Towards a microchip-based chromatographic platform. Part 1: Evaluation of sol-gel phases for capillary electrophoresis

Silica monolithic columns suitable for implementation on microchips have been evaluated by ion-exchange capillary electrophromatography. Two different silica monoliths were created from the alkyl silane, tetramethyl orthosilicate (TMOS), by introducing a water-soluble organic polymer, poly(ethylene oxide) (PEO), with varying molecular weights into the prehydrolyzed sol. Silica monoliths created using 10 kDa PEO were found to have a much more closed gel structure with a smaller percentage of pores in the μm size range than gels created using 100 kDa PEO. Additionally, the size of the mesopores in the 100 kDa PEO monolith was 5 nm, while those in the 10 kDa PEO gel were only 3 nm. This resulted in a strong dependence of the electroosmotic flow (EOF) on the ionic strength of the background electrolyte, with substantial pore flow through the nm size pores observed in the 10 kDa PEO gel. The chromatographic performance of the monolithic columns was evaluated by ion-exchange electrophoresis, with ion-exchange sites introduced via dynamic coating with the cationic polymer, poly(diallyldimethylammonium chloride) (PDDAC). Separating a mixture of inorganic anions, the 10 kDa PEO monolithic columns showed a higher effective capacity than the 100 kDa PEO column.

Keywords: Ion-exchange capillary electrophoresis / Perfusive flow / Pore flow / Silica monolithic column / Sol-gel phase

1 Introduction

In recent years, there has been an increasing trend towards miniaturization of analytical processes, beginning with the development of capillary electrophoresis (CE) as a faster alternative to high-performance liquid chromatography (HPLC) and more recently, with the development of even faster microchip electrophoresis (ME). While there are many benefits of miniaturization, such as reduced sample size, increased portability, and a significant reduction in analysis time, there are also many technical challenges to be faced in developing a miniaturized device [1]. While microchip fabrication was initially a major issue, there are now many standard approaches to constructing such devices in a variety of materials including silicon, glass, and various polymeric substrates [2]. Due to the technical challenge of manipulating fluid flow on such devices, many of the early analytical applications on microchips have used electrophoresis for separation, and while impressive results have been obtained, the analytical potential is restricted.

Recently, there has been an increased trend to incorporate a chromatographic phase into a microdevice to draw upon the vast knowledge and experience from traditional HPLC methods. While there are several such approaches to introducing a chromatographic phase, technically the simplest to fabricate is to use the channel walls themselves, giving rise to open-tubular columns [3–5]. The drawback to this approach, however, is the low column capacity and as such even more stringent requirements are placed on an already overtaxed detection system due to the need to inject low concentrations to avoid column overloading. The more traditional HPLC approach of packing chromatographic particles has been implemented both for electrophromatography [6, 7] and solid-phase extraction [8, 9], however, many technical challenges must be overcome to provide a reproducible and stable packed system inside the microchannel. An alternative and elegant approach developed by Regnier and co-workers [10], involved fabrication of the chromatographic phase during microchip construction by etching pillar-structures in the substrate to create a micro-column. While this provided some impressive results,
An alternative approach to column fabrication based on “polymerizing” the phase inside a column has arisen because of technical difficulties in fabricating capillary columns for micro-HPLC and capillary electrophoresis (CEC) and should be perfect for implementation on a microchip. In this case, a mixture of precursor materials is placed inside the device with polymerization typically initiated by exposure to heat or light. Monolithic columns created by this process are predominantly organic or inorganic in nature. Several different organic monoliths have been found useful [12], but inorganic monoliths have been dominated by silica phases created from sol-gel chemistry [13]. While organic monolithic columns have been used in microchips for solid-phase extraction [9] and electrophoresis [14], silica sol-gel phases have only been used by our group for solid-phase extraction [8].

Silica sol-gel phases have found use in HPLC for many years, with the first report by Tanaka et al. in 1993 [15]. Since then, the same group has published extensively in this area [16–20], focusing on silica monoliths created from tetramethyl orthosilicate (TMOS) and poly(ethylene oxide) (PEO); chromatographic functionality was introduced by reaction with octadecylsilane after polymerization. Using this basic formulation, they have systematically evaluated the conditions required to produce silica monoliths with the optimum properties for chromatography, and readers are directed to a recent review for a complete discussion of this work [13]. Outside this group, there are few reports on the use of sol-gel phases for HPLC or CEC. Chen and Hobo [21, 22] have used silica monoliths for chiral separations, however, the basic formulation employed was that derived by Tanaka’s group with the only difference being the material used for post-polymerization modification of the surface. Malik and Hayes [23] have used sol-gel monolithic columns for CEC in which chromatographic functionality was added by including an appropriately modified precursor. A silica alkoxide possessing octadecyl and quaternary ammonium functionality was used to provide a reversed EOF as well as reversed-phase chromatographic sites. This approach avoids the time-consuming step of post-polymerization modification as the chromatographic functional groups are included in the precursor mixture. Adopting a similar approach, Dulay et al. [24] reported the use of sol-gel phases constructed from methacryloxy-propyltrimethoxysilane. Unlike other published methods, polymerization was initiated by exposure to light, enabling formation of the monolith to be accurately controlled, by controlling which parts of the column were exposed to the light source. Recently, we demonstrated the purification of DNA from biological samples using microchips filled with sol-gels (Wu et al., in preparation). This approach was found to be much simpler than packing microchips with silica particles and provided better extraction performance. The introduction of chromatographic sites would extend the capabilities of this system to enable rapid separations to be performed.

The aim of this work was a preliminary investigation into the nature of silica-based monolithic columns for eventual implementation of a chromatographic phase on the microchip platform. The recipe developed by Tanaka’s group was used as a starting point, and the influence of varying the molecular weight of the polymer on the physical and electrochromatographic properties was examined. Having already demonstrated the ability to create sol-gel phases in microchips for DNA purification, a simple alternative to Tanaka’s method was investigated for introducing chromatographic functionality, namely by dynamic modification of the silica surface with the cationic polymer, poly(diallyldimethylammonium chloride). A mixture of inorganic anions was separated by ion-exchange CEC to determine the electrochromatographic properties of gels made with different molecular weight polymers.

## 2 Materials and methods

### 2.1 Instrumentation

All separations were conducted using a Hewlett-Packard 3DCE (Agilent Technologies, Waldbronn, Germany) with 75 μm ID (375 μm OD) polyimide-coated fused-silica capillary (Polymicro, Phoenix, AZ, USA) with a length of 33.5 cm, 26.5 cm to the detector, unless otherwise noted.

### 2.2 Chemicals and reagents

Analytical-grade tris(hydroxymethyl)aminomethane (Tris), 10 kDa PEO, and TMOS (99%) were all obtained from Acros Organics (New Jersey, NJ, USA). Poly(diallyldimethylammonium chloride) (PDDAC) with a molecular mass of 200–350 kDa obtained as a 20% w/v solution and 100 kDa PEO were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid was obtained from Fischer Scientific (Pittsburgh, PA, USA) and diluted to ~1 M in water for subsequent use. All background electrolytes (BGEs) were prepared by dilution from a stock solution comprising 500 mM Tris titrated to pH 8.05 with HCl and filtered through a 0.2 μm filter prior to use.
Nitrite, nitrate, iodide and bromide were obtained as the sodium salt and were all of analytical grade. Samples were prepared as a 1 mM stock solution in water and diluted in water to 0.1 mM for injection.

2.3 Column fabrication

Sol-gel precursor mix was hydrolyzed by a modification of the procedure of Tanaka et al. [16–19]. Briefly, 1.06 g PEO (10 or 100 kDa) was dissolved in 10 mL of 0.02 M HCl. 4 mL of TMOS was added with stirring at 0°C and allowed to react for 30 min. This mixture was then degassed using a syringe to create a slight vacuum, and forced through a capillary previously conditioned overnight with 1 M NaOH rinse followed by 1 h at 50°C, a 0.1 M HCl rinse for 10 min, an ethanol rinse for 5 min, then drying with air. Using a disposable 3 mL plastic syringe and a home-made glue-gun apparatus, the sol-gel solution was allowed to flush through the capillary for 10 min before the ends were placed in 0.5 mL plastic micro-centrifuge tubes filled with sol-gel solution then sealed using 5 min epoxy. The assembly was placed in an oven and allowed to gel overnight at 50°C. To avoid destruction of the gel, detection windows were fabricated by burning the polyimide at the appropriate position prior to introduction of the sol-gel precursor. Prior to use, columns were cut to length and rinsed with BGE at 34 bar using an HPLC pump before being mounted in the CE instrument and being conditioned by applying successively increasing voltages, in 2 kV increments, until a stable current was allowed to flush through the capillary for 10 min before the ends were placed in 0.5 mL plastic micro-centrifuge tubes filled with sol-gel solution then sealed using 5 min epoxy. The assembly was placed in an oven and allowed to gel overnight at 50°C. To avoid destruction of the gel, detection windows were fabricated by burning the polyimide at the appropriate position prior to introduction of the sol-gel precursor. Prior to use, columns were cut to length and rinsed with BGE at 34 bar using an HPLC pump before being mounted in the CE instrument and being conditioned by applying successively increasing voltages, in 2 kV increments, until a stable current was obtained for at least 5 min. Chromatographic functionality was introduced by dynamically adsorbing the cationic polymer PDDAC to the surface by adding low concentrations (< 0.1% w/v) to the BGE as described in Section 3.

2.4 Characterization of the silica monoliths

In order to preserve as much of the microstructure of the gels as possible for characterization by nitrogen sorption porosimetry and scanning electron microscopy (SEM), the gels were processed to form aerogels. Aerogels are air-filled gels produced by the sol-gel process then supercritically dried. Gelation was followed by three successive ethanol exchanges over a total of three days. The liquid ethanol was then exchanged with liquid carbon dioxide followed by supercritical drying to obtain the final aerogel samples. Immediately after supercritical drying, the gels were weighed and the geometric dimensions measured manually for density measurements. Nitrogen sorption porosimetry was performed using a Micrometrics ASAP 2010 (Accelerated Surface Area and Porosimetry System) on approximately 0.1 g aerogel samples after an 18 h evacuated bake at 250°C. The adsorption isotherm data were used to determine the internal surface area and cumulative pore volume, using the method of BET (Brunauer, Emmett, and Teller), and the average pore diameter using the BJH (Barrett, Joyner, Halenda) formula. Scanning electron micrographs were taken using a JEOL 6400 after vacuum sputtering with gold/palladium.

3 Results and discussion

Examination of the literature on sol-gel monolithic columns establishes the Japanese group led by Tanaka as the leaders of the field. They have used silica monoliths extensively, starting with molded columns for HPLC [17–20] and more recently capillary columns for micro-HPLC and CEC [16]. The advantages of these columns for HPLC have been extensively shown with superior performance and lower back pressure than conventionally packed columns. A reduction in back pressure by a factor of 20 when comparing sol-gel columns with 5 μm spherical particles means that more rapid and efficient separations can be obtained using the same pressure than when using a packed column. This makes the use of monolithic columns ideal for implementation on microchips where interfacing can be problematic and high-pressures may shatter the device.

Water-soluble polymers have previously been used in sol-gel formulations to produce macroporous (IUPAC definition: pores with a width exceeding 50 nm) samples by inducing a phase separation during gelation [16–20, 25, 26]. With TMOS used as the precursor, 10 kDa PEO is added to create a gel with large μm size macropores throughout the network. Several papers have dealt with modification of the recipe to tune the properties of the final gel, such as varying the silica (TMOS) content, varying the polymer content, and by treating the gel with NH₄OH after gelation. Of interest here, are the major variations in macropore size obtained by small variations in the polymer content [17, 19]. For example, varying the polymer content from 0.94 to 1.06 g (per 10 mL total) results in a change in the macropore size from 3.46 to 1.26 μm. This suggests that the system is highly sensitive to small variations in starting composition, and that by varying other factors similar dramatic changes may be seen.

3.1 Influence of polymer molecular weight on gel structure

One of the obvious parameters that has not been varied to date is the molecular weight of the polymer. Given that considerable differences in the gel can be obtained by varying the amount of polymer, we were curious as to
whether varying the molecular weight would have similar effects. To examine this, we selected 100 kDa PEO, the same type of polymer employed by Ishizuka et al. [16], but with each polymer molecule comprising approximately 10 times more repeat units than was used by Ishizuka et al. Surprisingly, when initial gels were created using the same formulation as that for the 10 kDa PEO (i.e., the same mass amount of PEO), pure white gels similar to those obtained using the lower molecular weight PEO were obtained suggesting that light was being scattered by the large pores resulting from this formulation. In contrast, gels created using the same molar amount of 100 kDa PEO provided opaque gels suggesting the gel comprised small pores which were unsuitable for electrochromatography. Examination of the two white gels (obtained using the same mass amount of both molecular weight polymers) by SEM establishes a significant difference between the gels created with different molecular weight PEO, as illustrated in Figs. 1A and B. It is readily apparent when comparing these two gels that their structures are substantially different. The 100 kDa PEO gel comprises a more open network structure of distinct branches than the 10 kDa PEO gel which is much more globular in appearance and has a more solid packed structure. It is clear that there are many pores in the 100 kDa PEO gel, and on average, they are approximately 1–2 μm in diameter; the 10 kDa PEO gel contains relatively few μm size pores, with the majority of pores significantly less than the 1 μm level. As a result of the increase in solid structure size, we suspect that the influence of PEO molecular weight upon gel structure is related to the point at which phase separation occurs in the prehydrolyzed sols and will be dealt with in a forthcoming publication. Other important physical properties of the two gels are shown in Table 1. As anticipated, the hydrodynamic flow properties of the two gels in capillaries are substantially different. The pressure required for a flow rate of 250 μL/h through the 100 kDa PEO gel was less than 30 bar, while that for the 10 kDa PEO gel was 130 bar. This is a significant increase in back pressure and suggests that the 100 kDa PEO gel may be more amendable to use on a microchip, particularly when pressure-driven flow is to be employed. Also of interest is the similarity between the surface areas of the two different gels. Both gels provide surface areas near 270 m²/g, which, considering the immense visual difference between the two gels, is quite surprising. It is also interesting to note, that the surface area of these gels is lower than that reported by the sol-gel columns made by Minakuchi et al. [19], where surface areas as high as 330 m²/g were reported. Given that we do not condition our gels with NH₄OH after gelation to increase the size of the mesopores (IUPAC definition: pores with a width between 2 and 50 nm), this difference in surface area is not surprising. Using BJH adsorption analysis to determine the population of mesopores, there is a major difference between the two, with the 10 kDa PEO gel having a much smaller pore volume

**Table 1.** Physical properties of two silica monoliths using 100 kDa PEO and 10 kDa PEO

<table>
<thead>
<tr>
<th>Property</th>
<th>100 000 PEO silica monolith</th>
<th>10 000 PEO silica monolith</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²/g)</td>
<td>279</td>
<td>279</td>
</tr>
<tr>
<td>Pore volume (cm³/g)</td>
<td>0.157</td>
<td>0.336</td>
</tr>
<tr>
<td>Pore diameter (nm)</td>
<td>2.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Backpressure (bar) for flow rate of 250 μL/h</td>
<td>130</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>0.224</td>
<td>0.161</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>89.4</td>
<td>92.3</td>
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than that of the 100 kDa PEO gel, with pore sizes of 3 nm and 5 nm, respectively. Given the large differences between physical structure of the gels (number of macro pores, size of mesopores and total porosity), it was anticipated that these gels would have substantially different electrochromatographic properties, namely, flow properties in an electric field, and effective capacity.

3.2 EOF characterization

It has been known for many years that an electrical double layer is established near charged surfaces, and when placed in an electric field, the ions in this layer move, resulting in a fluid flow called electroosmotic flow (EOF) [27]. In empty capillaries, EOF is only generated from the capillary walls, but when the capillary is filled with charged particles (such as those employed for chromatography) EOF is generated in both the interparticulate spaces and also in the pores of the material. The later effect, called “pore flow” or “perfusive flow”, has been of great interest over the last few years as it can improve the separation in two ways. Firstly, it has been shown that pore flow supplements the interparticle flow in packed CEC columns and provides a higher EOF and thus more rapid separations. Secondly, the flow profile is more evenly distributed over the cross section of the capillary resulting in a decrease in plate height due to a reduction in the eddy diffusion term in the van Deemter equation [28–35].

The underlying key to successful pore flow is the relationship between the diameter of the pores ($d_p$), and the double layer thickness from the surface (given by $\delta$). A convenient way to influence pore flow is to change the double layer thickness, which is related to the ionic strength by the following equation:

$$\delta = \left( \frac{\varepsilon_0 \varepsilon_r RT}{2IF^2} \right)^{0.5}$$

(1)

where $\varepsilon_0$ is the permittivity of a vacuum, $\varepsilon_r$ is the dielectric constant, $R$ is the universal gas constant, $T$ is the temperature, $I$ is the ionic strength of the electrolyte, and $F$ is the Faraday constant [29, 34]. It can clearly be seen that there is an inverse square relation between the double layer thickness and the ionic strength, with an increase in ionic strength resulting in a decrease in thickness. For CE in which the diameter of the capillary is much greater than the double layer thickness and the ionic strength, with an increase in ionic strength results in a decrease in thickness. For CEC in which the diameter of the capillary is much greater than the double layer thickness, increasing the ionic strength decreases the EOF due to compression of the electrical double layer according to the inverse square relationship shown in Eq. (1). This effect is also seen in CEC, but the problem is complicated when porous material is used. In systems where there is double layer overlap within the pores, an increase in ionic strength will reduce the double layer thickness, and increase the overall flow due to the increased flow through the pores. This effect will continue until a certain ionic strength is reached at which stage compression of the double layer will result in a decrease in EOF as in conventional CE. Variation of ionic strength is therefore a simple way to evaluate whether there is pore flow in the CEC system.

To evaluate the EOF properties of the two sol-gel CEC columns, the concentration of Tris-HCl (pH 8.05) was varied from 5 to 50 mM, with acetone used as an EOF marker. For comparison, measurements were also performed using the same BGE in an empty capillary. Figure 2a
shows the variation of EOF with Tris-HCl concentration for the three different columns. For the empty capillary, the reduction in EOF with increasing ionic strength followed an inverse-square relationship as expected from theory. However, the situation is more interesting when the two sol-gel columns are considered. In the 10 kDa PEO sol-gel capillary, the EOF increases markedly as the concentration of BGE is increased from 5 to 40 mM Tris-HCl, after which it decreases as expected from theory. This pattern was seen at several different pH values and also when the EOF was reversed by dynamically coating the surface with a cationic polymer (not shown). The most likely explanation for this increase in EOF is due to an increase in pore flow resulting from compression of the double layer as the ionic strength increases. This effect has been previously reported using several different pore sizes [28, 29, 35], but generally occurs at much lower ionic strengths than we employed here. As such, we believe this increase in flow arises from the nanometer size mesopores, and not the larger macropores.

The variation of the EOF with BGE concentration for the 100 kDa PEO gel is not as dramatic as that observed for the 10 kDa PEO gel, with the EOF reduced in an almost identical fashion to that of the empty capillary. There is a very slight deviation at 30 mM, which we tentatively attribute to a change in pore flow corresponding to the 5 nm mesopores. This effect is not as significant as that in the 10 kDa PEO with the flow contribution from pore flow in these columns estimated to be almost 100% for the 10 kDa PEO column but only 10% when the 100 kDa PEO was used. This is due to the greater number of large μm size pores in the 100 kDa PEO gel and as such, the relative contribution to the overall flow from the 5 nm micropores is not as substantial.

To examine the relationship between EOF and double layer thickness, the double layer thickness was calculated in the various concentration BGEs according to Eq. (1) and the values of the constants given by Dearie et al. [29]. Figure 2b shows variation in linear flow rate with double layer thickness for the two sol-gels examined in this study, and data for two capillaries packed with 5 μm silica with 8 nm and 30 nm pores published by Cikalo et al. [28]. While there are differences in the conditions we used and those employed by Cikalo et al., such as the addition of acetonitrile and the pH, this has been accounted for when calculating double layer thickness. As such, there will be differences in the EOF magnitude between the two systems. Given that we are examining changes in EOF with double layer thickness, however, it is appropriate to compare the two systems and draw some preliminary conclusions. First, the linear velocity for all four columns shows an initial increase in magnitude with decreasing double layer thickness (increasing ionic strength), which reaches a maximum (indicated by the arrows) and then decreases. This increase and subsequent decrease is expected from theory and has been discussed in more detail above regarding the profile of the 10 kDa PEO sol-gel. Second, for material containing mesopore sizes of 3, 5, and 8 nm, the highest value of EOF appears to occur when the double layer is half that of the mesopore size, i.e., when 2δ = d_p. This point corresponds to a transition point: when 2δ > d_p, double layer overlap occurs and EOF is reduced; when 2δ < d_p, the double layer has been compressed an appropriate amount such that overlap no longer occurs and the EOF now behaves in a similar fashion to a relatively large diameter capillary. We wish to stress at this stage, that it is not possible to make any final conclusions regarding the position of the maximum in EOF with regard to pore size, but the data suggests that there is some correlation. More work is clearly necessary in this area, particularly considering the position of the maximum corresponding to the 30 nm 5 μm silica.

Finally, it should be noted that we have only dealt with flow rates here and not addressed the issue of efficiency. It is anticipated from the original work by Pertorius et al. [27], that when the size of the flow channel is similar to the size of the double layer overlap, the flow profile will be more parabolic thanplug-like. While we generally obtained quite low efficiencies (typically 50 000 plates) no attempts were made to investigate this as numerous columns with different mesopore size would be required for a complete study.

3.3 Chromatographic characterization

Having demonstrated the substantially different flow properties obtained using the two different sol-gel columns, the next stage was to examine the chromatographic properties of these gels. To do this, it was necessary to introduce chromatographic functionality onto the material, as the potential applications available on unmodified silica are generally limited. While there are many different methods for introducing functionality onto silica monoliths, the most common is reaction of the silica post-gelation with silanizing reagents. However, one of the problems associated with this approach is the need to dry out the silica matrix due to shrinkage. To avoid this problem, functionality may be included in the precursor mixture by selecting appropriate silica derivatives [23, 24], however, this is often complex and may require complete redevelopment of the sol-gel process.
A simpler method that has recently become popular in CEC is the use of dynamic and adsorptive coatings. These approaches have found extensive use in CE for controlling EOF and surface adsorption of large molecules like proteins [36, 37]. The advantage that these approaches have over other methods for CEC is that functionalization occurs after column fabrication ensuring similar surface properties in packed and unpacked sections of columns. Furthermore, the surface is modified simply by including the coating material in the BGE, or by flushing prior to use. The use of dynamic and adsorbed coatings for CEC has been recently reviewed by Zou and Ye [38].

To modify the surface of the silica sol-gel, we decided to use the cationic polymer, PDDAC to provide ion-exchange chromatographic sites. This polymer has been used extensively in CE, for both surface coatings [36, 37, 39] and as an ion-exchange phase in electrokinetic chromatography [40–44], and should provide a simple and effective means to evaluate the chromatographic properties of the different gels. However, it is known that as an adsorptive coating it has a limited lifetime and therefore may be more functional as a dynamic coating. To evaluate its potential as both a dynamic and adsorptive coating, the amount of PDDAC was varied and the mobility of four common inorganic anions measured. Chromatographic interaction of the ions with PDDAC, either as a pseudophase or adsorbed onto the surface of the sol-gel, will result in a reduction in observed mobility of the ions. Figure 3 shows the change in mobility with decreasing PDDAC concentration in an empty (a) and 100 kDa PEO sol-gel capillary (b).

In the empty capillary, since there is no solid phase except the capillary wall, PDDAC will essentially function as a pseudophase and will act in a similar fashion to micelles. Thus, reducing the concentration of PDDAC in the empty capillary will result in a reduction in the amount of PDDAC with which the ions can interact and thus, an increase in mobility will result. When no PDDAC is added to the electrolyte, I- migrates with Br- indicating a small amount of residual interaction with PDDAC adsorbed on the capillary wall; this clearly indicates its ability to function as a semipermanent coating. Due to the low stationary phase to mobile phase ratio, the amount of retention of I- is only marginal. In contrast, reducing the PDDAC content in the BGE for the 100 kDa PEO sol-gel capillary results in only a slight change in mobility, and, with no PDDAC added, there is still considerable retention. Thus, retention is dominated by PDDAC adsorbed onto the surface of the monolith and not PDDAC acting as a pseudophase. This clearly indicates the increased surface area of the sol-gel capillary when compared to the empty capillary and the potential of these columns to provide chromatographic separations. However, when PDDAC was removed from the BGE, it was found that as an adsorptive coating, stability was poor, with generally only 2 h of operation obtainable before reconditioning was required. A similar result was observed for the 10 kDa PEO monoliths (data not shown).

To compare the chromatographic performance of the two sol-gel columns, a BGE comprising 30 mM Tris-HCl and 0.025% w/v PDDAC was selected. The concentration of BGE was selected on the basis of EOF properties to provide reasonable flow in both columns, while the polymer was included to provide stability to the adsorbed coating. Separation of the 4 ions, Br-, I-, NO3-, and NO2- and the EOF (visualized with acetone) in the two different sol-gel columns and an empty capillary is shown in Fig. 4. For simplification, the ions are numbered according to their CE migration order. It can be seen that the observed
mobility of I\(^{-}\) decreases in the order empty < 100 kDa PEO < 10 kDa PEO, indicating that the 10 kDa PEO monolith has a much higher effective capacity than the 100 kDa PEO monolith. Given that the surface area of these two columns is almost identical, the difference in retention can only be attributed to difference in the gel structure. As the 10 kDa PEO monolith has a much more closed pore structure, it is not surprising that this displays more retention than the 100 kDa PEO monolith, which has much larger pores and a more open structure. It is interesting to note that the separation order of Br\(^{-}\) and NO\(_3\)\(^{-}\) is reversed in the 10 kDa PEO column when compared to the empty capillary. These two ions can be difficult to separate by ion-exchange chromatography, and successful separation is typically only achieved when secondary interactions (such as π–π interaction) are present. This suggests that PDDAC is capable of providing such secondary interactions, and it is currently undergoing investigation for other types of separations. The efficiency for I\(^{-}\), the most retained analyte was approximately 50,000 plates, much lower than typically obtained in CEC. This is most likely due to parabolic flow due to pore flow in the nm size pores given that the double layer thickness is comparable to that of the pore size. One possible way this may be avoided is by postgelation treatment using NH\(_4\)OH in a manner similar to the procedure employed by Ishizuka et al. [17].

4 Concluding remarks
Changing the molecular weight of the water-soluble polymer PEO used in the sol-gel precursor mixture significantly alters the physical and electrochromatographic properties of the silica gel. Changing the polymer from 10 kDa PEO to 100 PEO significantly changes the structure of the gel with the latter having a more open gel structure and more \(\mu\)m size pores than the 10 kDa PEO gel. The 100 PEO monolith also had larger micropores than the 10 kDa PEO monolith with sizes of 5 and 3 nm, respectively. As a consequence, significant pore flow was observed in the 10 kDa PEO gel, with a maximum in EOF corresponding to an ionic strength at which the double layer was approximately half the pore diameter. Comparing with EOF values reported from the literature, suggests that the highest EOF will be obtained when the above conditions are true, and may be an alternative method to BET measurements for pore size quantification. Chromatographic performance of the silica monolithic columns was evaluated by separating inorganic anions by ion-exchange capillary electrochromatography. Chromatographic interaction was found to be much greater in the 10 kDa PEO monolithic column than in the 100 kDa PEO column due to the denser structure of the silica monolith.

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