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## Single-column method of chelation ion chromatography for the analysis of trace metals in complex samples

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### Abstract

A single-column chelation ion chromatographic system for the preconcentration and separation of trace transition metals is described. The system includes standard chromatographic equipment with a post-column reagent system based on the reaction with 4-(2-pyridylazo)resorcinol followed by photometric detection at 495 nm. Iminodiacetic acid bonded to 5  $\mu\text{m}$  silica (Diasorb IDA) was used as a chelating stationary phase. The strong complexing ability in combination with good kinetics of complexation and ion-exchange selectivity of iminodiacetic functional groups allow both preconcentration of Mn, Co, Cd, Zn, Ni and Cu from waters of high salinity and efficient separation with the same column. The retention characteristics of alkaline-earth and transition metal ions on Diasorb IDA silica (250 $\times$ 4 mm I.D.) column was investigated for a variety of eluents including nitric acid, maleic, malonic, citric, dipicolinic, picolinic, tartaric and oxalic acids. The influence of ionic strength on retention of metal ions involving high nitrate and chloride concentrations was also evaluated. The baseline separation of preconcentrated metals was achieved using a three-step gradient elution scheme which involved first, flushing of the column loaded with the sample with 0.5 M KCl–0.5 mM HNO<sub>3</sub> for 10 min, followed by 80 mM tartaric acid for 20 min and finally 10 mM picolinic acid for 20 min.

**Keywords:** Chelation ion chromatography; Iminodiacetic acid-silica; Metals

### 1. Introduction

The determination of trace metals in complex matrices remains one of the most complicated areas of analytical chemistry. In the last decade, chelation ion chromatography (CIC) has become one of the useful analytical tools in solving this problem. However, most studies using CIC involve the preconcentration of trace metals on a pre-column packed with chelating ion exchanger, followed by another matrix isolation column and finally ion

chromatographic determination using a standard high-performance anion- or cation-exchange column, depending on the type of complexes formed in the eluent [1–3]. This approach was successfully used for the analysis of seawater [1,2], biological fluids and tissues [1–3], industrial wastewaters and inorganic reagents [2]. Clearly, the equipment required is rather complex, involving a combination of several concentration and separation columns, pumps and switching valves under computer control. A proportionally increased number of different reagents is also required for preconcentration, elution and separation steps. This significantly complicates the analysis and increases its cost.

A recent improvement in the chemistry and op-

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timization of the structure of chelating ion exchangers allows the use of a single column, both for the preconcentration of trace metals and for their separation. The use of a single column provides some practical advantages. Firstly, it eliminates the use of a specially designed chelating column linked to a series of separation columns. Secondly, it is not necessary to use additional eluents for flushing and regenerating of these columns as well as valves and pumps for purging of them. It simplifies the whole chromatographic system and decreases the possible effect of impurities in the different eluents on the final results of analysis.

High stability and separation efficiency with optimal selectivity for alkaline, alkaline-earth and transition metals have been demonstrated for chelating ion exchangers with iminodiacetic acid (IDA) functionality. Several types of high efficiency chelating substrates, including IDA bonded to silica [4–6] and to hydrophilic polymer matrix [7], octadecylsilica dynamically coated with N-dodecyliminodiacetic acid [8] and a number of chelating substrates obtained by impregnation of polymeric resins with such dyes as Xylenol Orange [9–11], Phthalein Purple [12] and Methylthymol Blue [9,12] have been successfully applied for the separation of metal ions. Some of the above mentioned polymeric chelating materials have been used for analysis of many environmental and industrial samples having high ionic strength, such as seawaters [11,13], concentrated brines [11,14], mineral waters [12], laboratory reagents [11] and effluent streams.

The main drawback of polymeric resins is the relatively lower efficiency of separation compared to silica based matrices. Because of this, a four-step concentration gradient must normally be used for the satisfactory separation of a mixture of nine alkaline-earth and transition metals [11]. However, IDA bonded to silica demonstrates a higher efficiency that allows isocratic separation of up to eight metal ions [15]. The stronger complexing ability of IDA bonded silica also allows the use this sorbent in acid media where it is very stable.

This paper describes the development and use of a simplified mode of CIC involving IDA bonded silica as a tool for the analysis of water samples with complex matrices.

## 2. Experimental

### 2.1. Apparatus

An isocratic ion chromatographic system consisting of a LKB 2150 HPLC titanium pump (Bromma, Sweden), a Rheodyne 7010 polyether ether ketone (PEEK) liquid six port injection valve (Rheodyne, Cotati, CA, USA) fitted with 100  $\mu$ l or 1 ml PEEK sample loop and post-column detection system. The latter includes a Constametric III HPLC pump (LDC, Riviera Beach, FL, USA) used for delivery of 4-(2-pyridylazo)resorcinol (PAR) post-column reagent, a zero dead volume poly(tetrafluoroethylene) (PTFE) tee followed by a 1.4 m $\times$ 0.3 mm I.D. PTFE reaction coil and a Dionex spectral array detector (Dionex, Sunnyvale, CA, USA) set at 495 nm. The post-column reagent is a mixture of  $1.2 \cdot 10^{-4}$  M PAR, 2.6 M  $\text{NH}_4\text{OH}$  and 0.85 M  $\text{NH}_4\text{NO}_3$  which gives a pH of 10.2. The separations with conductivity and fluorometric detection were performed with a LKB Conductolyser 5300 (LKB) and a Hitachi F-1050 fluorescence spectrophotometer (Hitachi, Tokyo, Japan), respectively.

### 2.2. Columns

The stainless-steel column (250 $\times$ 4 mm) packed with 6  $\mu$ m particle size silica bonded with iminodiacetic acid was purchased from JV Bio-Chemmack (Moscow, Russian Federation). The efficiency of the column calculated from peak benzene retained in pure hexane was equal to  $1.2 \cdot 10^4$  theoretical plates per meter.

### 2.3. Reagents

All reagents were of analytical grade unless specified. Oxalic acid, dipicolinic acid, picolinic acid, maleic acid, malonic acid, citric acid (Aldrich, Gillingham, UK) and sulfosalicylic acid, tartaric acid (BDH, Poole, UK) were used for preparation of eluents. Other common reagents were supplied by BDH except PAR which was obtained from Fluka (Glossop, UK). 1000 ppm metal stock solutions were Spectrosol obtained from BDH. Solutions were prepared using deionised water from Milli-Q system

(Millipore, Bedford, MA, USA) and stored in poly-(propylene) bottles (BDH).

### 3. Results and discussion

The strong complexing ability of iminodiacetic acid bonded to silica has been used for the pre-concentration of transition metals from dilute solutions, biological fluids and tissues [16]. It was shown that the complete extraction of most of the transition metals except manganese(II) can be achieved in batch experiments at pH 4 and higher. It was also shown that the good mechanical and hydrolytic stability of this sorbent in weak acid and neutral media, in conjunction with good exchange kinetics, allowed the efficient chelating ion-exchange separation of a number of metal ions [4,6,15,17]. It was reasoned that such a combination of properties could be applied to the determination of trace transition metals in samples with complex matrices by single-column chelation ion chromatography. It was also expected that the preconcentration and separation of metals on the same column could be achieved.

#### 3.1. Elimination of sample matrix and preconcentration of trace metals

The high salinity of brines, seawater and industrial samples is due in part, to the presence of high levels of alkali and alkaline-earth metal ions. So, to determine trace transition metals, the excess of alkaline-earth metals must be separated as they usually strongly influence the chromatographic resolution of preconcentrated transition metal ions. The clear separation of alkaline-earth metals from transition metals is also important for another reason, in that a large excess of alkaline-earth metals will give a significant photometric response during post-column reaction detection, in spite of being relatively insensitive to PAR.

Chelating ion exchangers with covalently bonded iminodiacetic acid functional groups have been shown to react with alkaline-earth metal ions by simple ion-exchange at pH values below 4 and by chelation-exchange at pH values above 6 [18]. Between these values, both mechanisms take place.

So it is possible to achieve a simultaneous separation of alkali, alkaline-earth and transition metal ions under isocratic conditions after optimising the pH of the eluent (Fig. 1). It should be noted that the high efficiency of the column packed with iminodiacetic acid bonded to silica is comparable with traditional ion chromatographic columns.

The role of ion exchange in the sorption of alkaline-earth metal ions can be drastically reduced in the presence of high concentrations of simple electrolytes. Several elution systems have been previously developed, exploiting this effect, for the removal of excess alkaline-earth metals from preconcentrated transition metals (Table 1). Usually, ammonium acetate or potassium nitrate were used for this purpose. However, it was shown in a recent paper that the complexing ability of iminodiacetic acid bonded to silica is higher than that for other chelating ion exchangers with the same functional groups [15]. Therefore, further optimization of the nature of the simple electrolyte, its concentration and pH was found to be necessary when using the IDA

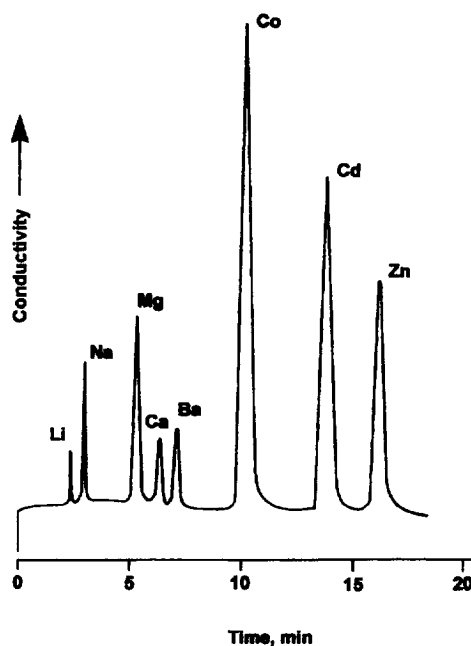


Fig. 1. Separation of a mixture of alkali, alkaline-earth and transition metal ions. Mobile phase: 10 mM sulphosalicylic acid; flow-rate 1.0 ml/min; conductivity detection. Sample volume: 50  $\mu$ l.

Table 1

Recommended conditions for the group separation of alkaline-earth metals from transition metals on chelating exchangers containing iminodiacetic functional groups

Chelating ion exchanger	Recommended eluents		Ref.
	Concentration	pH	
Chelex 100	1 M CH <sub>3</sub> COONH <sub>4</sub>	5.0–5.5	[19]
MetPac CC-1	2 M CH <sub>3</sub> COONH <sub>4</sub>	5.5	[1,2]
Tosoh Chelate 5PW	0.4 M KCl	5.0	[8]
	1 M KNO <sub>3</sub>	6.0	[11,12]
Xylenol Orange impregnated resin	1 M KNO <sub>3</sub>	6.5	[11–13]
	1 M CH <sub>3</sub> COONH <sub>4</sub>	6.0	[15]

bonded silica. Initially, the retention behaviour of 0.5 M KNO<sub>3</sub> eluents containing varied concentrations of nitric acid for the elution of alkaline-earth metals solution was determined (Fig. 2a). It can be seen that the alkaline-earth metals can be quantitatively eluted by 0.5 M KNO<sub>3</sub> containing 1–3 mM HNO<sub>3</sub> (pH 2.3 to 3.0), while transition metals and lead are strongly retained on the column. Despite the usefulness of this mixture for the preconcentration and separation of traces of transition metals, it can be of limited value for the analysis of samples with increased concentration level of chloride, such as estuarine and seawater samples. This is because the known relatively strong complexing ability of chloride ions towards Zn(II), Cd(II) and Pb(II) and to a lesser extent the other transition metals, may lead to incompleteness of sorption of these metals and to errors in their determination. Therefore, the use of potassium chloride in the eluent for the removal of alkaline-earth metals, while retaining transition metals, needed to be investigated and optimised to allow the analysis of samples high in chloride. A similar study of retention behaviour for alkaline-earth metals, transition metals and lead was carried out for 0.5 M KCl eluents with varied concentrations of nitric acid (Fig. 2b). When compared to the 0.5 M KNO<sub>3</sub> eluents, a decrease in retention times for all metal ions and a reversed elution order for some metals was observed. Nevertheless, the elution of alkaline-earth metals and separation from preconcentrated transition metals was still possible by using a mixture of 0.5 M KCl and 0.1 mM HNO<sub>3</sub> (pH 3.9). Thus, in further experiments involving samples high in chloride, the flushing of the chromatographic

column with this eluent for 10 min following sample injection was considered acceptable as the first step of a gradient elution scheme. In certain situations however, some manganese could be lost because its retention is very close to those of the alkaline-earth metals.

### 3.2. Selection of eluent and optimization of step gradient programme for the separation of transition metals

After the preconcentration and initial separation achieved with a potassium chloride–nitric acid mixture, the chromatographic bands of transition metal ions are retained in the chromatographic column in the following order from the start: iron(III) > copper > nickel > lead > cobalt > zinc > cadmium > manganese. This is not the exact expected retention order when considering the stability constant sequence for metal complexes of IDA, because zinc, cadmium and lead are strongly affected by competition from the chloride ion in the eluent. A step increase of the concentration of nitric acid in 0.5–1.0 M potassium salts was used in previous studies for the further separation of retained metals producing an elution pattern corresponding to the above mentioned retention order [11,13]. However, in practice, when considering the possibility of the analysis of seawater it would contain at least one order of magnitude more zinc than cobalt and cadmium. This would produce definite problems for the detection of small concentrations of cobalt on the tail of the zinc peak. It would be preferable therefore, if the next gradient step after the potassium chloride–1 mM

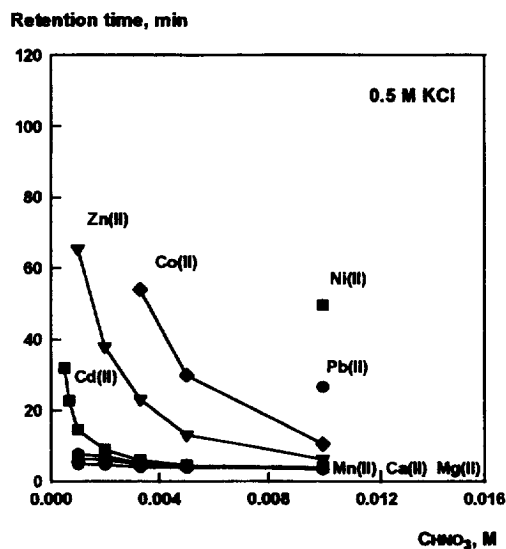
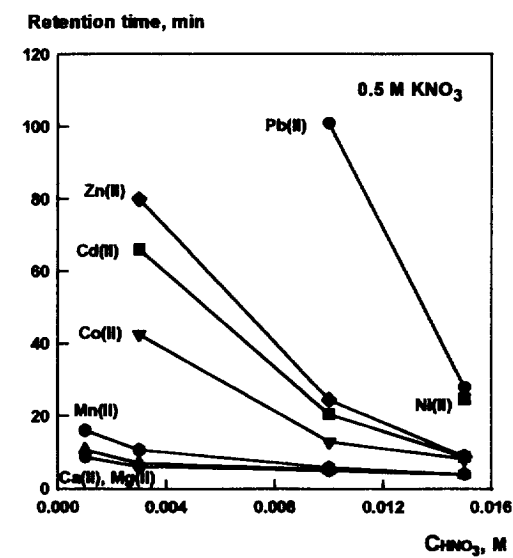


Fig. 2. Influence of nitric acid concentration on the retention times of alkaline-earth and transition metal ions in presence of (a) 0.5 M KNO<sub>3</sub> and (b) 0.5 M KCl; flow-rate 0.7 ml/min.

HNO<sub>3</sub> eluent, produced a separation and elution order: Cd>Co>Zn. The retention characteristics of a number of different organic acids were tested (Table 2), but only oxalic and tartaric acid provided a good selectivity for the separation of cadmium, cobalt and zinc. However, poor solubility and the possibility of precipitation of oxalates of transition metals in the

chromatographic column strongly restricted the application of oxalic acid as an eluent. So, the flushing of the chromatographic column with 80 mM tartaric acid was chosen for the elution of cadmium, cobalt and zinc as a second step of the gradient programme.

It should be noted that nickel, lead and copper were retained on the column after 1 h elution with 0.1 M tartaric acid (Table 2), so the next step of the gradient programme must include the application of stronger complexing eluents, or the use of strong acids, to destabilise these metal complexes held on the surface of the IDA-silica and speed up the elution. According to previous data [4–6] the use of dipicolinic or picolinic could be effective for the elution and separation of nickel, copper and lead. These acids behave like strong eluents, especially for nickel and copper, due to formation of very stable complexes. Initial experiments showed that dilute dipicolinic acid was too strong an eluent for copper and nickel and did not provide a reasonable separation of these metals. However, 10 mM picolinic acid gave a reasonable separation of nickel and copper in 20 min.

Finally, taking all the above points into account, a three-step gradient elution programme (Table 3) providing a good separation of two alkaline-earth and six transition metals (Fig. 3) was developed. It was demonstrated that the silica gel bonded IDA column gave a good separation of cadmium from other transition metals as compared to the dye-coated column developed earlier [11,13]. Thus, samples high in chloride and alkaline-earth metals can be concentrated on the column and the transition metals eluted and separated using the same column. It should be noted that there is a limit to the “capacity” of the column and elution system and it is considered that the maximum volume of sample containing high concentrations of alkaline earth metal cations is about 20 ml at pH 3.9.

#### 4. Conclusion

This study shows that it is not always necessary to use a combination of different chromatographic columns for the CIC determination of trace metals in complex samples. A single column can be used to achieve both preconcentration and high efficiency

Table 2  
Retention times (min) of transition metal ions on iminodiacetic acid bonded silica in organic acid eluents

Metal	Oxalic acid	Maleic acid	Malonic acid	Tartaric acid	Citric acid
Mn(II)	3.7	3.9	7.3	11.1	10.6
Co(II)	3.8	4.1	11.4	15.3	18.0
Cd(II)	4.2	5.03	18.1	27.7	30.0
Zn(II)	4.29	5.0	18.8	21.8	28.9
Ni(II)	4.38	12.9	>45	>60	>80
Pb(II)	5.51	37.9	–	>60	>45
UO <sub>2</sub>	11.03	32.03	41.7	–	>35
Ci(II)	21.79	–	–	>60	>40

Column: 250×4 mm; concentration of eluent: 0.1 M; flow-rate: 0.8 ml/min.

Table 3  
Three-step gradient elution programme for separation of transition metal ions

Eluent	Time (min)	Eluted metal ions	Column bed	Top of the column
0.5 M KCl, pH 3.9, HNO <sub>3</sub>	10	Mg<Ca,Sr<Ba<Mn	Cd<Zn<Co	Ni<Cu
80 mM Tartaric acid	20	Cd<Co<Zn	Ni<Cu	
10 mM Picolinic acid	20	Ni<Cu		

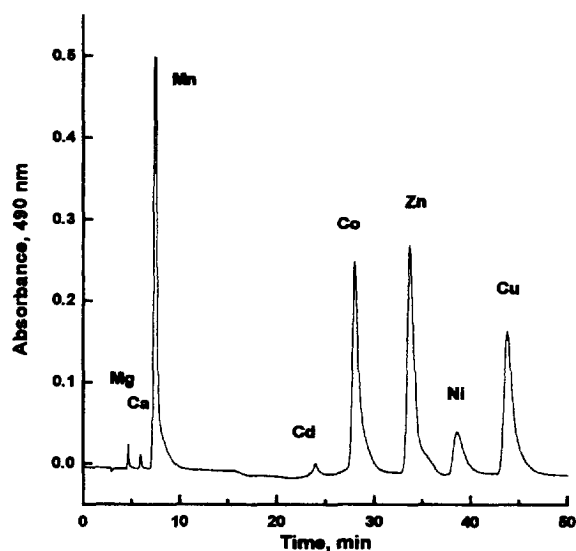


Fig. 3. Separation of a mixture of alkaline-earth and transition metal ions. Three step gradient: 0–10 min 0.5 M KCl– $5 \cdot 10^{-4}$  M HNO<sub>3</sub>, 10–30 min 0.08 M tartaric acid and 30–50 min 0.01 M picolinic acid; flow-rate 0.8 ml/min; detection 490 nm, 0.2 AUFS, post-column reagent with PAR–NH<sub>4</sub>OH–HNO<sub>3</sub>. Sample volume 100  $\mu$ l, concentrations of metals 4  $\mu$ g/ml Mn<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup> and Zn<sup>2+</sup>, 9  $\mu$ g/ml Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup> and 13  $\mu$ g/ml Ni<sup>2+</sup>.

separation. The IDA-silica column produced even more efficient separations than previous studies using resin columns, due to better exchange kinetics and smaller particle size. The wide flexibility of these high-performance CIC columns was also demonstrated, where choice of eluent in the gradient programme can be used to produce a retention order suited to the concentration profiles of the trace elements in the sample. The metal chelating properties of the IDA-silica column proved to be surprisingly strong and retention of transition metals in high chloride media, similar to that of sea water, could be achieved even at a pH as low as 4. Further studies are under way to evaluate the applicability of the developed chelation ion chromatography system for the analysis of trace metals in sea and estuarine water.

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