wide range of applications.

*Keywords:* Capillary columns; Coated capillaries; Polyamide resins; Inorganic anions

## 1. Introduction

The direction of electroosmotic flow (EOF) in a fused-silica capillary is counter to the migration of anions, so that their relative rates will determine the net direction in which the anions move. Highly mobile inorganic anions may have a net migration away from the cathodic detection end of the capillary, thus remaining undetected. EOF modifiers (usually cationic surfactants) are used to dynamically coat the fused-silica capillary wall, making the EOF anodic. If the detection end of the capillary is then made anodic, anion migration will be in the same direction as the EOF, allowing fast separation to be achieved. In binding a suitable polymer to the inner

wall of the capillary to give a more permanent positive charge, the separation of highly mobile anions may be achieved without the need for flow modifiers.

Coatings may be applied to the inner walls of fused-silica capillaries used in capillary electrophoresis (CE) for a number of additional reasons. The manipulation of EOF can be achieved in order to improve overall reproducibility, or suppressed to provide appropriate conditions for techniques such as isotachopheresis or capillary gel electrophoresis. Electrostatic interactions between the silica wall and analytes in the capillary reduce separation efficiency, and adsorption onto the walls may prevent the migration of analytes through the capillary. Coatings may be applied in order to reduce such analyte-wall interactions. Polymers may be used to coat capil-

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laries, imparting positive [1,2], negative [3–7] or neutral [8–10] net charge, which may or may not be affected by the electrolyte pH. Positive coatings have been used previously [1,2] to achieve some of the effects mentioned above.

High-molecular-mass polyethyleneimine [1] has been used to achieve a bonded positive charge. The polymer was adsorbed to the capillary wall and then crosslinked using the agent, ethyleneglycol diglycidyl ether (EDGE). The coating was found to be stable over the pH range 2–12, with little change in EOF, and was used successfully for protein separations. 3-Aminopropyltrimethoxysilane [2] has also been used to coat capillaries, giving a net positive charge and anodic EOF with buffers of pH ca. 5.0. When this capillary was used to separate metal cations with a buffer containing 2-aminopyridine, improved separation efficiency and resolution were seen, compared to cation separations in fused-silica and neutral-coated capillaries, although the total analysis time was increased. Working at pH 5, the coating was stable, giving EOF that were unchanged after 50 runs or more.

In this work we have explored the use of a reactive polyamide resin to provide an immobilised net positive charge on the capillary wall, and the separation of inorganic anions using such a coating is demonstrated. Two advantages of this particular polymer are its simple application and ease of derivatisation, making it potentially extremely versatile. The results presented here are preliminary, thus an outline of intended further work is also proposed.

## 2. Experimental

### 2.1. Materials

The reactive polyamide resin was obtained from an undisclosed commercial source as a 12.5% aqueous solution containing various functional groups including azetidinium ions. The inorganic anion standards were obtained from FSA Laboratory Supplies (Loughborough, UK) and the fused-silica capillary tubing was from Composite Metal Services (Hallow, UK). All other reagents were purchased from Sigma-Aldrich (Poole, UK).

### 2.2. Apparatus and electrophoresis

A Dionex capillary electrophoresis system (CES1) was used. Standard rinse procedures were used between each run, i.e. 9 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 s. The supply voltage was negative to give anodic flow. Indirect UV detection at 250 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. The running buffer was 2.25 mM pyromellitic acid (PMA) and 1.6 mM triethanolamine (TEA), prepared using degassed distilled deionised water, and the pH was adjusted to 7.9 with sodium hydroxide (1 M).

The preparation of coated capillaries was performed using a Varian 3300 Gas Chromatograph (Varian, Walton-on-Thames, UK).

### 2.3. Preparation of coated capillaries

The fused-silica capillaries were treated with 1 M NaOH for 10 min, then with deionised distilled water for 30 min. The capillaries were dried in a gas chromatography oven at 100°C for 1 h, with nitrogen running through the capillary at a pressure of 275 kPa. The coating solution was injected into the capillary with a syringe and the capillary was left overnight, coiled horizontally. The coating solution consisted of 1.25% polyamide in deionised water or 90:10 (v/v) methanol–water. The capillary was then re-connected to the gas chromatography oven and the excess coating material was pushed out under nitrogen pressure. The nitrogen flow was continued at 275 kPa and the capillary heated to 100°C for a further 4 h to crosslink the resin and immobilise it on the capillary wall. Before use, the capillary was rinsed for 10 min with deionised distilled water and then for a further 10 min with running buffer. The window for the on-column detector was made in the capillary by burning off a small (ca. 0.5 cm) section of the outer coating with a match or butane lighter, then wiping off the residue with methanol.

### 3. Results and discussion

#### 3.1. Choice of capillary coating

The water-soluble, reactive polyamide resin (see Fig. 1) used in this study has several features which make it potentially useful as a coating for CE capillaries.

Firstly, its application to the capillary wall is simple to achieve, not requiring high temperature or pressure or the separate application of a crosslinking agent. The resin consists of a polyamide backbone containing tertiary and quaternary amines. Some of the quaternary amine groups are reactive four-membered ring azetidinium cations. The resin is used as an aqueous solution; on drying, the azetidinium ions form crosslinks with tertiary amines to yield an insoluble resin.

In the above manner, the drying process can be used to immobilise the resin on a suitable substrate.

Secondly, the crosslinked resin has a permanent net positive charge due to the presence of quaternary amine groups in the polymer backbone; this positive charge may be increased at low pH due to the protonation of tertiary amines also present. As outlined in Section 1, a positively charged capillary wall can yield benefits in reducing wall-analyte interactions, increasing resolution and efficiency for cation separations and eliminating the need for flow modifiers in the separation of highly mobile anions.

Thirdly, the resin is reactive, allowing easy derivatisation to modify coating properties. It is a property of these polymers that a small degree of

crosslinking will immobilise the coating on the silica capillary, so unreacted azetidinium ions may also be used to introduce other functional groups. This derivatisation is simple, the reagent is added to the resin in its soluble form and reacts with the azetidinium on drying, in the same way that crosslinking occurs. The most suitable reagents are those containing amines or other nucleophiles. It may be possible to add small bi-functional amines to the coating solution in order to increase the degree of crosslinking, whilst aromatic or alkyl-chain amines may be used to increase overall hydrophobicity. Proteins could potentially be used to impart a degree of chiral selectivity to the capillary wall and dyes could be used to alter the absorption or fluorescence properties of the capillary. Production of a derivatised capillary coating has yet to be attempted but will be one of the subjects of future investigation using this resin.

#### 3.2. Anion separation

The baseline separation of six inorganic anions was successfully achieved using the polyamide-coated capillary (Fig. 2). The six anions, in elution order, were bromide, chloride, sulphate, nitrate, fluoride and phosphate, each at a concentration of 10 ppm. The buffer consisted of 2.25 mM PMA and 1.6 mM TEA, at a pH of 7.9. This buffer is commonly used with hexamethonium hydroxide added as a flow modifier, to perform similar separations in fused-silica capillaries.

The elution order of the anions indicates that a positive coating has been achieved. When inorganic anions are separated in a fused-silica capillary (negatively charged) without the use of EOF modifier, the anion migration and EOF will be counter-directional. Thus, the anions will only be detected if a high pH buffer yielding high cathodic EOF is used, so that the mobile anions are swept towards the cathodic detector. The elution order under those conditions [12] is essentially reversed compared to that seen here. When separated in the presence of EOF modifier and with an anodic detector, the anions elute in the same order as is seen here, indicating that we have achieved a positively charged capillary wall without the need for EOF modifier.

The baseline, although unsteady, is reproducible

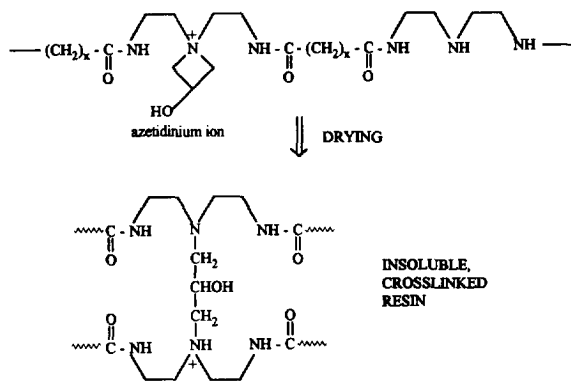


Fig. 1. Chemistry of the reactive polyamide resin.

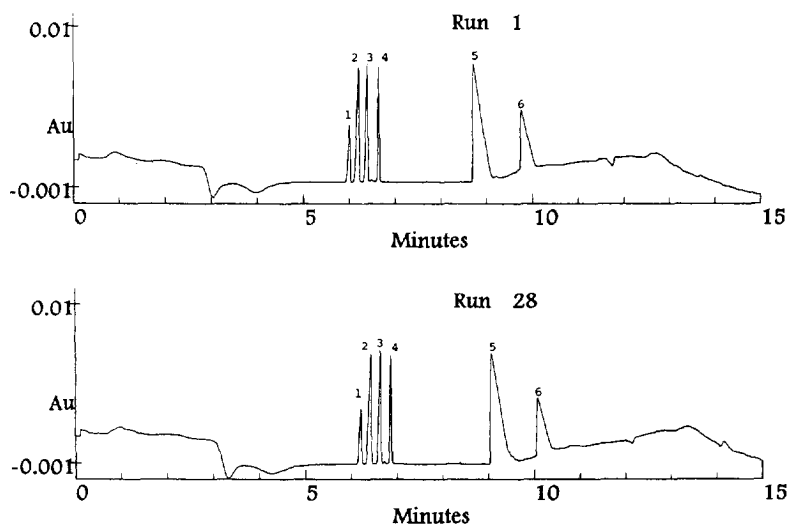


Fig. 2. Separation of six inorganic anions. Capillary zone electrophoretic separation of six inorganic anion standards. Capillary, polyamide resin-coated (coating solution 1.25% polyamide in deionised water), 375  $\mu\text{m}$  O.D., 75  $\mu\text{m}$  I.D., 60 cm length (55 cm to detector); Buffer, 2.25 mM PMA, 1.6 mM TEA, pH 7.9 (adjusted with 1 M NaOH); Control, -20 kV, detector anodic; Injection, gravity, 100 mm, 30 s; Detection, indirect UV, 250 nm; peaks (all 10 ppm): 1=bromide, 2=chloride, 3=sulphate, 4=nitrate, 5=fluoride and 6=phosphate.

from run to run, and gives the same shape when the buffer alone is run, with no sample injection. There is very little change in migration time from run-to-run (Table 1), although no tests have been performed of more than 30 repeat runs.

### 3.3. Coating stability and reproducibility

The reactivity of the polyamide resin is one problem which needs to be addressed and will be the subject of future investigation. The azetidinium ion is highly susceptible to nucleophilic attack [11]. When the coating solution was prepared in a methanol-water mixed solvent to reduce the time taken to

thoroughly dry the resin, it was found that the methanol reacted slowly with the azetidinium via a ring-opening reaction, reducing the ability of the resin to crosslink. Thus, in subsequent experiments water alone was used as solvent, providing a more stable coating. It is thought that the presence of nucleophilic solvents in the running buffer may prove to be detrimental to the stability of the coating. The same may also be true of amines in the buffer solution. The TEA used in this study is necessary to buffer the PMA electrolyte to the required pH, and the authors intend to investigate the use of alternative electrolytes such as chromate. The reactivity of the resin has also presented a problem regarding capillary storage; to date a suitable storage medium has not been found, and the performance of the capillaries is reduced or destroyed when filled with either buffer or water. These problems may be alleviated by the addition of agents to increase the proportion of crosslinking in the resin, or to eliminate, by end-capping reactions, the residual azetidinium ions.

The reproducibility from capillary-to-capillary has been disappointing. The baseline is not exactly reproduced, although the basic shape is similar, while the R.S.D. of anion migration time is as high as 30% ( $n=5$ ). Several capillaries produced using the

Table 1  
Migration time stability for inorganic anions

Peak	Migration times	
	Mean (min)	R.S.D. (%)
Bromide	6.09	1.35
Chloride	6.29	1.41
Sulphate	6.49	1.49
Nitrate	6.73	1.36
Fluoride	8.87	1.58
Phosphate	9.90	1.36

Conditions as for Fig. 2.

method described yielded zero current when the voltage was applied under the conditions detailed in Fig. 2. This was not caused by a blockage in the capillary, but by bubbles entering the capillary when the destination and source vials were rinsed. When the capillary alone was rinsed with buffer, the problem did not occur. At this stage no reasonable explanation has been found for this phenomenon, but it is thought that the problem must be of physical rather than chemical origin.

#### 3.4. Further work

Once the coating stability has been improved and bubble formation within the capillary eliminated, there are a number of further studies which we would like to perform. It is important to assess the variation of EOF within the capillary as a function of pH, since other researchers have found that the EOF may switch direction at a given pH, due to incomplete coverage of the surface silanol groups. It is desirable that coated capillaries be stable and therefore useful over as large a pH range as possible, and therefore work will be undertaken to fully assess the pH-stability of the resin coating. The coating appears stable at the pH of 7.9 used in this study, but data at more extreme pH values has yet to be obtained. It is also intended to use the capillary to separate alternative analytes including cations and proteins, and to attempt chiral separations by including a protein in the coating.

From preliminary studies using scanning electron microscopy, it appears that this technique can potentially yield useful information regarding coating thickness and coverage. It is intended that these lines of inquiry will be pursued further and reported in detail in a subsequent paper.

#### 4. Conclusions

From the preliminary results presented here, it can be seen that the reactive polyamide resin can be

applied to a fused-silica capillary to yield a positively charged, bonded coating which gives good run-to-run reproducibility for the separation of highly mobile inorganic ions. The coating is simple to apply and may be easily derivatised to alter the capillary surface properties. Once the problems described have been rectified, it is hoped that the capillary coating may prove useful in a variety of CE separations.

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