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Dynamic chelation ion chromatography of transition and heavy metal ions using a mobile phase containing 4-chlorodipicolinic acid

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Abstract

The chromatographic behaviour of selected transition and heavy metal ions, the lanthanides, uranium and aluminium, on a neutral polystyrene–divinylbenzene (PS–DVB) stationary phase (7 μm Hamilton PRP-1) dynamically modified with 4-chlorodipicolinic acid, was investigated to evaluate retention characteristics. Complicated retention factor against pH plots were found for these metals demonstrating changes in retention order. It was concluded that complexation between the metal ions and the ligand adsorbed on the resin was strongly influenced by the decrease in dynamic loading with increase in pH, coinciding with changes in the metal-to-ligand ratio in the mobile phase. Possible reversed-phase interactions between metal–chlorodipicolinic acid complexes and the hydrophobic PS–DVB stationary phase also could not be ruled out. An eluent of 0.25 mM chlorodipicolinic acid, 1 M potassium nitrate at pH 2.2 was suitable for the separation of seven transition and heavy metal ions in under 20 min on a 250 \times 4.6 mm column (with 50-mm guard column), determined in a certified water sample with good accuracy ($R^2 \geq 0.994$) and reproducibility (RSD 1–4.2%). Pb(II), Cd(II) and Cu(II) were additionally analysed in <10 min in a more complicated certified rice flour matrix, using the same eluent but adjusted to pH 1.5, again with good accuracy ($R^2 \geq 0.998$) and reproducibility (RSD 0.48–1.38%). © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; Chelation; Metals; 4-Chlorodipicolinic acid

1. Introduction

A liquid chromatographic system incorporating a hydrophobic stationary phase together with complexing agents added to the mobile phase, provides varying possibilities for the separation and determination of metal ions. Depending upon the properties

of the chosen ligand, different separation mechanisms can occur. Two related chromatographic systems are well established as analytical separation tools.

(i) Reversed-phase HPLC of metal chelates with in situ or on-column complexation. In this case, the affinity of the hydrophobic stationary phase towards the ligand is much less than the corresponding affinity towards metal chelates under identical separation conditions. The separation mechanism is based on a difference in the hydrophobicity of the eluting complexes. A wide range of organic reagents have

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been added to the mobile phase as complexing agents, including 8-hydroxyquinoline [1], 4-(2-pyridylazo)resorcinol (PAR) [2], 1,10-phenanthroline [3], quinaldic acid [4], picolinic acid [5] and mandelic acid [6], and investigated for the separation of various groups of metal ions by either ion interaction or reversed-phase HPLC. A further selection of reagents can be found in reviews by Sarzanini [7], Timerbaev and Bonn [8], and Karcher and Krull [9]. To avoid any interference from having an excess of ligand present in the mobile phase, and possible modification of the stationary phase surface, the separation conditions should allow only the formation of 1:2 and 1:3 chelates, which are retained more strongly than the free ligand itself. Metal chelates that are only partially resolved using this technique, can often be baseline separated by the addition of organic solvents to the mobile phase, acting as eluent modifiers.

(ii) A different separation mechanism takes place when the chelating agent is strongly adsorbed at the surface of a hydrophobic substrate. This has been found for a range of chelating dyestuffs added to the mobile phase for the separation of transition, heavy and alkaline earth metals, as reviewed by Paull and Haddad [10]. The dyes were found to form a dynamic layer on neutral stationary phase sorbents, effectively forming a chelating ion-exchanger. This approach is one of a group of techniques used for forming a chelating substrate to achieve metal separations, and has been termed chelation ion chromatography or high-performance chelation ion chromatography, since a high salt content is usually present in the eluent to suppress ion-exchange interactions and ensure chelation ion-exchange is the dominant retention mechanism. Chelation ion chromatography separations are based on the formation and dissociation of metal complexes on the surface of a chelating stationary phase, separation resulting from the differences in conditional stability constants between the metal complexes. Further details on this type of separation process are given in a comprehensive review by Jones and Nesterenko [11].

Recent investigations have found that a dynamic chelating layer is established when small aromatic carboxylic acids are present in a high salt containing eluent, when using neutral polystyrene-divinylbenzene (PS-DVB) and C_{18} silica sorbents [12]. Studies

have shown that under certain conditions the complexing agents can be adsorbed onto the stationary phase producing competitive chelation between the solid substrate and the eluent. This mixed-mode separation is a less popular approach due to the complex separation characteristics, although there are more possibilities to vary the separation selectivity depending upon whether the chromatographic conditions favour a reversed-phase or chelation ion-exchange separation mechanism.

Dipicolinic acid has shown particular promise for producing efficient separations of groups of metals, when dynamically sorbed to small particle size (5–10 μm) PS-DVB, exemplified by the separation of high valence metals including U(VI), Hf(IV), Zr(IV), Bi(III) and V(V) [13], which is typically difficult to achieve with liquid chromatographic techniques. At low pH (<2), the system has been shown to be very stable to ionic strength and large concentrations of matrix metals, producing reproducible retention of analytes over a long period of column usage [12]. The discovery that U(VI) could be separated from potentially interfering metal ions including Fe(III), Th(IV) and the lanthanides [14], led to the development of a method for determining this actinide in sediments and seawater [15]. Unfortunately, the degree of loading of this heterocyclic acid to PS-DVB matrices is insufficient to provide adequate resolution of the lower charged transition and selected heavy metals including Pb(II) and Cd(II) [12]. An attempt to increase the dynamic loading on the neutral polystyrene substrate was undertaken by modifying dipicolinic acid with a π -electron accepting functional group, in this case chloride. It was envisaged that the addition of this ion to the pyridine ring would result in a stronger adsorption of dipicolinic acid to the π -electron donating resin support. Initial results were very promising, culminating in the separation of seven metals in under 8 min on a 100-mm column length, though with an unusual broad, symmetrical peak shape for Ni(II) which normally shows strong tailing due to the slow kinetics of dissociation with this metal [16].

This paper presents a more detailed and expanded investigation into the metal retention properties of neutral PS-DVB dynamically modified with 4-chlorodipicolinic acid, together with the analysis of both

a certified water sample and rice flour to verify the suitability of this phase for the high-performance separation and determination of metal ions.

2. Experimental

2.1. Instrumentation

The isocratic ion chromatographic system consisted of a Dionex GP40 gradient pump (Dionex, Sunnyvale, CA, USA) set to deliver 1 ml min^{-1} , a Rheodyne 9010 polyether ether ketone (PEEK) liquid six-port injection valve (Rheodyne, Cotati, CA, USA). A $100\text{-}\mu\text{l}$ PEEK sample loop was used for all standards and samples. The analytical column was a PEEK ($100 \times 4.6 \text{ mm}$ or $250 \times 4.6 \text{ mm}$ I.D.) casing, packed with $7 \text{ }\mu\text{m}$ PRP-1 PS–DVB resin (Hamilton, Reno, NV, USA). The postcolumn detection system comprised a Constametric III HPLC pump (LDC, Riviera Beach, FL, USA), to deliver the postcolumn reagent (PCR), a zero dead volume PTFE tee and a $1.4 \text{ m} \times 0.3 \text{ mm}$ I.D. PTFE reaction coil. Detection was achieved using a spectral array detector (Dionex). To use the background subtraction technique devised by Jones [17], the detection wavelength for the transition and heavy metal complexes was 520 nm and 445 nm for the isosbestic point.

The void volume used to calculate k' values was found by injecting pure water into a potassium nitrate eluent monitored at 230 nm .

2.2. Reagents

All reagents were of AnalaR grade (BDH, Poole, UK) with the exception of 4-(2-pyridylazo)-resorcinol (PAR) and zinc–EDTA (Fluka, Glossop, Derbyshire, UK), Arsenazo III (95% dye content) (ICN Biomedicals, Aurora, OH, USA) and pyrocatechol violet (PCV) and dipicolinic acid (Aldrich, Gillingham, UK). Metal stock solutions were prepared from Spectrosol standards (BDH) using Milli-Q water (Millipore, Milford, MA, USA), and stored in polypropylene bottles (BDH).

The postcolumn reagents used during these studies were 0.1 mM PAR, 0.125 M disodium tetraborate

and 0.2 M sodium hydroxide (pH 10.5) for the transition, heavy metals and the lanthanides (520 nm), 0.12 mM PAR, 0.2 mM Zn–EDTA and 2 M 35% ammonia for the alkaline earths (520 nm), 0.15 mM arsenazo III in 1 M nitric acid for uranium (654 nm) and 0.004% PCV buffered to pH 6.9 with 2 M hexamine and nitric acid for aluminium (580 nm).

The mobile phase consisted of aqueous 1 M potassium nitrate adjusted to the appropriate pH with nitric acid, and containing an appropriate concentration of 4-chlorodipicolinic acid.

2.3. 4-Chlorodipicolinic acid synthesis

The synthesis involved a one-step reaction of 0.5 mol chelidamic acid (Aldrich) with phosphorus pentachloride (Aldrich) in chloroform (Fluka), in accordance with the procedure described by Bradshaw et al. [18]. The chloroform was distilled from the reaction mixture and crude product was precipitated by the addition of cold water. The final product was filtered on a porous glass filter, washed with cold water and dried.

2.4. Rice flour sample digestion

The rice flour sample, National Research Centre Certified Reference Material, GBW 08502 (LGC, Middlesex, UK) was prepared for analysis using microwave digestion. A known amount of accurately weighed sample ($\sim 0.5 \text{ g}$) was added to a PTFE bomb together with 1 ml 30% hydrogen peroxide (Fluka) and 4 ml nitric acid (Aristar, BDH), capped and left overnight. The predigested material was microwaved at 750 W using a commercial microwave, for five 2-min cycles. The vessel was uncapped and the material evaporated to incipient dryness using a waterbath. The residue was transferred to a volumetric flask with small aliquots of eluent, made up to a final volume of 2 ml and analysed within 2 h . A blank run was also performed to estimate system contamination, and found to be negligible. The moisture content of the flour was determined by oven drying ($115 \text{ }^\circ\text{C}$) 0.5 g of the material for 4 h , before cooling in a vacuum desiccator, and found to be 2% .

3. Results and discussion

3.1. Dynamic modification of PS–DVB with 4-chlorodipicolinic acid

Dynamic modification of a small particle sized neutral stationary phase with a relatively low molecular mass complexing molecule is a quick and effective method of producing a high efficiency chelating ion-exchange material. The technique entails adding a low concentration of a chelating molecule to the mobile phase in order to establish an equilibrium with a sorbed layer on the stationary phase [12]. A low concentration is normally chosen, consistent with maintaining a stable adsorbed layer, to minimise potential postcolumn detection interferences arising from having a competing chelating agent present in the mobile phase. A high concentration of potassium nitrate is additionally added to suppress ion-exchange interactions [11]. It has previously been shown that eluent ionic strength has a negligible effect upon the degree of loading of heterocyclic acids onto neutral PS–DVB resins [12].

Eluent pH, however, does have an important role in determining the degree of dynamic modification, as shown by a plot of retention factors (k') versus eluent pH at ambient temperature for both dipicolinic acid and chlorodipicolinic acid on the neutral resin (Fig. 1). Dipicolinic acid contains both basic and acidic functional groups, namely, the pyridyl nitrogen and carboxyl oxygens. Therefore its maximum adsorption onto a neutral hydrophobic resin surface would be expected to lie close to the molecule's isoelectric point (pI). Taking into account the three protonation constants for dipicolinic acid, for the species $(H_3)^+$, $(H_2)^0$ and $(H)^-$, corresponding to the pK_a values 0.46, 2.16 and 4.76 [19], and considering that the isoelectric point can be expressed as $pI = (pK_1 + pK_2)/2$ [20], the isoelectric point for this acid should lie at pH 1.3, which agrees well with the maximum sorption of this molecule to the resin as shown in Fig. 1. The third protonation constant for chlorodipicolinic acid is not known, but based on the fact that its two known pK_a values are 1.7 and 3.75 [21], its isoelectric point should also fall within a similar region, which is shown by the plot given in Fig. 1.

At pH 1.3, the retention of chlorodipicolinic acid

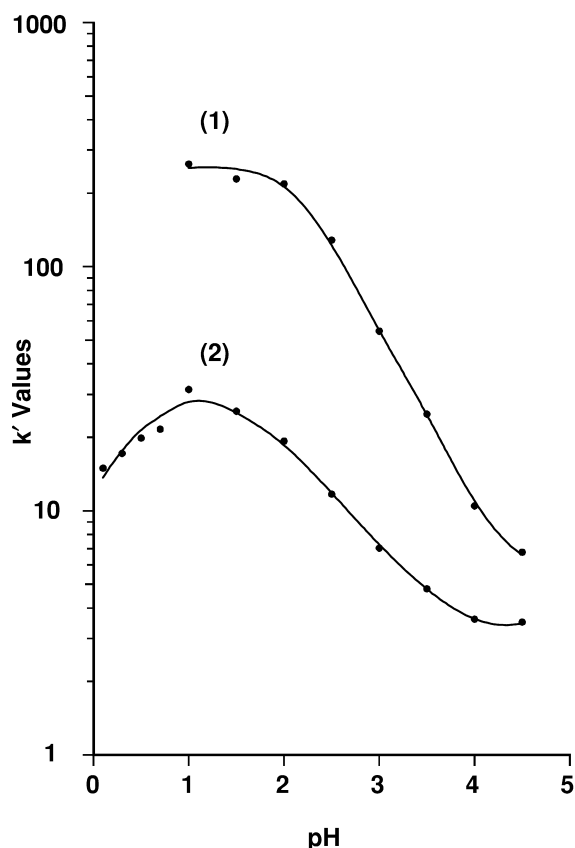


Fig. 1. Dependence of retention factors (k') for (1) chlorodipicolinic acid and (2) dipicolinic acid on the mobile phase pH. Mobile phase: 1 M potassium nitrate adjusted with nitric acid.

($k' = 229$) is almost ninefold greater than the retention of dipicolinic acid ($k' = 26$). As the retention of these heterocyclic acids is related to the distribution coefficients of the chelating molecules between stationary and mobile phases, it can be deduced that a higher capacity chelating ion-exchange material is obtained by using the more π -electron accepting molecule in the eluent. The steady drop in retention of these molecules with increasing pH beyond the isoelectric point, can be related to an increasing percentage of the -1 then -2 species of acid in solution, chlorodipicolinic acid having a k' value of only 6.7 at pH 4.5, when the -2 species is becoming dominant.

To provide a more meaningful estimation of the degree of dynamic modification, the phase ratio was calculated to give the amount of chlorodipicolinic

acid loaded onto the resin as a function of pH. Using the stationary phase volume (resin mass/density: 0.4 g cm^{-3} for Hamilton PRP-1) and the column dead volume, the phase ratio was calculated to be close to 1:1. Taking into consideration the constancy of concentration of the modifier in the mobile phase, the k' value can be used to estimate the concentration of acid sorbed onto the stationary phase, and hence the effective dynamic loading. Using a concentration of 0.25 mM chlorodipicolinic acid in the eluent, chosen to obtain the appropriate analyte selectivity as will be explained in the following section, the effect of eluent pH on the degree of dynamic modification is shown in Fig. 2. Referring to this figure, the estimated loading was 0.060 mmol/g at pH 1.3, which is a significant adsorbed concentration, but much lower than those resins with chemically bonded groups. Unfortunately, using

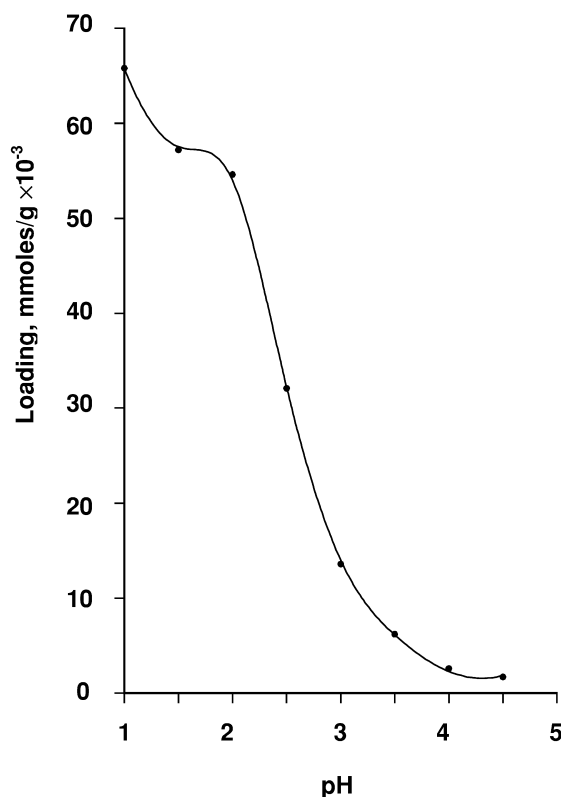


Fig. 2. Loading of chlorodipicolinic acid onto neutral $7 \mu\text{m}$ PRP-1 PS-DVB, using an eluent containing 1 M potassium nitrate and 0.25 mM chlorodipicolinic acid; pH adjustments made using nitric acid.

metal breakthrough curves to estimate column capacity, as has been used with fabricated chelating dye columns [22], was unfeasible due to the likelihood of equilibrium disturbance affecting the extent of dynamic loading. Nevertheless, the chlorodipicolinic acid column has been found to be an effective substrate for trace metal separations as detailed in the following section.

3.2. Metal retention properties of the modified resin

In an earlier study [13], the effect of chlorodipicolinic acid concentration (varied from 0.5 to 1 mM) upon the retention of selected transition and heavy metal ions, was investigated using a 100-mm column length. For this study, an attempt to improve the selectivity and performance of the dynamic system was made by increasing the column length to 250 mm , and further investigating the effect of chlorodipicolinic acid concentration in the eluent on metal retention characteristics as a function of pH. More detailed retention factor plots were produced over a wider pH range, and additional metal ions studied, namely U(VI), Fe(III), Al(III), La(III) and Lu(III), typically present in significant concentrations in many environmental and geological samples. From these studies, it was found that a concentration of 0.25 mM was sufficient to provide resolution of transition and heavy metals on the 250-mm column, the rather complicated k' plots being shown in Fig. 3.

Clearly, a rather complex situation is present in dynamically modified systems compared to that involving coated or bonded phases. Normally, for coated and bonded chelating phases with no complexing agent in the eluent, straight line $\log k'$ plots against pH are obtained [11]. However, with dynamic systems the loading of the chelating agent on the substrate is not constant and starts to fall substantially above a certain pH (Fig. 1). This would be expected to cause the $\log k'$ plots to flatten out and even go negative if the pH is high enough. However, a further complicating factor is the degree of complexation in the mobile phase. The complexation on the resin substrate can only be 1:1, whereas in the mobile phase it can be 2:1 or even 3:1 with the lanthanides. Therefore, as the pH is raised

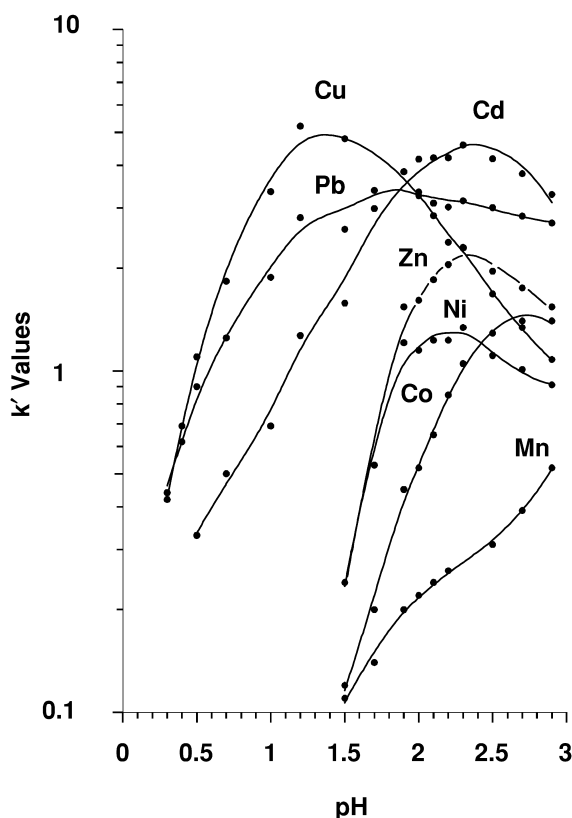


Fig. 3. Dependence of retention factors for selected transition and heavy metal ions as a function of eluent pH. Eluent as given in Fig. 2. Detection conditions as given in the text.

the more stable 2:1 complexes will start to form in the eluent and become more and more competitive with the 1:1 complexes on the resin. The formation of higher ligand-to-metal ratios would be expected to reinforce the drop in loading of the dynamic layer and this is indeed supported by the large negative slopes seen for some of the metals. The lack of a maximum for manganese is likely to be due to the relatively low $\log \beta_2$ value, so little 2:1 complex is present even at pH 3.

An additional consideration is the possibility of some reversed-phase interactions between the neutral forms of metal complexes in the mobile phase and the substrate, as discussed in the Introduction. Notwithstanding the validity of the hypothesis proposed above, the rapid drop in capacity of the sorbed layer must produce maxima in the $\log k'$ plots and the varying slopes give rise to changes in selectivity that

can be potentially exploited. Thus, using the k' plots as a guide, a near baseline separation of seven transition and heavy metal ions in under twenty min was possible at pH 2.2 as shown in Fig. 4. However, a 50-mm guard column needed to be added to the existing 250-mm column to achieve the required resolution.

The retention of selected higher valency metal ions often present in many sample types was also investigated at a chlorodipicolinic acid concentration of 0.25 mM; the log retention factors vs. pH plot is given in Fig. 5. With the exception of Al(III), and to some degree La(III), the higher valent metal ions are relatively strongly retained, especially U(VI), in comparison with the +2 metals, which was similarly found with a dynamically modified dipicolinic acid column [15]. The straight line plots observed for

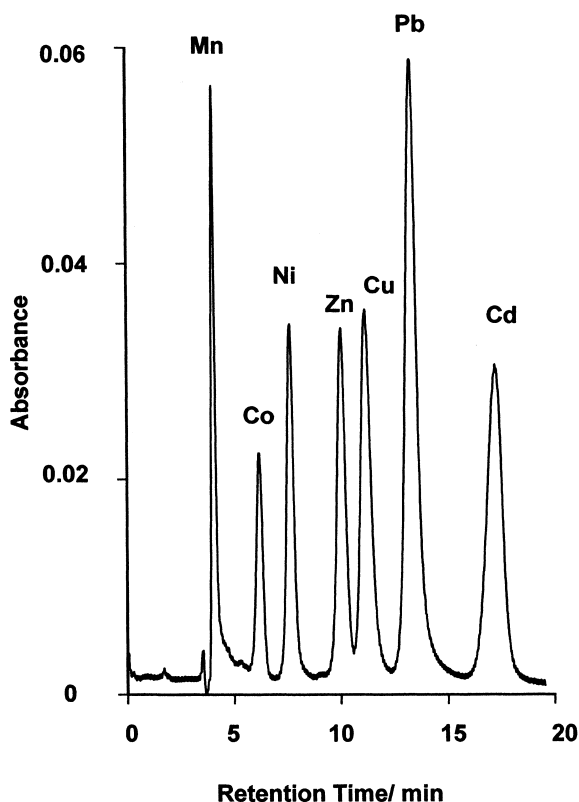


Fig. 4. Separation of Mn(II) 0.5 mg l⁻¹, Co(II) 0.5 mg l⁻¹, Ni(II) 0.5 mg l⁻¹, Zn(II) 2 mg l⁻¹, Cu(II) 1 mg l⁻¹, Pb(II) 10 mg l⁻¹ and Cd(II) 20 mg l⁻¹ on a 300×4.6 mm PRP-1 7 μm PS-DVB column. Eluent: 1 M potassium nitrate, 0.25 mM chlorodipicolinic acid and 6.25 mM nitric acid (pH 2.2). Detection: PAR at 520 nm.

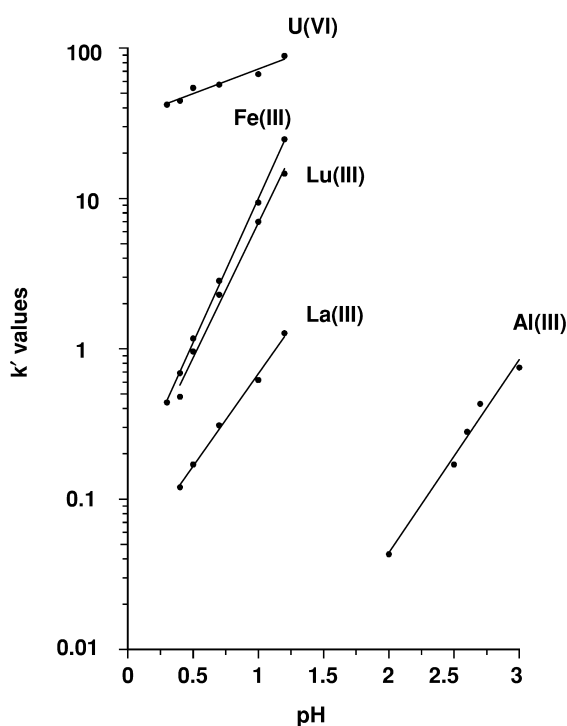


Fig. 5. Dependence of retention factors for U(VI), La(III), Lu(III), Fe(III) and Al(III) as a function of eluent pH. Eluent as given in Fig. 2. Detection conditions as given in the text.

these metals would infer that chelation ion-exchange is dominant in the retention of these ions over the pH range studied, when using chlorodipicolinic acid as a complexing agent in the mobile phase, with little influence from complexation in the mobile phase. As anticipated from the stability constants for the analogous dipicolinic acid, the alkaline earth metals were unretained on this substrate over the whole pH range examined.

3.3. Analytical performance of the resin

Using an optimised eluent of 1 M KNO_3 , 6.5 mM HNO_3 (pH 2.2) and 0.25 mM chlorodipicolinic acid, together with a 250-mm column length and 50-mm guard column, a calibration of seven transition and heavy metal ions over the range $5 \mu\text{g l}^{-1}$ to 5mg l^{-1} was possible. The standards were prepared in a matrix-matched solution to minimise equilibrium disturbances. This was deemed necessary due to the appearance of system peaks that interfered in the

Table 1

Calibration characteristics using a $250 \times 4.6 \text{ mm } 7 \mu\text{m}$ PRP-1 PS-DVB column, and an eluent of 1 M potassium nitrate, 6 mM nitric acid and 0.25 mM chlorodipicolinic acid; flow-rate 1 ml min^{-1} , postcolumn detection with PAR at dual wavelengths of 520 and 445 nm

Metal ion	R^2	RSD (%) (100 μg) (Pb(II) 200 μg)	Limit of detection ($\mu\text{g l}^{-1}$)
Manganese	0.9988	1.02	3.5
Cobalt	0.9997	4.26	3.5
Nickel	0.9981	3.50	14
Zinc	0.9986	4.31	5.5
Copper	0.9994	1.12	12.5
Lead	0.9987	1.51	82
Cadmium	0.9997	4.13	15.5

detection of Mn(II) and Zn(II) when injecting unmodified water samples. The calibration results are given in Table 1. The reproducibility—relative standard deviation (RSD)—was calculated with repeat injections ($n=6$) of a $100 \mu\text{g l}^{-1}$ standard, with the exception of Pb(II) at $200 \mu\text{g l}^{-1}$, because of poorer detection sensitivity. The limit of detection (LOD) was calculated as twice baseline peak-to-peak noise, and metal concentrations determined using peak area measurements. The regression coefficients were good (>0.998), as was the reproducibility ($\text{RSD} < 5\%$). The detection limits were also good ($3.5\text{--}82 \mu\text{g l}^{-1}$), especially considering chlorodipicolinic acid was present in the mobile phase, which could compete with the metallochromic ligand PAR in the post-column reagent.

To assess the analytical performance of the system for a relatively simple matrix, a National Water Research Institute standard reference soft water sample, TMDA 54.2 (LGC) was analysed. This water contains low concentrations of alkali and alkaline earth metals, Ca(II) 9.2 mg l^{-1} , Mg(II) 2.1 mg l^{-1} , Na 3.1 mg l^{-1} and K 0.4 mg l^{-1} , which were found not to disturb the dynamic equilibrium. The water sample was diluted twofold using an eluent-matched solution, and analysed using standard addition curves. The reproducibility was calculated from repeat injections ($n=6$). The results are given in Table 2, a chromatogram of the water sample given in Fig. 6. The results for each metal agreed well with the certified values, and the system reproducibility was good ($<5\%$ RSD).

As can be seen from Fig. 6 however, problems

Table 2

Analytical results obtained using the certified TMDA 54.2 water sample; conditions as given in Table 1

Metal ion	R^2	RSD (%) (diluted sample)	Certified ($\mu\text{g l}^{-1}$)	This method ($\mu\text{g l}^{-1}$)
Manganese	0.9998	1.55	346±32.7	362±5.6
Cobalt	0.9996	3.52	276±21.9	268±9.4
Nickel	0.9944	3.00	325±30.3	350±10.5
Copper	0.9987	1.07	460±41.9	477.5±5.1
Lead	0.9967	4.22	531±54.4	506±21.4
Cadmium	0.9996	1.54	165±16.1	173±2.7

arose when using this system. A reproducible system peak interfered with, and prevented the detection of Zn(II), which was attributed to a phase equilibrium shift, temporarily displacing chlorodipicolinic acid from the resin backbone, upon injecting a sample plug onto the column. In addition, the system was adversely susceptible to matrix metals present in the sample plug, which would temporarily swamp the

system, resulting in a disturbed baseline shift until re-equilibration was reached.

As discussed earlier, it is envisaged that retention at pH 2.2 is strongly influenced by complexation in the mobile phase, being easily disturbed by the sample matrix. Separation at this pH therefore is highly susceptible to background metals and high concentrations of ions present in the sample plug, making this system suitable for relatively clean aqueous samples only.

Based on earlier studies [12,13,15], it was believed a reduction in pH would allow complexation with the chlorodipicolinic acid on the resin surface to become the most dominant, metals being essentially separated only by a chelation ion-exchange process. In addition, a reduction in pH close to the acids isoelectric point would increase the concentration of chlorodipicolinic acid present on the resin. A pH of 1.5 was chosen, based on the retention factor plots (Fig. 3) and the dynamic loading curve (Fig. 2). At this pH, the system behaved in a markedly different way, in that no baseline shifts or disturbances were observed upon injecting Ca(II) and Mg(II) up to 1000 mg l^{-1} onto the column, supporting the above hypothesis.

Unfortunately, a disadvantage of using this lower pH was a loss of column selectivity, with most of the metal ions co-eluting in the void volume. Fortunately, Cd(II), Pb(II) and Cu(II), of special interest for pollution studies, could however still be baseline resolved away from matrix ions in the void volume. A 100-mm column was now sufficient for their separation, as shown in Fig. 7. It is interesting to note the change in elution order for these three metals, being Cd(II) < Pb(II) < Cu(II) at pH 1.5, in contrast to Cu(II) < Pb(II) < Cd(II) at pH 2.2, as predicted by the log k' plots in Fig. 3.

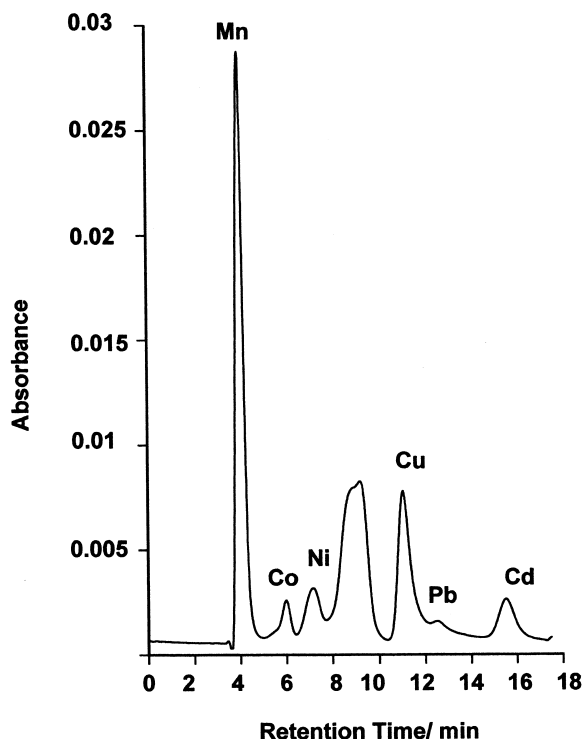


Fig. 6. Chromatogram of transition and heavy metal ions in the certified soft water sample TMDA 54.2 on the $300 \times 4.6 \text{ mm}$ PRP-1 $7 \mu\text{m}$ PS-DVB column. Eluent: 1 M potassium nitrate, 6 mM nitric acid and 0.25 mM chlorodipicolinic acid. Detection: PAR at 520 nm .

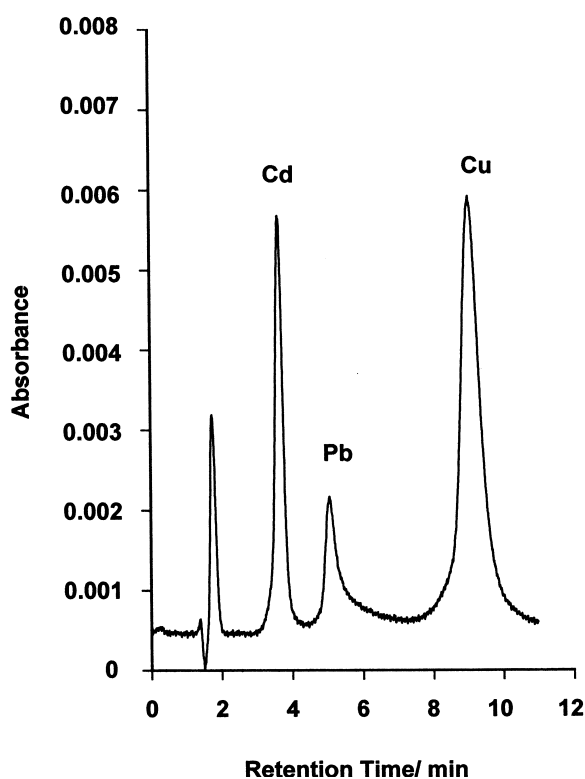


Fig. 7. Separation of Cd(II) $100 \mu\text{g l}^{-1}$, Pb(II) $500 \mu\text{g l}^{-1}$ and Cu(II) $200 \mu\text{g l}^{-1}$ on the $100 \times 4.6 \text{ mm}$ PRP-1 $7 \mu\text{m}$ PS-DVB column. Eluent: 1 M potassium nitrate 30 mM nitric acid and 0.25 mM chlorodipicolinic acid ($\text{pH } 1.5$). Detection: PAR at 520 nm .

To ascertain system performance at this lower pH, the other eluent characteristics remaining unaltered, a calibration was performed as detailed earlier. The results are given in Table 3 with reproducibility values calculated from repeat injections ($n=6$). Due to the much shorter retention times, lead and cadmium peak shapes were significantly sharper, correspondingly improving the detection sensitivity for

these two metals. The multiple wavelength background subtraction method devised by Jones was also used [17], lowering the detection limits even further, most notably for Cd(II). For all three metals, detection limits compare favourably with existing ion chromatography methods [23–25], especially as analyte preconcentration was not undertaken. With a detection limit of $<1 \mu\text{g l}^{-1}$, it should be possible to determine Cd(II) directly in drinking waters and other high purity samples.

To assess the effect of a more complex sample matrix on the system analytical performance, a certified rice flour, GBW 08502 was analysed. Food samples generate problems due to lipids and proteins that generally require high dissolution temperatures for complete digestion. It was decided that closed vessel microwave digestion was suitable, based on reviews of the technique [26,27]. In addition, Cd(II), Cu(II) and Pb(II) have been determined in rice flour using microwave digestion prior to ICP-MS detection [28], and Pb(II) and Cu(II) by nitric acid wet-ashing prior to HPLC separation as hexamethyl dithiocarbamate complexes [29].

Replicate sample analysis was undertaken ($n=5$), using standard addition curves to take into account possible system bias. The reproducibility of the method was calculated with the repeat injection ($n=6$) of a sample. The results are also presented in Table 3, and show for each metal good agreement with the certified values. A chromatogram of the rice flour sample is given in Fig. 8.

4. Conclusions

Using dynamic modification as a means of coating a neutral high efficiency resin with a metal complex-

Table 3

Calibration characteristics using a $100 \times 4.6 \text{ mm}$, $7 \mu\text{m}$ PRP-1 PS-DVB column, and an eluent of 1 M potassium nitrate, 30 mM nitric acid and 0.25 mM chlorodipicolinic acid; flow-rate, 1 ml min^{-1} ; postcolumn detection with PAR at dual wavelengths of 520 and 445 nm

Metal ion	Range ($\mu\text{g l}^{-1}$)	R^2	RSD (%)	Limit of detection ($\mu\text{g l}^{-1}$)	Rice flour	
					Certified ($\mu\text{g l}^{-1}$)	This method ($\mu\text{g l}^{-1}$)
Cadmium	1–1000	0.9987	0.48 (100 μg)	0.8	20 ± 3	19.1 ± 0.5
Lead	5–5000	0.9980	1.38 (500 μg)	39	750 ± 100	727.6 ± 41.5
Copper	5–5000	0.9995	0.70 (200 μg)	5.5	2600 ± 300	2670 ± 100

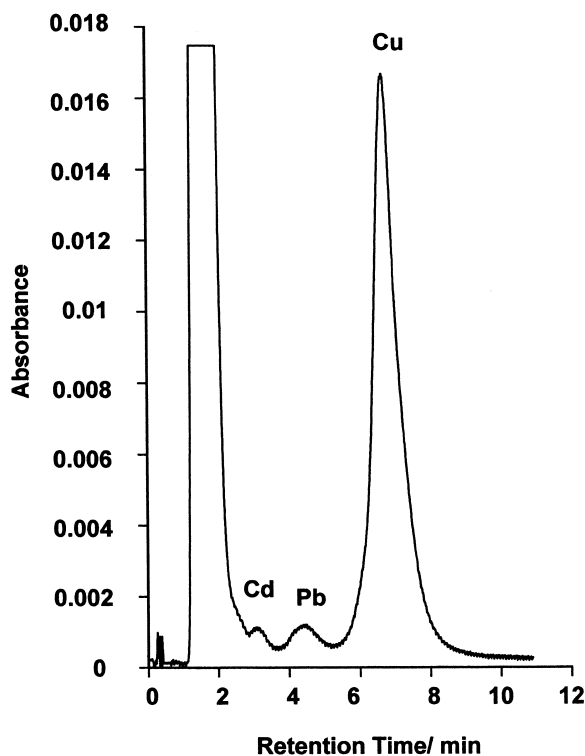


Fig. 8. Separation of Cd(II), Pb(II) and Cu(II) from matrix interferences in the certified rice flour GBW08502 on the 100×4.6 mm PRP-1 $7 \mu\text{m}$ PS-DVB column. Eluent: 1 M potassium nitrate, 30 mM nitric acid and 0.25 mM chlorodipicolinic acid. Detection: PAR at 520 nm.

ing agent, a method has been devised to determine transition and heavy metal ions in complex sample matrices. Through manipulation of eluent pH, and optimising the ligand concentration in the mobile phase, seven metal ions could be determined in dilute aqueous samples within 20 min, and Cd(II), Pb(II) and Cu(II) in under 10 min in a rice flour matrix, dependent upon analysis requirements. Detection limits and system reproducibility were good and compare well with existing chromatographic methods for the determination of these metals.

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