Ionic strength, pH and temperature effects upon selectivity for transition and heavy metal ions when using chelation ion chromatography with an iminodiacetic acid bonded silica gel column and simple inorganic eluents

Wasim Bashir, Brett Paull*

National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland

Abstract

An investigation into the selectivity of an iminodiacetic acid (IDA) modified silica gel column for transition and heavy metal ions using non-chelating inorganic eluents has been carried out. A number of eluent parameters were investigated to determine the exact retention mechanism taking place and to control selectivity. The parameters studied were eluent ionic strength and the nature of the inorganic salt used, eluent pH and eluent temperature. The results obtained showed how despite certain metal ions exhibiting similar stability constants with the bonded IDA groups, careful control of each of the above parameters, in particular eluent chloride ion concentration and eluent temperature, could result in large changes in selectivity. Optimal conditions for the isocratic and gradient separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) were determined. An isocratic method using a 0.035 M KCl, 0.065 M KNO$_3$ (pH 2.5) eluent was successfully applied to the determination of Mn(II), Cd(II), Co(II) and Zn(II) at concentrations between 20 and 121 μg/l in a freshwater certified reference material (NIST 1640). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ionic strength; pH effects; Temperature effects; Stationary phases, LC; Mobile phase composition; NIST 1640; Transition metals; Heavy metals; Iminodiacetic acid bonded silica

1. Introduction

The chelation ion chromatography (CIC) of metal ions using high-performance grade chelating stationary phases is a specialised ion chromatographic technique that offers alternative selectivities to those achievable when using simple sulfonated or carboxylated ion-exchange stationary phases. As metal ion retention is based upon the relative conditional stability constants of each metal and the immobilised chelating ligand, retention and selectivity is predominantly controlled through eluent pH, although, as in simple ion-exchange chromatography, a range of eluent parameters can be used to obtain desired changes in selectivity. In addition to this, CIC has the added advantage of there being a large and varied number of chelating ligands available to functionalise the stationary phase, each offering unique metal ion selectivity. Two approaches to the production of chelating stationary phases have been investigated in

*Corresponding author. Tel.: +353-1-700-5060; fax: +353-1-700-5503.

E-mail address: brett.paull@dcu.ie (B. Paull).

recent years, namely, (a) permanently or dynamically coating polymeric and silica-based reversed-phase phases with hydrophobic chelating ligands, and (b) covalently bonding chelating groups to either polymeric resins or silica gels. A number of review and comparative articles on the principles, various modes and applications of CIC have been compiled which discuss in detail the above approaches and highlight the versatility of this ion chromatographic technique [1–5].

Of all the studies carried out to date on the CIC of metal ions (with the exception of some recent work using aminophosphonic acid functionalised silica [6]), those using stationary phases functionalised with carboxylic acid containing ligands have proven to be the most successful in terms of selectivity, efficiency and number of applications. This would appear to be the case whether the stationary phase contained co-valently bonded chelating groups [7–11], was permanently coated with chelating ligands [12,13], or dynamically coated using an eluent containing such a ligand [5,14]. Results from the above studies have shown one ligand, namely iminodiacetic acid (IDA), to be particularly suitable for this specific mode of ion chromatography, due mainly to the following two factors; firstly, stability constants for alkaline earth metal ions and common transition/heavy metal ions with the IDA ligand are not prohibitively high as to require excessively strong eluents. Secondly, the complexation/dissociation kinetics of the IDA–metal ion complex seem to be sufficiently rapid to produce separation efficiencies comparable with simple ion-exchange.

Several studies have been carried out using iminodiacetic acid bonded silica gel stationary phases for the separation of metal ions. These include the separation of alkali and alkaline earth metals [10,11,15–17], lanthanide series metal ions [9] and transition and heavy metal ions [2,7,8,18]. However, the separation of the latter group of metal ions using IDA-silica has received little attention when compared with the number of studies carried out with this group of metal ions on simple ion-exchange stationary phases. Bonn et al. [7] were amongst the first to investigate the use of IDA-silica for the separation of alkaline earth and transition metal ions. Using organic chelating eluents such as citric, tartaric and dipicolinic acids the study showed how

under acidic conditions a separation of Mg(II), Fe(II), Co(II), Cd(II) and Zn(II) could be achieved in just under 30 min. A separation of Co(II), Cd(II) and Zn(II) spiked into a seawater matrix was also shown, although due to the strong complexing nature of the eluent and the use of conductivity detection, sensitivity was poor. Later work by Elefterov et al. [18] also employed dipicolinic acid as an eluent with an IDA-silica column, this time using direct UV absorbance of the eluting metal–ligand complexes for detection. A separation of Zn(II), Pb(II), Co(II) and Cd(II) was shown, although once again sensitivity was relatively poor with detection limits ranging from 0.4 to 1.2 mg/l. More recently Nesterenko and Jones [2,8] further investigated the use of various complexing eluents for both the isocratic and gradient separations of alkaline earth and transition metal ions. Using a three step gradient, beginning with a KCl–HNO$_3$ eluent, followed by tartaric acid and finally picolinic acid, the separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II), Zn(II), Ni(II) and Cu(II) was obtained, although the overall run time was excessively long at just under 50 min.

The following paper describes a more detailed study into the retention behaviour of common transition metal ions on IDA-silica using simple inorganic salts as eluents. The omission of chelating ligands such as tartaric and picolinic from the eluents used in this study allowed the true selectivity of the IDA-silica gel to be observed. Optimum conditions for the isocratic separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II) and Zn(II) were investigated, together with a pH step gradient method which allowed the separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) in under 10 min. Due to the non-chelating nature of the eluent, sensitive post-column reaction detection with 4-(2-pyridylazo)resorcinol (PAR) was possible, resulting in detection limits for of between 4 and 10 μg/l for the above transition metal ions in a reference freshwater sample.

2. Experimental

2.1. Instrumentation

A Dionex Model GPM2 Gradient Pump Module

(Sunnyvale, CA, USA) was used to deliver the eluent (1.5 ml/min). A manual sample injection valve, Model 7125, (Rheodyne, Cotati, CA, USA), fitted with a 250 µl injection loop was used for introduction of the samples. The IDA-silica packed analytical column (8 µm particle size, 130 Å pore size, 250×4.0 mm I.D.) used was supplied by BioChemMack (Moscow, Russia). A pressure-driven Dionex Reagent Delivery Module (RDM) was used for introduction of the post-column reagent (1.5 ml/min), which was mixed at room temperature with the eluent using a 0.5 m polyether ether ketone (PEEK) reaction coil (0.01 in. I.D.; 1 in.=2.54 cm). A Model SPD-6AV Shimadzu UV–Vis detector (Kyoto, Japan) was used at 495 nm to monitor the resultant chromatograms. These were recorded using Dionex AI450 chromatographic software. For the determination of column outlet pH, the flow from the RDM was turned off and discrete samples taken from the detector outlet every minute. Each sample pH was then measured using a standard laboratory pH meter.

2.2. Reagents

The eluent and post-column reagent (PCR) were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, USA). The PCR ligand, PAR, and sodium hydroxide were purchased from Sigma–Aldrich (Gillingham, UK) and used without further purification. Potassium nitrate was obtained from Merck (Darmstadt, Germany). Sodium nitrate was purchased from Riedel-de Haen (Sleeze–Hannover, Germany) and sodium chloride and potassium chloride obtained from Fluka (Buchs, Switzerland). Final eluent conditions for the isocratic analysis of samples were 0.065 M KNO_3–0.035 M KCl adjusted to pH 2.5 using dilute HNO_3. The post-column reagent solution was 0.5 M ammonia, 0.4 mM PAR, adjusted to pH 10.5. The pH of the NIST reference standard material was increased to pH 2.5 using measured quantities of dilute NaOH prior to injection. All solutions prepared were filtered through a 0.45-µm filter and degassed using sonication. Low-level standard solutions were generally prepared freshly each day from stock solutions (1000 mg/l) stored in 1% nitric acid.

3. Results and discussion

3.1. Stationary phase selectivity

In previous studies involving IDA-silica stationary phases it has been shown that considerable retention of alkali and alkaline earth metal ions can be achieved through simple ion-exchange interactions with the dissociated carboxylic acid groups of the IDA ligand (pK_{a1}=1.76, pK_{a2}=2.70 [17]). For the alkaline earth metal ions this ion-exchange interaction is the predominant means of retention between pH 2 and 5, as complexation under such acid conditions has been calculated previously to be insignificant [7]. However, within the above pH range any ion-exchange interactions can be minimised through the use of high ionic strength eluents. In this work the retention behaviour alkaline earth and transition metal ions was firstly evaluated with simple 0.5 and 1.0 M KNO_3, $NaNO_3$, KCl and NaCl eluents. The pH was controlled with the addition of HNO_3 between the investigated pH range of 2 to 3. Under all conditions studied Cu(II) and Fe(III) were completed retained on the column. As expected Ca(II) and Mg(II) were unretained under the above conditions as complexation was not significant and ion-exchange interactions were suppressed. The retention behaviour observed for Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) is shown in Fig. 1. It was clear from the results obtained that there was very little difference in selectivity when using either the sodium or potassium form of either the nitrate (Fig. 1A and B) or chloride salt (Fig. 1C and E). However, there were significant differences between the selectivity shown with the nitrate eluent and that of the chloride (e.g., Fig. 1A and C). With the nitrate eluent the retention of Pb(II) rapidly increased above pH 2.3, such that it was completely retained (>100 min) at pH 2.6. With the chloride eluents the retention of Pb(II) was significantly reduced. This was also noticeable with Cd(II), which co-eluted with Co(II) when using a nitrate eluent, irrespective of eluent pH, and which co-eluted with the slightly retained peak for Mn(II) when using the chloride eluents. The above observation was as expected when considering the stronger tendency of chloride ions to form chloro complexes with both Pb(II) and Cd(II) relative to the remaining metal ions ($K_{1(ZnL)}$=
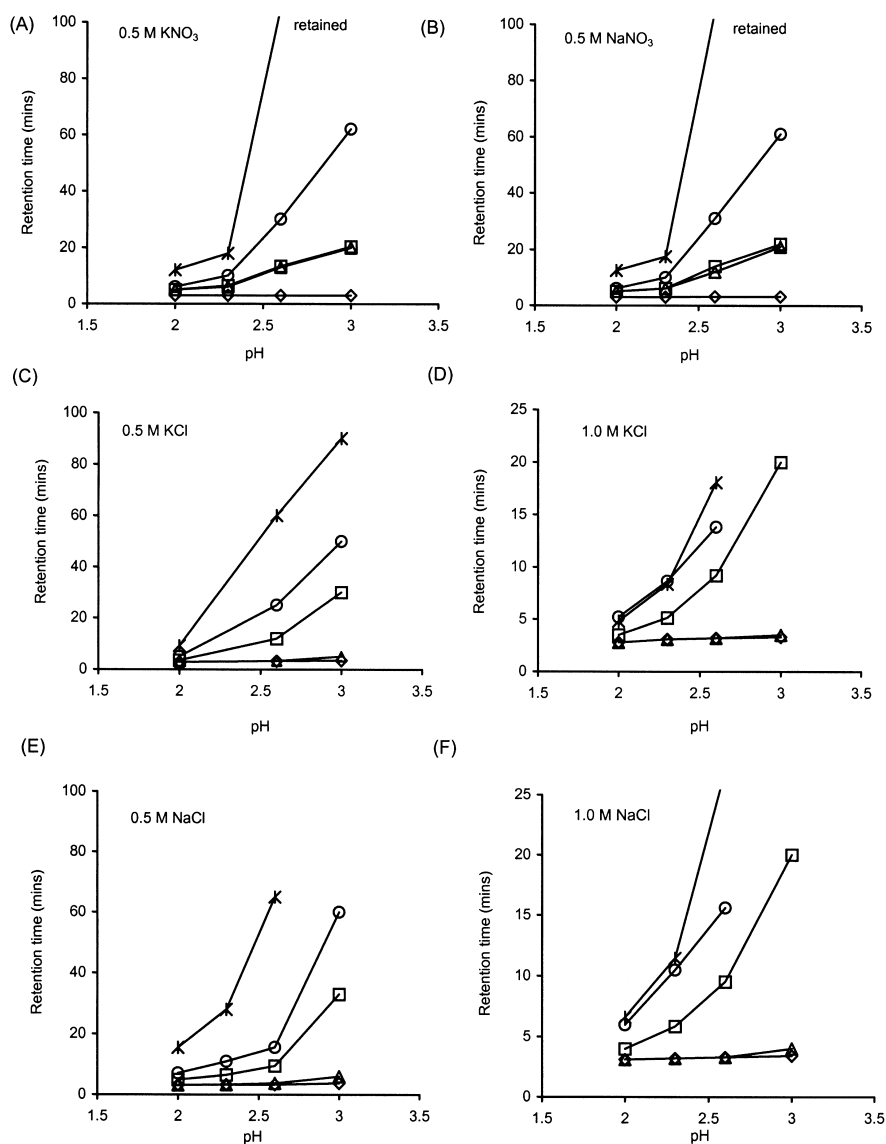
Fig. 1. Effect of pH upon retention of Mn(II) ◇, Cd(II) △, Co(II) □, Zn(II) ○ and Pb(II) ✳ in 0.5 and 1.0 M KNO$_3$, NaNO$_3$, KCl and NaCl eluents.

0.11, $K_{1(PbL)}$=1.18, $K_{1(CdL)}$=1.38, medium=1 M NaClO$_4$, 25°C [19]). Increasing the concentration of the chloride eluent from 0.5 to 1.0 M resulted in a further decrease in the retention of Pb(II) in relation to Zn(II), Co(II) and Mn(II), such that Pb(II) and Zn(II) co-eluted at pH<2.3. As Zn(II) and Co(II) showed little decrease in retention when the concentration of the eluent was increased, it can be

assumed that retention for these metal ions by ion-exchange interactions (under these conditions) was not significant. Using the 1.0 M chloride eluent both Mn(II) and Cd(II) were unretained at pH<2.5.

Fig. 2a and b show chromatograms resulting from the injection of a 1 mg/l mixture of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II) (5 mg/l) when using a 0.5 M KNO$_3$ eluent (a) and a 0.5 M NaCl eluent (b),
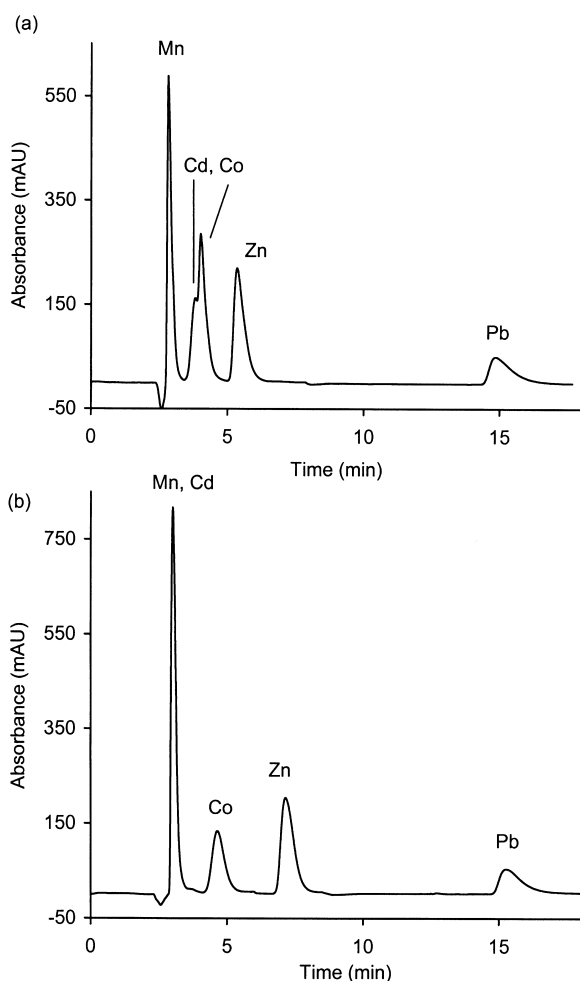
Fig. 2. Chromatogram showing (a) the separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II), using a 0.5 M KNO$_3$ eluent (pH 2.0) and (b) the separation of the same metal ions using a 0.5 M NaCl eluent (pH 2.0). Standard concentration = 1 mg/l for Mn(II), Cd(II), Co(II) and Zn(II), 5 mg/l for Pb(II).

both adjusted to pH 2.0 with HNO$_3$. As can be seen from the two chromatograms shown, the isocratic separation of all five metals was not possible, with either Cd(II) and Co(II), or Cd(II) and Mn(II) co-eluting. However, the above conditions resulted in excellent selectivity for Pb(II) which was well resolved from all the other metal ions injected and eluted as a reasonably sharp peak giving a sensitive detector response. By further increasing the pH of the chloride eluents to 2.6–3.0, Mn(II) and Cd(II) could be separated but eluted too close to the void to

be of use in real samples. In addition, the retention of Zn(II) and Pb(II) was too great (>40 min) at the increased pH.

As mentioned above, reducing the concentration of the eluent below 0.5 M would result in the unwanted retention through ion-exchange of alkaline earth metals, and also the excess retention of Pb(II) (unless very acidic conditions were used, pH<2.0). To investigate this a 0.1 M KCl eluent (pH 2.0) was prepared and retention times for above transition metal ions and Ca(II) and Mg(II) were determined. It was found that under these conditions Ca(II) and Mg(II) were indeed beginning to exhibit retention and that Mn(II) partially co-eluted with Ca(II). Increasing the pH to 2.6 resulted in the improved resolution of Ca(II) and Mn(II), but also increased the retention of Zn(II) to >25 min. In addition the peak for Cd(II) eluted closely to that of Ca(II) and Mn(II) when using the chloride eluent, which would interfere with the determination of Cd(II) in real samples where Ca(II) was present in excess. To remedy this the concentration of KCl in the eluent was reduced to 0.035 M and replaced with KNO$_3$ at 0.065 M, keeping the overall concentration of the eluent at 0.1 M. This resulted in an increase in the retention of Cd(II) relative to the remaining metal ions, meaning it now eluted free from possible interferences from excess alkaline earth metals. A slight reduction in eluent pH to 2.5 resulted in the optimum isocratic separation of the above alkaline earth and transition metals as shown in Fig. 3.
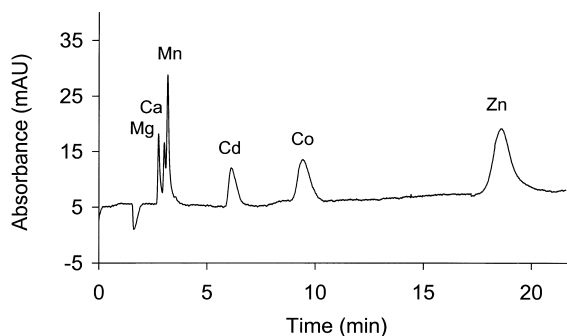

Fig. 3. Chromatogram showing the optimised separation of Mg(II), Ca(II), Mn(II), Cd(II), Co(II) and Zn(II). Eluent conditions: 0.035 M KCl, 0.065 M KNO$_3$ (pH 2.5). Standard concentration = 0.3 mg/l for Mg(II) and Ca(II), 0.05 mg/l for Cd(II), Co(II) and Zn(II) and 0.02 mg/l Mn(II).

3.2. Temperature effects

In previous work with IDA-silica stationary phases the effect of temperature upon the retention of alkali and alkaline earth metal ions [11,17] and the lanthanide series metals [9] has been studied and explained. It is generally agreed that temperature can be used to improve selectivity depending upon the nature of the dominant retention mechanism acting upon the analyte ions. For example, alkali and alkaline earth metal ions are predominantly retained through simple ion-exchange interactions with both carboxylate groups and to a lesser extent uncapped surface silanol groups. It would therefore be expected that the retention of these metals would be affected differently by a change in column temperature than transition metal ions, which under the conditions used in this work, were likely to be exhibiting retention due to a combination of ion-exchange and complexation, with the latter being dominant. It has been shown previously that where the main retention mechanism is complexation, an increase in column temperature results in a clear increase in analyte retention [9,10,14]. Therefore, the effect of column temperature upon the retention of both alkaline earth metals [Ca(II) and Mg(II)] and transition metal ions [Mn(II), Cd(II), Co(II) and Zn(II)] was investigated. The temperature of the column was varied from 15 to 50°C (other conditions as in Fig. 3), with the results shown in Fig. 4. As can be seen from Fig. 4, there appears to be a clear correlation between the degree of retention (and thus complexation) and the effect of temperature. Both Zn(II) and Co(II), which were quite strongly retained by the IDA-silica column, exhibited similarly rapid increases in retention as the column temperature was increased. With Cd(II) and Mn(II) the effect of temperature was less pronounced, although a clear increase in retention was observed [in the case of Cd(II), it may be expected that the affect of temperature would have been more significant if a non-chloride containing eluent were used].

With the alkaline earth metal ions, Mg(II) was totally unaffected and Ca(II) actually showed a slight decrease in retention. These results indicated that even though Mn(II) eluted close to both Ca(II) and Mg(II) the mode of retention was different, in that unlike Mg(II) and Ca(II), Mn(II) showed retention
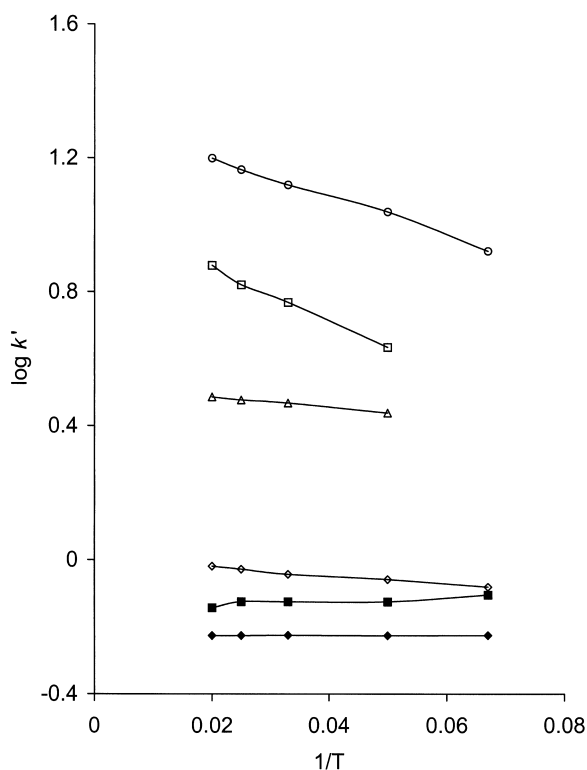

Fig. 4. Effect of temperature upon retention of alkaline earth and transition metal ions. Ca(II) ■, Mg(II) ♦, Mn(II) ◇, Cd(II) △, Co(II) □, Zn(II) ○. Other eluent conditions as in Fig. 3.

behaviour which suggested that under the conditions used complexation was indeed playing a significant role in retention.

To illustrate how the above difference in behaviour could be exploited when analysing a real sample, a certified reference freshwater sample (NIST 1640) was obtained which contained trace amounts of Mn(II) (0.121 mg/l) and excess Mg(II) and Ca(II) (5.82 and 7. 05 mg/l, respectively). The sample was injected over the temperature range shown in Fig. 4 and the resultant chromatograms (at 15, 20, 25 and 50°C) are shown in Fig. 5. As can be seen, by using an elevated column temperature, Mn(II) can be readily resolved and thus quantified in a real sample containing excess Ca(II) and Mg(II).

3.3. Gradient elution

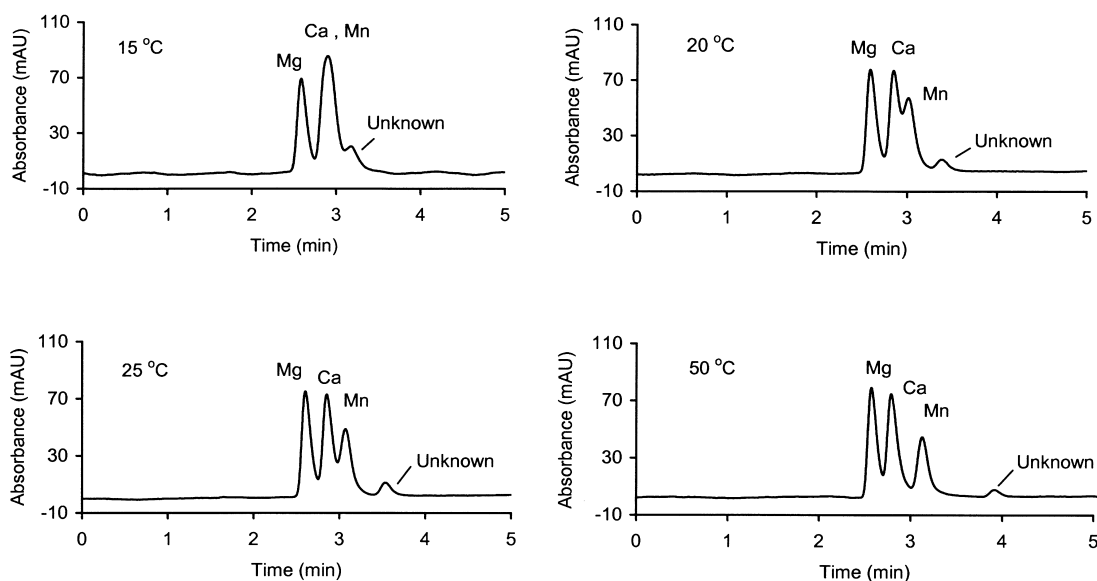From the above results it was clear that both the

Fig. 5. Chromatograms showing the effect of column temperature on the resolution of Mg(II), Ca(II) and Mn(II) at 15, 20, 25 and 50°C. Other eluent conditions as in Fig. 3.

use of lower ionic strength eluents and elevated column temperatures led to long retention times for ions such as Zn(II) and more importantly Pb(II). When using any eluent which resulted in the resolution of Mn(II) and Cd(II) (either as in Fig. 3 or simply 0.1 M KCl at pH 2.6–3.0), the retention of Zn(II) was greater than desired and Pb(II) was totally retained. Therefore, in order to obtain the resolution of Mn(II) and Cd(II) from each other and from the void, and also to decrease the retention time of Zn(II) and Pb(II), the investigation of concentration and pH gradients was the next logical step. Initially a simple concentration gradient was investigated rapidly increasing from 0.1 to 1.0 M NaCl (pH 2.3) following the elution of Co(II) at 10 min, to speed the elution of Zn(II) and Pb(II). However, as shown previously, when using 1.0 M NaCl under isocratic conditions, Pb(II) and Zn(II) were retained for over 10 min. Therefore, the above gradient was not strong enough, even at 1.0 M NaCl, to facilitate the fast elution of Zn(II) and Pb(II) from the column, and this resulted in Zn(II) eluting after 18 min and Pb(II) eluting as a broad peak after approximately 35 min.

It was therefore necessary to investigate a pH gradient to obtain the desired separation of all five

metal ions. As in previous studies using chelation ion chromatography [8,12,13], a pH step gradient was used to attempt the rapid elution of Zn(II) and Pb(II). To achieve this, a 0.1 M NaCl eluent (pH 2.6) was switched to 0.1 M NaCl (pH 1.6) following the elution of Mn(II), Cd(II) and Co(II) (10 min). At pH 1.6 under isocratic conditions both Zn(II) and Pb(II) were only marginally retained, and so when using the above gradient both metals should have been rapidly eluted from the column as the lower pH eluent moved through the system. However, it was found that the step decrease in eluent pH did not result in the rapid elution of Zn(II) and Pb(II) from the column, which instead eluted from the column after >25 min. It was clear from this result that the IDA itself was buffering the sudden pH change within the column and that for a pH gradient to be successful the buffering capacity of the column would have to determined and overcome. To graphically illustrate the strong buffering capacity of the IDA-silica column, the column outlet pH was monitored during a typical pH step gradient. Fig. 6a shows the column outlet pH against eluent volume for an IDA-silica column (V_0=1.1 ml) previously equilibrated with an eluent of pH 2.6, and switched at the column inlet to an eluent of pH 1.6 at a
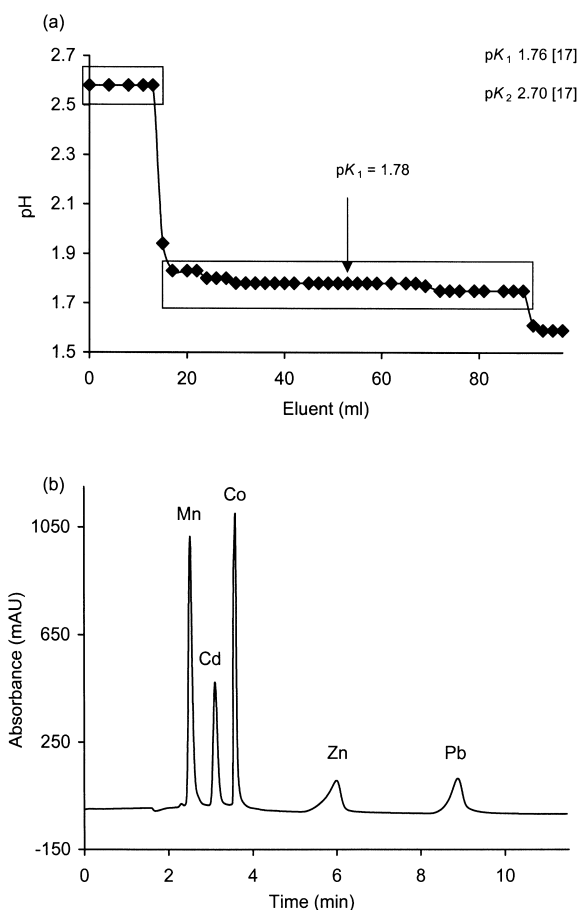
Fig. 6. (a) Graph showing column outlet pH against eluent volume following a pH step gradient from pH 2.6 to pH 1.6 at $E=0$. (b) Step gradient separation of Mn(II), Cd(II), Co(II), Zn(II) and Pb(II). Eluent conditions: 0.1 M NaCl (pH 2.6) switched to 0.1 M NaCl (pH 2.6) at time=3 min prior to standard injection.

flow-rate of 1.5 ml/min (volume=0). As can be seen from the figure, two distinct buffering regions are evident, corresponding to the pK_{a1} and pK_{a2} of IDA, which under the conditions investigated meant the outlet pH only reached that of the eluent after 90 ml (or 60 min). The results shown were used to calculate the approximate capacity of the column, which was found to be ~185 μmol of "active" IDA (which corresponds closely to the 130 μmol/g reported earlier by Nesterenko and Jones for similar IDA-silica [2]).

However, despite the strong buffering capacity of the column, the pK_{a1} value for IDA was sufficiently acidic (1.78) to allow the elution of Zn(II) and Pb(II) from the column and so the gradient could be used provided the timing of the drop in eluent pH was correct. With a column equilibrated to pH 2.6 it was found that switching to the lower eluent 3 min prior to sample injection caused a drop in column pH approximately 5 min after injection and eluted both Zn(II) and Pb(II) as well resolved and relatively sharp peaks (again using a flow-rate of 1.5 ml/min). Fig. 6b shows the resultant chromatogram, showing all five metals well resolved, removed from the void and eluted in under 10 min. It was found that the developed gradient method was reproducible provided the column was fully equilibrated with the higher pH eluent at the start. Following each gradient run the length of this re-equilibrium stage was dependent upon the length of time the column was exposed to the pH 1.6 eluent. Therefore, it was vital to monitor the outlet pH after each gradient run to ensure the column had fully re-equilibrated to the starting pH before the next injection could be made. However, it was found that this added considerably to the total analysis time and seriously limited the practicality of the gradient method.

3.4. Applications

To illustrate that the unique selectivity exhibited by chelation ion chromatography can indeed be utilised in the analysis of real samples, a certified reference freshwater sample (NIST 1640) was analysed for trace levels of Mn(II), Cd(II), Co(II) and Zn(II). As mentioned above the certified reference material (CRM) consisted of a typical freshwater matrix containing Mg(II) and Ca(II) at ~6 and 7 mg/l, and K and Na at ~1 and 30 mg/l, respectively. Standard analytical performance indicators (linearity, precision, accuracy and detection limits) were determined using the sample itself under the isocratic eluent conditions optimised in Section 3.1. Under these conditions it was not possible to determine Pb(II) in the CRM sample, this being an unfortunate disadvantage of the developed method.

All four metal ions gave a linear response ($R^2 >$ 0.999) over the investigated range (0–250 μg/l). For

Cd(II) both a standard calibration and a standard addition calibration were carried out. This resulted in an R^2 value of 0.9999 for the standard calibration ($n=5$, 50–250 µg/l) and an R^2 value of 0.9993 for the standard addition calibration ($n=3$, 20–100 µg/l). Precision was determined using five repeat injections of the CRM and calculated for retention time, peak area and peak height. The method proved highly reproducible with RSD values for the retention times of all six metals ranging between 0.7 to 2.9%. The same precision was reflected in the RSD values for peak areas (1.1–2.0%) and peak heights (0.6–1.1%). Detection limits for Mn(II), Cd(II), Co(II) and Zn(II), determined using a 250 µl injection of the CRM and calculated using 3× baseline noise, were found to be 4.2 (±1) µg/l for Co(II), 4.6 (±1) µg/l for Mn(II), 5.4 (±1) µg/l for Cd(II), and 9.0 (±2) µg/l for Zn(II). The excellent sensitivity exhibited by the method was in part due to the absence of any complexing ligands within the eluent that would otherwise compete with the PCR and reduce detector response. Finally, accuracy was assessed with the determination of the above four metal ions in the CRM. The results obtained using standard addition are shown in Table 1, calculated using both peak area and peak height. As can be seen from the results, considering the trace concentrations of the four metals, the accuracy of the method was found to be satisfactory. The chromatogram obtained for the NIST 1640 sample is shown overlaid with a spiked NIST sample in Fig. 7. The excellent selectivity for Cd(II) is clear from the chromatograms shown, making the developed method suitable for the determination of Cd(II) in samples containing
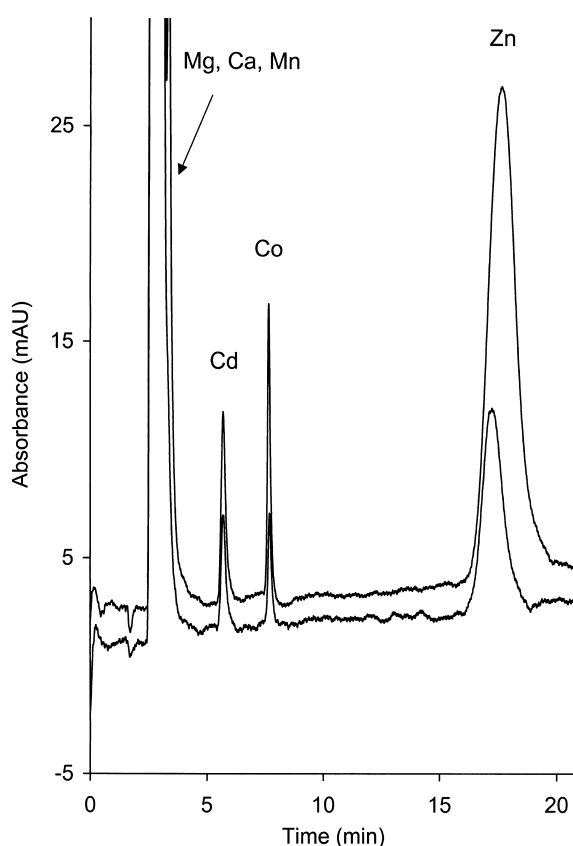

Fig. 7. Chromatogram of NIST 1640 freshwater sample overlaid with spiked sample. Eluent conditions as in Fig. 3.

excess alkali/alkaline earth metals and common transition metals such as Zn(II).

4. Conclusion

Iminodiacetic acid bonded silica gel offers a viable alternative to simple cation-exchange resins for the determination of trace transition metal ions in real samples. Its unique selectivity can be manipulated to produce well resolved separations of several alkaline earth and transition metal ions when using only simple inorganic eluents, with the resultant chromatograms exhibiting peak efficiencies comparable to those obtainable with modern cation-exchange resins.

Table 1
Analysis of NIST 1640 standard reference material

Metal ion	Certified value (mg/l)	Amount determined[a] (mg/l)	Amount determined[b] (mg/l)
Mn(II)[c]	0.121±0.001	0.095	0.093
Cd(II)	0.022±0.001	0.028	0.028
Co(II)	0.020±0.003	0.018	0.016
Zn(II)	0.053±0.001	0.065	0.063

[a] Calculated using peak height plot.
[b] Calculated using peak area plot.
[c] Analysis carried out at 50°C.

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