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Fast ion chromatography of inorganic anions and cations on a lysine bonded porous silica monolith

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Abstract

A 0.46 cm × 10.0 cm silica monolith column was modified through the in situ covalent attachment of lysine (2,6-diaminohexanoic acid) groups. Due to the zwitterionic nature of the resultant stationary phase, the modified monolithic column contained both cation and anion exchange capacity. In the case of cation exchange, the capacity was found to be relatively low at between 5 and 6.5 μmoles Me^{2+} per column. However, as expected, the lysine monolith exhibited a higher anion exchange capacity at 12–13 μmoles A^- per column (at pH 3.0), which was found to be dependent upon column pH, due to the dissociation of the weak acid carboxylic acid groups. High-performance separations of transition metal cations and inorganic anions were achieved using the modified monolith, with the effects of eluent concentration, pH and flow rate evaluated. Using elevated flow rates of up to 5 mL/min the separation of nitrite, bromate, bromide, nitrate, iodide and thiocyanate was possible in approximately 100 s with peak efficiencies of between 50 and 100,000 N/m and retention time %RSD of under 0.3%.

Keywords: Monolithic silica; Lysine; Cations; Anions; Ion chromatography

1. Introduction

The recent commercial introduction of both silica and polymer monolithic columns into the LC market has motivated scientists from both academia and industry to study their performance and application [1,2]. The unique properties of monoliths, in particular their tolerance to high flow rates while maintaining excellent peak efficiencies, and the rapid speed of chromatographic separations that can be achieved at acceptable backpressures, make the monolithic column format superior in some applications to the more commonly used packed columns. According to Sinz and Cabrera, "monolithic silica technology has opened the doors on a new era of dramatically higher sample throughput and added new opportunities to improve separation performance" [3]. However, due to the fact that monolith column technology is still relatively new, the number of different stationary phase chemistries and separation modes remains much smaller than that available for packed columns [4]. For example, both polymer and silica based monolithic columns have overwhelmingly been applied to reversed-phase separations only, and until recently have received only limited attention in other modes of LC, most notably ion exchange and ion chromatography (IC) [5]. Much of this attention is based within bioanalysis, where work has been carried out using short polymer based monolithic columns and discs for the rapid isolation of large biomolecules from complex samples [6]. However, recently a limited number of workers have been exploring the potential use of monolithic phases for rapid high-performance separations of smaller ionic species, including inorganic anions and cations [7–12], and an early review of these early studies has recently been compiled [5].

In IC the approach taken to-date to produce high-performance monolithic ion exchangers is to either dynamically modify or permanently coat reversed-phase monoliths with liquid ion exchangers [13–16]. However some potential problems exist with this approach, namely the possible

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variations in coating stability and intolerance of the coated phase to changes in eluent composition and column temperature. To solve these difficulties a different approach was employed by Sugrue et al., who colvalently bonded a chelating ion exchanger (iminodiacetic acid) to a bare silica monolith using on-column modification and applied the new column to the rapid separation of alkali, alkaline earth and transition metal ions [17,18]. In addition to the above studies some interesting work has been reported by Pack and Risley who investigated the direct cation exchange capacity of unmodified silica monoliths when used in so-called hydrophilic ion chromatography mode [10].

Recently, good selectivity and ion chromatographic performance has been demonstrated for a number of amino acids chemically bound to silica gels. Depending on the structure of the amino acids attached to the silica surface, the prepared ion exchanger could have cation, anion or zwitterion exchange properties based upon the occurrence, location and pK_a of oppositely charged groups within the molecule [19–21]. In work by Nesterenko et al. a number of ion-exchange stationary phases were prepared by the immobilisation of amino acids of different structure (Asp, Glu, Val, Try, Pro, Hypro, Arg and Lys) onto silica gel [22,23]. In aqueous solutions, such amino acids behave as inner salts and are generally both weak acids and weak bases. At low pH the conjugate acid is the predominant form, whereas at intermediate pH, amino acids exist in equilibrium between the neutral molecules and the zwitterionic form. For example, using an acidic 3 mM perchloric acid eluent with a lysine bonded silica gel column Elefterov et al. demonstrated the simultaneous separation of alkali and alkaline earth metal cations based upon weak cation exchange at the carboxylic acid site of the lysine molecule [24].

In the work presented here, a bare silica monolith has been covalently modified with lysine using on-column modification to produce a high-performance monolithic zwitterionic ion exchanger. The new phase was investigated for both its anion and cation exchange capacity and the effect of eluent pH upon this capacity and ion selectivity determined. The modified monolith exhibited unique and unusual selectivity compared to that seen previously with both lysine modified silica gels and standard ion exchangers. The lysine monolith was also evaluated for its performance at elevated flow rates for its application to fast IC.

2. Experimental

2.1. Equipment

An Applied Biosystems 400 Solvent Delivery System (Foster City, CA, USA) was used to deliver the eluent for the majority of the work involving low to moderate flow rates, while for the work requiring flow rates greater than 4.9 mL/min or the use of a pH gradient, a Waters Model 600E Multisolvent Delivery System (Waters, Milford, MA, USA) was used. Samples were injected manually using a Rheodyne injector fitted with a 20 μL injection loop. A 1050 series, Hewlett Packard (Palo Alto, CA, USA) UV–vis spectrophotometric detector was used for the detection of anions and cations. A Gilson pump, Model 302 (Anachem, Luton, UK) was used for the introduction of the post-column reagent (PCR) for transition metal work, which was mixed at room temperature with the eluent using a 0.5 m PEEK reaction coil (0.01 in. i.d.). For suppressed conductivity detection a Dionex conductivity detector (Dionex Series 4500i) was used in conjunction with a CSRS Ultra 4 mm membrane suppressor (Dionex). Data acquisition was at a rate of 10 Hz with processing of chromatograms performed using a PeakNet 6.0 chromatography workstation (Dionex).

2.2. Reagents

All chemicals used were of analytical reagent grade, and were supplied by Sigma–Aldrich (Tallaght, Dublin, Ireland). All eluents and standard solutions were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA), and were filtered through a 0.45 μm filter and degassed by sonication. For indirect photometric detection, λ = 279 nm, a phthalate eluent (phthalic acid (Aldrich), pH adjusted to 6.5 dilute NaOH) was employed. Both KCl (Aldrich) and phosphate buffer solutions were used as eluents for direct photometric detection, λ = 225, 214 nm. Phosphate buffer solutions (1–50 mM) were prepared using monobasic-, dibasic sodium phosphate (Aldrich) and phosphoric acid (85%, Riedel-de Haën, Hanover, Germany) in the appropriate ratios to give the required pH values. For conductivity detection, a nitric acid eluent was employed (Fisher Chemicals, AGB Scientific, Dublin 11). Dilute solutions of NaOH and HCl were used to adjust the pH of the eluents. The PCR reagent used for the detection of cations consisted of a mixture of 0.4 mM 4-(2-pyridylazo) resorcinol and 0.5 M ammonia, adjusted to pH 10.5. Low-level standard solutions of metal cations and anions were generally made up freshly each day from stock solutions (1000 mg/L).

2.3. Column preparation

A PEEK lined bare monolithic silica column (Performance SI) of 10 cm length and 4.6 mm i.d. was purchased from Merck KGaA (Damstadt, Germany). According to the manufacturer the silica monolith has a surface area of 300 m^2/g and a bimodal porous structure. The mesopores constitute 80% of the measurable pores with pore diameters centered around 12 nm, and provide the high specific area of 300 m^2/g to the silica skeleton [25,26]. Before modification the surface of the silica monolith was activated. This was achieved by washing the column with hot distilled water (DW), through placing the column a thermostated water bath at 60 °C for 4 h [27]. The modification of the column with lysine groups was performed at 70 °C by recycling 80 mL of a water solution containing pre-mixed

γ-glycidoxypropyltrimethoxysilane and lysine through the column (both from Fluka Chemie GmBH, Buchs, Switerland), in accordance with the method first described by Gimpel and Unger [28], and more recently presented and discussed in detail by Lisichkin et al. [29]. The recycling system consisted of glass beaker containing the above reagents, a Waters Model 510 HPLC pump (Waters, Milford, USA) and the thermostated monolithic silica column. The reagent mixture was pumped at a flow rate 0.5 mL/min for 6 h. Then the column was then washed with 0.01 M nitric acid for approximately 1 h and equilibrated with the eluent before use.

3. Results and discussion

3.1. Cation selectivity—alkali and alkaline earth metal ions

Elefterov et al. [24] demonstrated on a packed bed lysine bonded silica gel column that when using a dilute perchloric acid eluent a linear dependence of $\log k$ versus $\log [E^-]$ was observed for alkali and alkaline earth metal ions, indicating that cation exchange was the predominant retention mechanism. In this work, at the concentration of perchloric acid used (pH 2.5–2.9), both the amino groups of the bonded lysine were assumed to be protonated (pK_a's being 9.2 and 10.7 [30]), giving the lysine molecule an overall charge of +1 (ignoring likely changes to the pK_a of the α-amino group through the attachment to the silica surface). With this in mind it is clear that the spatial accessibility of the carboxylic acid group within the bound molecule is such that the repulsion effect of the protonated amino groups is small enough to still allow cation exchange take place. According to Nesterenko et al. [23,24], when studying lysine bonded silica gels, the phase exhibited mainly cation-exchange properties because of the formation of a three-layer sandwich structure. This included a negatively charged layer of silanols, a positively charged layer of protonated amino groups coordinated to surface silanols via hydrogen bonding, and the negatively charged carboxyls at the outer layer. Such cation-exchange properties have also been observed for other diaminocarboxylic acids attached to silica, including 2,3-diaminopropionic, 2,5-diaminopentnoic acid (ornithine) and arginine [31]. In each case the outer layer of dissociated carboxyls exhibited a lower affinity to the alkaline earth metals than other known carboxylic or sulfonic cation exchangers (obviously due to existing repulsive effects), thus allowing the use of eluents with lower concentration, resulting in reduced analysis time, and the ability to separate $Li^+ < Na^+ < NH_4^+ < K^+ < Mg^{2+} < Ca^{2+} < Ba^{2+}$ in under 15 min.

Repeating the work of Elefterov et al. [24] with the lysine modified monolithic column over the pH range 2.5–3.3, using a dilute nitric acid eluent (with suppressed conductivity detection), very little selectivity or retention of alkali or alkaline earth metal ions was observed (under the pH range investigated, obviously the majority of the outer carboxylic acid groups should be dissociated, as for α-amino acids the pK_a values for the acid site are between 1.8 and 2.1). This immediately indicated either a much lower capacity column or a change in the charge distribution of the attached lysine molecule on the monolithic silica surface. The latter could be attributed to the less acidic properties of the silica matrix of the Chromolith column used and subsequent weakened ability to coordinate amino groups of bonded lysine molecule [10]. However, the limited selectivity shown by the new monolithic column for the alkali metal ions did match that shown by Elefterov et al., namely $Na^+ < NH_4^+ < K^+$, indicating some limited cation exchange capacity, although the retention order for the alkaline earth metal cations differed distinctly, with $Ba^{2+} < Mg^{2+} < Ca^{2+}$. Such a retention order for alkaline earth metal ions has been shown previously with alternative aminocarboxylic acid groups, where weak complexation has played a role in cation retention [29]. Lysine in acid-neutral solutions is known to bind cations through the α-amino and α-carboxylate groups to form M(HL)*n* complexes [30]. If this were indeed the case here it would suggest non-hindered access to both the carboxylic acid group and the amino group in the bonded ligand, which would obviously reduce apparent cation exchange capacity, particularly for the alkali metal ions, which are known to only interact with lysine through ion-exchange and not complexation. Fig. 1 shows the selectivity exhibited for the alkali and alkaline earth metal ions using the lysine monolith under weak acidic conditions.

3.2. Cation selectivity—transition and heavy metal ions

Initially the selectivity of the lysine monolith was evaluated for selected transition metal ions using simple KCl and HNO_3 eluents. The metal ions investigated included manganese(II), cobalt(II), cadmium, zinc and lead. Using eluent concentrations above 5 mM KCl (pH 2.5) resulted in the rapid elution of all except zinc and lead. The selectivity for lead shown by the lysine monolith was particularly strong, with lead being retained for >30 min when using an 80 mM KCl eluent (pH 2.5). This high selectivity for lead matches that noted by Elefterov et al. [24], indicating the lysine functionality was indeed governing selectivity being exhibited by the monolithic column.

Clearly complexation between the metal ions and the lysine group was resulting in the selectivity shown and so eluent pH was varied to manipulate selectivity. Table 1 shows the retention data for the metal ions using a 5 mM KCl eluent adjusted to pH 4.0, 4.5 and 5.1. Once again the retention order shown matches that seen previously with alternative aminocarboxylic acid phases [31], and is consistent with published stability constant data for M(HL) type lysine–metal complexes (calcium, $K_1 = 0.72$ (25 °C, 0.7 M KCl); manganese, $K_1 = 2.18$ (20 °C); cobalt, $K_1 = 3.62$ (25 °C, 1.0 M KCl); cadmium, $K_1 = 3.70$ (30 °C, 1.0 M KNO_3); zinc, $K_1 = 4.06$ (25 °C, 0.2 M KCl)) [30].

The data shown in Table 1 shows the strong pH dependence exhibited by the column and the strong affinity for

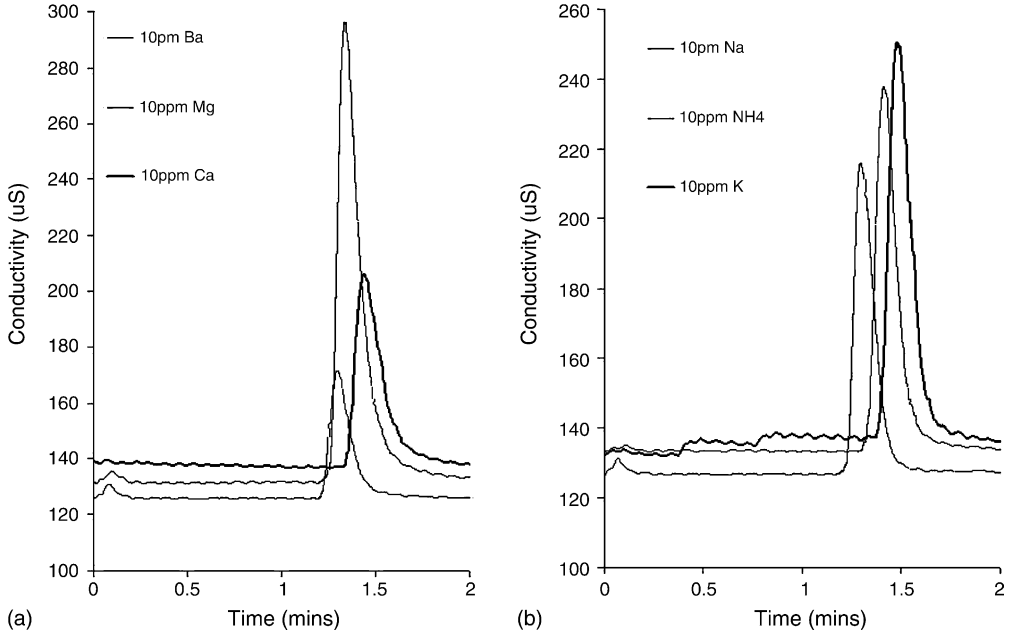

Fig. 1. Overlays of chromatograms for (a) alkali and (b) alkaline earth metal ion standards obtained on the lysine modified monolith. Conditions: eluent = 3 mM HNO_3; flow rate = 1 mL/min; detection = suppressed conductivity.

both zinc and lead. Peak shapes rapidly broadened with retention, particularly for zinc, once more indicative of surface complexation being the dominant retention mechanism. However, it is also possible that the retention shown may include secondary interactions with the many unreacted surface silanol groups, which could also explain some of the peak tailing seen. In general however, the rapid peak broadening is a typical product of the slow exchange kinetics associated with surface chelation compared to ion exchange interactions. Fig. 2 shows an overlay of chromatograms of low mg/L standards obtained using a 5 mM KCl eluent, pH 4.5 at a flow rate of 2 mL/min.

3.3. Cation capacity

During the above eluent pH study, it was noted that retention data varied with sample concentration, for example when injecting mixed standards. Combined with the poor peak shapes, this indicated a low overall capacity. Therefore, the column capacity was determined and compared to an unmodified silica monolithic column of similar dimensions (Merck Chromolith Si) to ascertain the capacity due to complexation with bound lysine groups under non-acidic conditions. Columns were first washed with 10 mM dipicolinic acid solution for 30 min to remove any bound cations. The columns were then washed with 1 M KCl for a further 30 min to convert the column to the potassium form, followed by deionised water for 30 min. This procedure was followed by passing a 1 mM solution of a selected transition metal through the columns at 1 mL/min and monitoring the column eluate, starting at the point the metal solution enters the column. Both cadmium and zinc solutions were used to determine capacity. The results obtained showed the bare silica monolith itself exhibited a very small ion exchange capacity equivalent to between 1.5 and 3.0 μmoles Me^{2+}, and this increased to 5.1–6.5 μmoles Me^{2+} for the modified column. The ion-exchange capacity of the bare silica monolith was obviously due to surface silanol activity, a reduced degree of which will also be present on the modified monolith. The low capacity exhibited by the modified monolith for divalent cations does not necessarily reflect the absolute amount of bound lysine groups. This is due to the unknown extent of steric hindrance on the bound lysine limiting the complexation of the above metal ions.

Table 1
Retention data for transition/heavy metal ions on lysine modified monolithic column—effect of eluent pH

Eluent pH	Mn(II)		Co(II)		Cd(II)		Zn(II)		Pb(II)	
	t_R (min)	k	t_R (min)	k	t_R (min)	k	t_R (min)	k	t_R (min)	k
4.0	1.2	0.15	1.9	0.81	3.4	2.24	4.8	3.57	>30	–
4.5	1.3	0.24	2.2	1.09	4.0	2.81	7.6	6.24	>60	–
5.1	1.3	0.24	4.5	3.29	5.9	4.61	30.5	28.05	>60	–

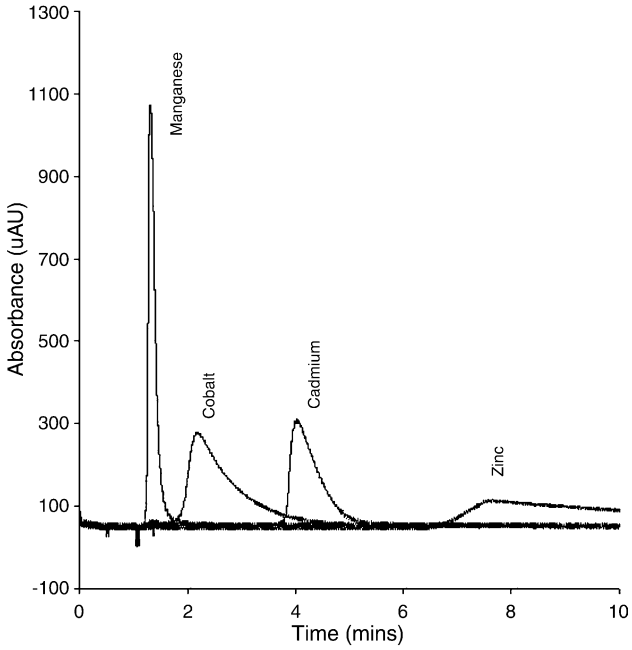

Fig. 2. Overlay of Mn(II), Co(II), Cd(II) and Zn(II) chromatograms obtained on the lysine modified monolith. Conditions: eluent = 3 mM KCl, pH 4.5; flow rate = 2 mL/min; detection = post-column reaction with PAR, absorbance at 495 nm.

3.4. Anion selectivity

The zwitterionic nature of lysine means the modified monolith should exhibit a degree of anion selectivity, due to the net positive charge on the molecule under acidic and neutral pH conditions. However, it has been previously postulated that the bound lysine results in the formation of a multi-charged layer on the silica surface, with an inner negatively charged silica surface from unreacted silanol groups, followed by a positive middle layer formed by the protonated amino groups, followed by a negatively charged external layer resulting from the carboxylic acid groups. If this is the case it is clear that under acid-neutral conditions the deprotonation of the carboxylic acid group ($pK_a = 2.16$) will also play a role in the observed selectivity due to repulsive effects, and this contribution toward selectivity should vary with eluent pH.

Previous studies have not reported significant anion selectivity on lysine bonded silica, instead all have focused on separation of cations [23,24,31,32]. Here anion selectivity was initially evaluated using a test mix of UV absorbing anions, namely nitrite, bromate, bromide, nitrate, iodide and thiocyanate. Initial experiments were carried out using dilute (0.5–3.0 mM) KCl eluents (pH 6.0) with direct UV detection at either 214 or 225 nm. Under these conditions, the lysine column offered only limited retention and selectivity, with all of the test anions eluting almost immediately at eluent concentrations above 3 mM. In an attempt to increase retention, the eluent pH was varied over the range 3–6.5. To achieve this, the eluent was changed from a KCl solution to a phosphoric acid/phosphate buffer solution. Fig. 3 (a) shows the effect upon retention of varying eluent pH (eluent = 1 mM phosphate buffer). As the elution strength of the eluent also varied towards the extremes of the pH range investigated, the

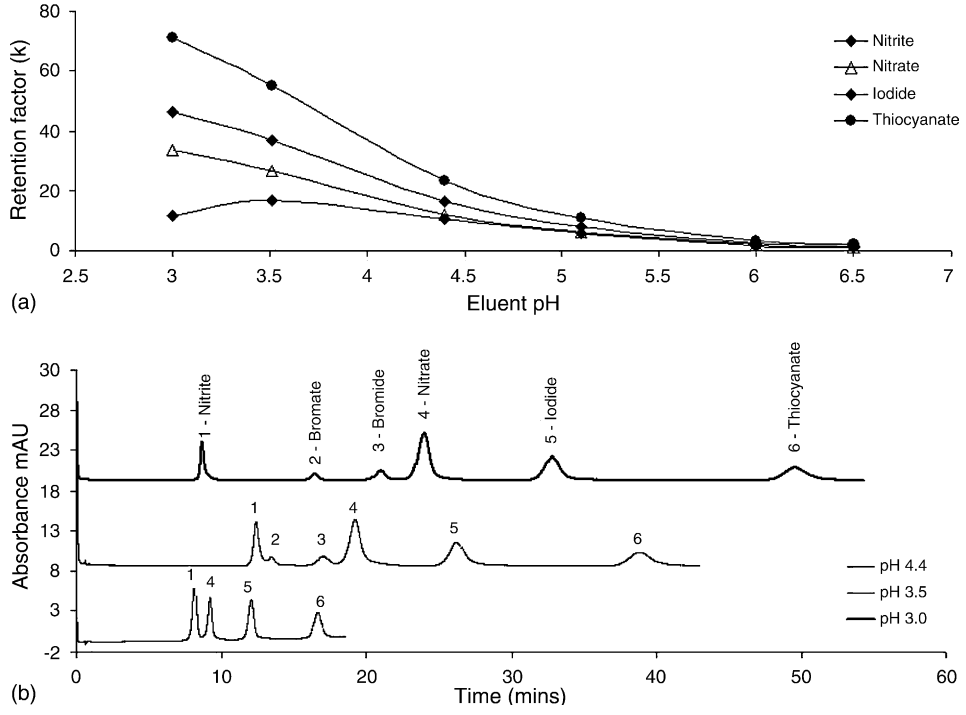

Fig. 3. Effect of eluent pH on anion capacity. (a) Eluent = 1 mM phosphate buffer; flow rate = 1 mL/min; detection = 214 nm. (b) Eluent = 2 mM phosphate buffer; flow rate = 2 mL/min; detection = 214 nm.

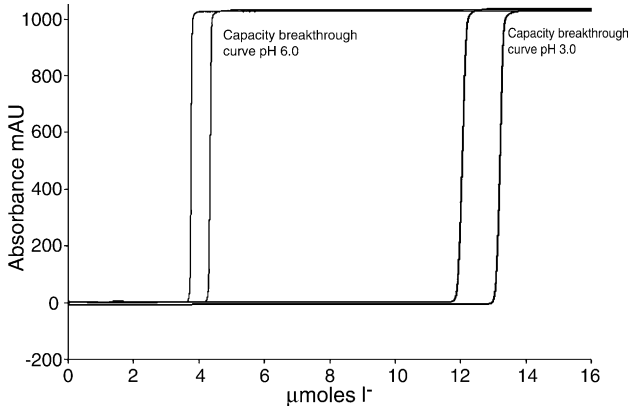

Fig. 4. Overlaid breakthrough curves performed on the lysine monolith using 1 mM KI at pH 3.0 and 6.0, delivered at 1 mL/min.

graph shown represents a combination of stationary phase and eluent effects. However, it is clear from the retention data that significant pH dependence is exhibited. It is also clear that the data observed does not reflect that which would be predicted based upon the dissociation constant of the carboxylic acid group. The unusual behaviour of nitrite can be explained by its pK_a of 3.15, resulting in the formation of nitrous acid and the resultant reduction in retention at that point. Fig. 3(b) shows overlaid ion chromatograms obtained at pH 3.0, 3.5 and 4.4, using a 2 mM phosphate eluent delivered at 2 mL/min.

3.5. Anion capacity

To evaluate the pH effect upon effective anion exchange capacity, the capacity of the monolith was again evaluated. The column was converted to chloride form with a wash with 1 M KCl, followed by distilled water. This was followed by passing a 1 mM KI solution through the monolith and recording the absorbance of the eluate. The procedure was carried out in duplicate, with all solutions adjusted to either pH 3.0 or 6.0. The resultant breakthrough curves are shown in Fig. 4. As can be seen from the graphs shown the effective capacity at pH 6.0 was found to be approximately 30% of that seen at pH 3.0. At pH 3.0, the capacity of the modified monolith was found to be equivalent to between 12 and 13 μmoles I^-. The difference in the effective capacities determined in this study, show how the lysine functionality and the terminal weak acid group produce a variable capacity anion exchanger, whereby eluent pH gradients can be used to reduce retention of strongly retained anions.

3.6. Eluent concentration

As can be seen from Figs. 3 and 4, at pH 3.0 the lysine column exhibited the best retention and resolution of the anion test mixture. To reduce run times the effect of eluent concentration was evaluated. The concentration of the phosphate eluent was varied over 2–50 mM ($n = 11$) whilst keeping pH

Table 2
Retention data for UV absorbing anions on the lysine modified monolithic column

Anion	t_R (min)	k	Efficiency, N	Peak asymmetry, A_s
Formate (80 mg/L)	1.7	1.4	288	2.10
Nitrite	3.2	3.6	4752	1.37
Bromate	5.2	6.4	5195	0.97
Iodate	5.2	6.4	5587	0.97
Chloride	5.4	6.7	4770	0.86
Chlorate	6.2	7.9	4516	1.15
Bromide	6.6	8.4	4712	0.93
Sulphite	7.4	9.6	4539	1.03
Nitrate	7.6	9.9	4523	1.01
Iodide	9.8	13.0	4233	1.12
Thiocyanate	14.9	20.3	4096	1.20

Eluent = 10 mM phosphate, pH 3.0; flow rate = 2 mL/min; $t_0 = 0.7$ min. Efficiency calculated from: $5.54(t_R/W_{50\%})^2$. Asymmetry values calculated from: $(RW_{5\%} + LW_{5\%})/2(LW_{5\%})$.

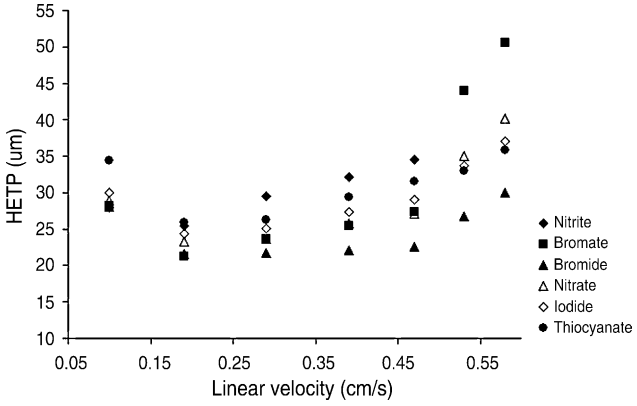

Fig. 5. van Deemter plot for anions on the 10 cm lysine modified silica monolith. Eluent = 20 mM phosphate eluent (pH 3.0).

constant at 3.0. A plot of log k versus log $[E^-]$ resulted in a linear response for al the anions investigated ($R^2 > 0.995$ in all cases) with slopes ranging from −0.8045 (nitrite) to −0.9101 (bromate). As expected these results indicated a pure anion exchange interaction was responsible for retention. Taking the optimal separation conditions (resolution and retention factor) from this study, the selectivity of the monolith for an increased range of common anions was determined, the results of which are included in Table 2. The lysine bonded column demonstrated good ion-exchange selectivity, especially for nitrate.

3.7. Peak efficiency

An advantage of monolithic phases is the ability to further reduce runs times through elevated eluent flow rates. To determine peak efficiency on the lysine monolith at elevated eluent flow rates, a 20 mM phosphate eluent was run at flow rates between 1.0 and 6.0 mL/min ($n = 7$) and peak efficiencies determined under each flow rate. The peak efficiency data for each of the test anions is presented as a van Deemter plot in Fig. 5. As can be seen from the curves shown, opti-

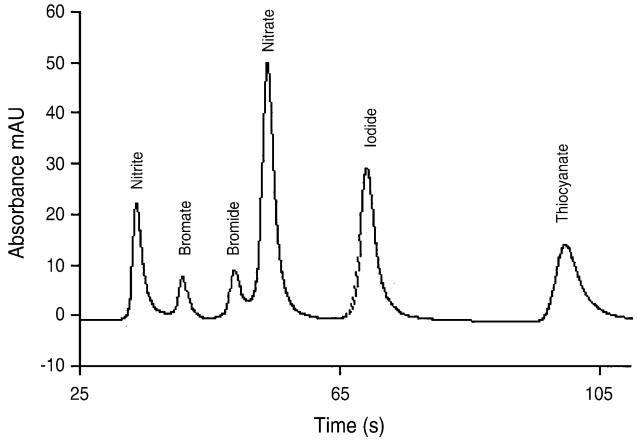

Fig. 6. Separation of 6 anions in 105 s using the lysine modified monolith. Eluent = 50 mM phosphate (pH 3.0); flow rate = 4.9 mL/min.

mum peak efficiencies on the lysine monolith were observed at flow rates of between 2.0 and 3.0 mL/min. At 2.0 mL/min, the column exhibited peak efficiencies between 47, 600 and 38, 460 N/m. At 5 mL/min these efficiency values decreased to between 33, 400 and 19, 770 N/m (excluding nitrite).

3.8. Rapid separations

Fig. 6 shows the rapid separation of six UV absorbing anions in a separation window of just 76 s. This was obtained using a 50 mM phosphate eluent (pH 3.0) and a moderate flow rate of 4.9 mL/min. The separation was achieved under so-called 'isofluentic' conditions, meaning a uniform flow rate was applied throughout the separation. The monolithic nature of the column also allows the investigation of flow gradient separations, which can under certain conditions be applied to speed the elution of strongly retained analytes whilst maintaining resolution of early and closely eluting peak [15,33]. Flow gradient separations were considered here using a range of eluent strength and flow gradient programs. In most cases, overall peak resolution and efficiency for flow gradient separations were comparable or better than isofluentic separations when using the same eluent. A comparison between ion chromatograms obtained with an 1 mL/min isofluentic run, and flow gradient runs (1.0–4.9 mL/min) over either the first 4 and 2 min of the chromatogram are shown within Fig. 7. This approach maintains excellent resolution of the early eluting peaks from the injection void whilst speeding the elution of the stronger retained anions.

3.9. Analytical performance

Clearly a concern with the use of elevated flow rates for rapid separation maybe the effect upon method precision and reproducibility. This is particularly true for dynamically coated monolithic phases, such as those used in previous studies [11,13–15,34]. Here, the bonded nature of the lysine monolith should improve such figures. The repeat injection ($n = 10$) of a 6 anion standard mixture was carried out under flow rates of both 2.0 and 4.0 mL/min. Precision data was calculated for both retention time and peak area and expressed as %RSD (see Table 3). Chromatograms resulting from the 1st, 5th and 10th standard injections are also shown overlaid as Fig. 8. As can be seen, retention time precision data was excellent, in all but one case being below 0.4% RSD, even at the elevated flow rate, showing the stability of the phase under such conditions. In all the column was used on a daily basis for >2-month period without significant signs of degra-

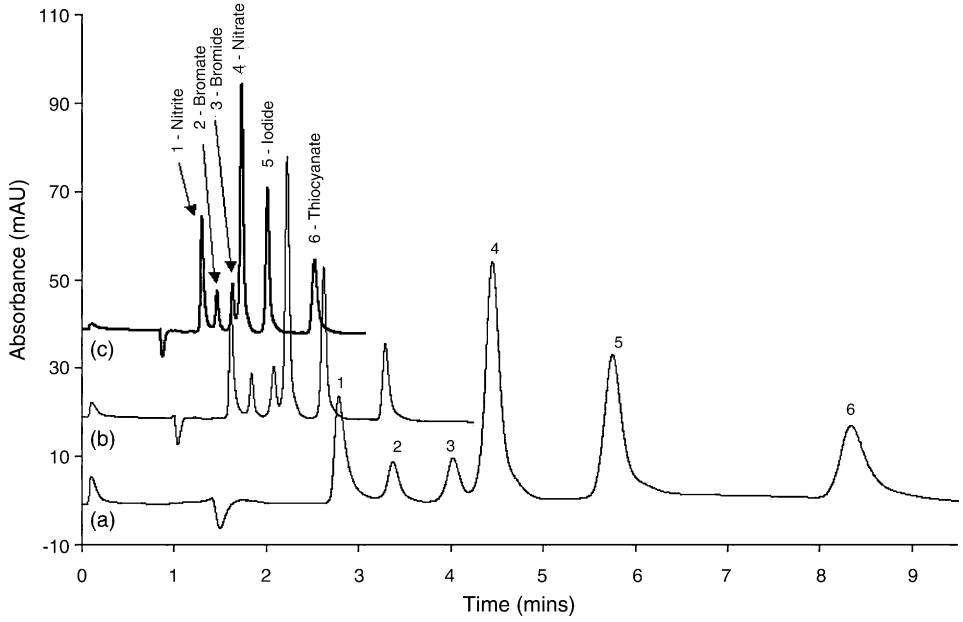

Fig. 7. Overlaid chromatograms obtained using the lysine modified monolith under: (a) isofluentic conditions at 1 mL/min; (b) flow gradient conditions from 1 to 4.9 mL/min over $t = 0$–4 min; (c) flow gradient conditions from 1 to 4.9 mL/min over $t = 0$–2 min. Eluent = same as Fig. 6.

Table 3
Summary of precision data for lysine modified monolithic column ($n = 10$)

	Nitrite	Bromate	Bromide	Nitrate	Iodide	Thiocyanate
2 mL/min						
t_R %RSD	0.38	0.23	0.31	0.88	0.29	0.36
Peak area %RSD	3.4	3.4	2.1	3.1	4.1	2.7
4 mL/min						
t_R inj. #1	0.700	0.848	1.011	1.112	1.434	2.062
t_R inj. #10	0.695	0.845	1.008	1.106	1.422	2.043
t_R %RSD	0.21	0.23	0.23	0.21	0.26	0.29
Peak area %RSD	5.4	4.9	5.0	4.0	2.0	4.5

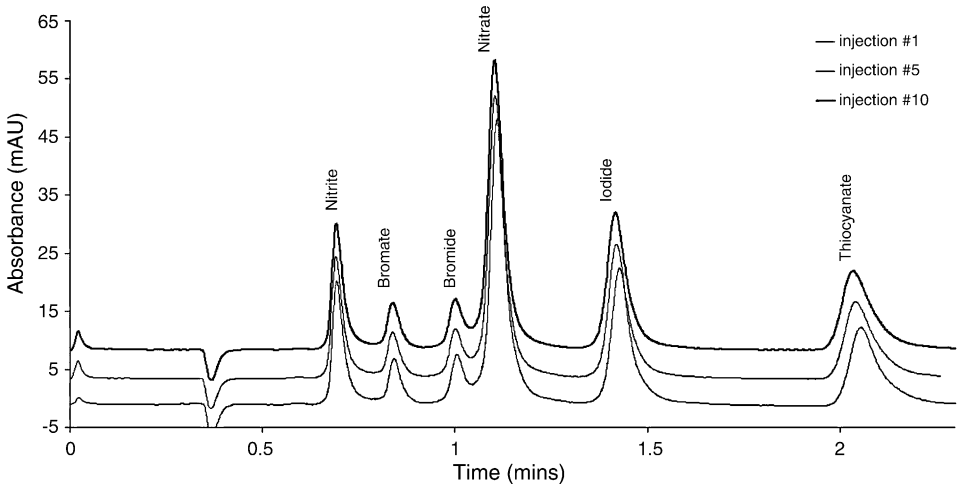

Fig. 8. Overlaid chromatograms from precision study carried out using the lysine modified monolith. Eluent = same as Fig. 6. Flow rate = 4.0 mL/min.

dation, with reproducibility studies carried out at the end of this period.

4. Conclusions

A novel bonded monolithic zwitterionic ion exchanger has been evaluated for both cation and anion selectivity. The column shows selectivity towards transition and heavy metal cations through complexation, whilst only limited retention of alkali and alkaline earth cations. However, the column exhibits excellent selectivity and efficiency for inorganic anion separations, and can be used at elevated eluent flow rates to achieve impressive rapid separations.

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