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## ION CHROMATOGRAPHY OF TRANSITION METALS ON AN IMINO-DIACETIC ACID BONDED STATIONARY PHASE

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### SUMMARY

A stationary phase synthesized by covalent binding of iminodiacetic acid to a porous silica support was investigated for the separation of transition metals by ion chromatography. Eluents containing carboxylic acids either alone or in combination with stronger complexing agents were studied in order to examine the elution mechanisms of alkali, alkaline earth and transition metal ions. It was found that both complexation reactions and ion-exchange mechanisms occurred, their rate being governed by the stability of metal complexes. These investigations were used to find optimum conditions for the determination of transition metal ions in alkali and alkaline earth metal-rich matrices, *e.g.*, sea water.

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### INTRODUCTION

The need for rapid and convenient methods for the determination of heavy metals in environmental and biological samples has induced a great deal of research work in the field of cation chromatography<sup>1–3</sup>. This method allows the separation and subsequent detection of a large number of bivalent transition metals in liquid samples without tedious sample cleanup procedures<sup>4–6</sup>. Short analysis times, simple handling and low cost of equipment and maintenance are assets that make ion chromatography (IC) very attractive for the screening and routine analysis of metal ions.

Stationary phases generally applied to the ion-chromatographic determination of transition metals are low-capacity cation-exchange materials with sulfonic acid functional groups<sup>1,7</sup>. These packing materials give good results if aqueous standard solutions are analysed, but frequently fail as soon as more complex real samples are injected. One problem often encountered during IC of environmental or biological samples is interference from other ionic constituents. For instance, in samples such as sea water the large system peak produced by the predominant ions  $\text{Na}^+$  and  $\text{Mg}^{2+}$  impedes the determination of trace levels of heavy metals. This shows that new stationary phases with higher selectivities for transition metal ions are required. Dicarboxylic acids show high affinities toward transition metals<sup>8–10</sup>, especially if a nitrogen atom is part of the chelating center<sup>9,11,12</sup>.

Iminodiacetic acid (IDA) gels have found extensive application as powerful chelating agents for the preconcentration of heavy metal ions from complex matrices<sup>13-17</sup>. In this study, a newly synthesized, pressure-stable, silica-based material with IDA functions covalently coupled to its surface was examined for use in cation chromatography.

## EXPERIMENTAL

### *Chromatographic conditions*

Analyses were performed on an HPLC system consisting of a Model 501 pump (Waters Assoc., Milford, MA, U.S.A.), a Model 7010 injection valve (Rheodyne (Cotati, CA, U.S.A.), equipped with a 100- $\mu$ l loop, and a Model 430 conductivity detector (Waters Assoc.). The peaks were integrated by a CR-2A integration system (Shimadzu, Kyoto, Japan). The column temperature was maintained at 25°C.

### *Column*

The stationary phase was a 7- $\mu$ m silica-based material with an average pore size of 300 Å (Nucleosil 300-7; Macherey Nagel & Co., Düren, F.R.G.). The silica was derivatized with  $\gamma$ -glycidoxypropyltrimethoxysilane. IDA was covalently coupled to the epoxy activated surface<sup>18,19</sup>. The stationary phase in a methanol slurry was packed into a 100  $\times$  4.6 mm I.D. stainless-steel column.

### *Materials*

The mobile phases were prepared by dissolving analytical-reagent grade L(+)-tartaric acid, citric acid monohydrate, ethylenediamine (EDA) and pyridine-2,6-dicarbonic acid (Merck, Darmstadt, F.R.G.) in deionized water. In some instances the pH was adjusted by addition of 1 M hydrochloric acid. Metal ion solutions were prepared from analytical-reagent grade chlorides or nitrates (Sigma, St. Louis, MO, U.S.A. and Fluka, Buchs, Switzerland). The stock solutions (1000 mg/l) were adjusted to pH 1 with nitric acid.

## RESULTS AND DISCUSSION

IDA exhibits a strong complexing ability for transition metal ions, which can be attributed to the carboxylic groups and the nitrogen atom. Free carboxylate groups act as ion-exchange sites, especially with ions that have low tendencies to form complexes, such as alkali or alkaline earth metal ions.

### *Chromatography of alkaline earth metal ions with an ethylenediamine-citric acid eluent*

The behavior of IDA columns as a normal cation exchanger was investigated for alkaline earth metal ions using an ethylenediamine-citric acid eluent system. In order to optimize the composition of the mobile phase, both the citric acid and the ethylenediamine concentrations were varied independently.

The (ethylenediammonium)<sup>2+</sup> concentration has a strong influence on retention time. Addition of citric acid increases the retention of cations owing to the decrease in pH caused by the acid dissociation (Fig. 1). This means that the complexation ability of the citrate ions is mainly governed by pH.

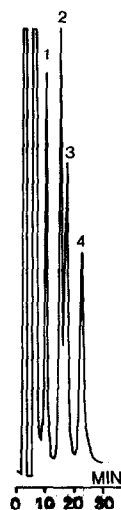
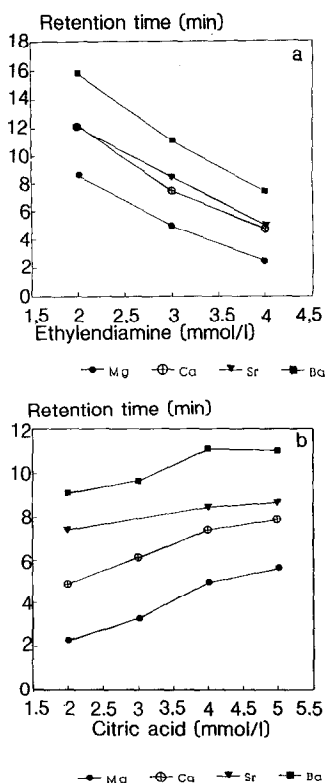


Fig. 1. Influence of ethylenediamine and citric acid concentration on retention times. (a) 4.0 mmol/l citric acid; (b) 3.0 mmol/l ethylenediamine. Stationary phase, 7- $\mu$ m porous silica coupled with iminodiacetic acid (IDA-silica); flow-rate, 1.0 ml/min; samples, 1 ppm of each ion; detection, conductivity. ●,  $Mg^{2+}$ ; ○,  $Ca^{2+}$ ; ▼,  $Sr^{2+}$ ; ■,  $Ba^{2+}$ .

Fig. 2. Separation of alkaline earth metals. Stationary phase, IDA-silica; mobile phase, 3.0 mmol/l ethylenediamine-4.0 mmol/l citric acid; flow-rate, 1.0 ml/min; detection, conductivity. Sample concentration: (1) 1.5 ppm  $Mg^{2+}$ ; (2) 5 ppm  $Ca^{2+}$ ; (3) 10 ppm  $Sr^{2+}$ ; (4) 20 ppm  $Ba^{2+}$ .

The chromatogram of a standard mixture of  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Sr^{2+}$  and  $Ba^{2+}$ , as depicted in Fig. 2, was obtained under optimized conditions, using an eluent consisting of 3.0 mmol/l EDA and 4.0 mmol/l citric acid.

#### *Chromatography of alkali, alkaline earth and transition metals with organic acid eluents*

Different organic acids were screened for their ability to elute alkali, transition and alkaline earth metals from the IDA column. Citric acid and tartaric acid showed very similar elution behavior, but the retention times achieved with citric acid were slightly longer owing to its lower degree of dissociation.

Retention times of alkali, alkaline earth and transition metal ions are plotted against the tartaric acid concentration in Fig. 3. The eluent concentration affects the retention of bivalent ions more markedly than that of monovalent ions. Although in the pH range investigated (2.5–3.0) there is evidence for ion-exchange processes taking place additionally (also between transition metals and partially protonated IDA

functions), the elution order reflects the affinity of metal ions to iminodiacetic acid as a complexing ligand. Alkali metal ions, which obviously do not form IDA complexes, are eluted first, followed by alkaline earth metal ions (plus  $Mn^{2+}$  and  $Fe^{2+}$ ), then by the transition metals  $Cd^{2+}$ ,  $Co^{2+}$  and  $Zn^{2+}$ . Ions that form IDA complexes of higher stability, such as  $Cu^{2+}$  and  $Ni^{2+}$ , remain on the column under these conditions. This fact and the long retention times of transition metals observed with citric or tartaric acid justified further efforts to find more efficient eluents for the IDA column.

One complexing agent that proved particularly useful for this purpose was pyridine-2,6-dicarboxylic acid (dipicolinic acid, DPA). Both DPA and IDA have three centers involved in complexation (Fig. 4), *i.e.*, the two carboxylate groups and the nitrogen atom, which are apparently very similarly arranged within the molecules.

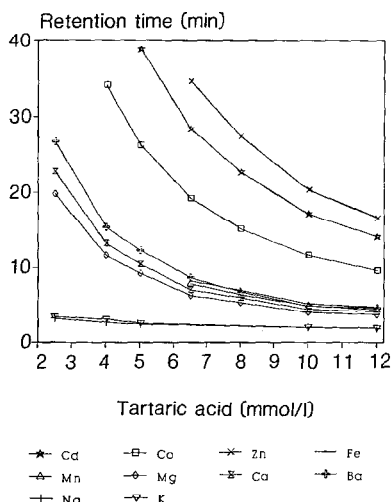


Fig. 3. Influence of tartaric acid concentration on retention times. Stationary phase, IDA-silica; mobile phase, tartaric acid (2.5–12.0 mmol/l); flow-rate, 1.0 ml/min; detection, conductivity.

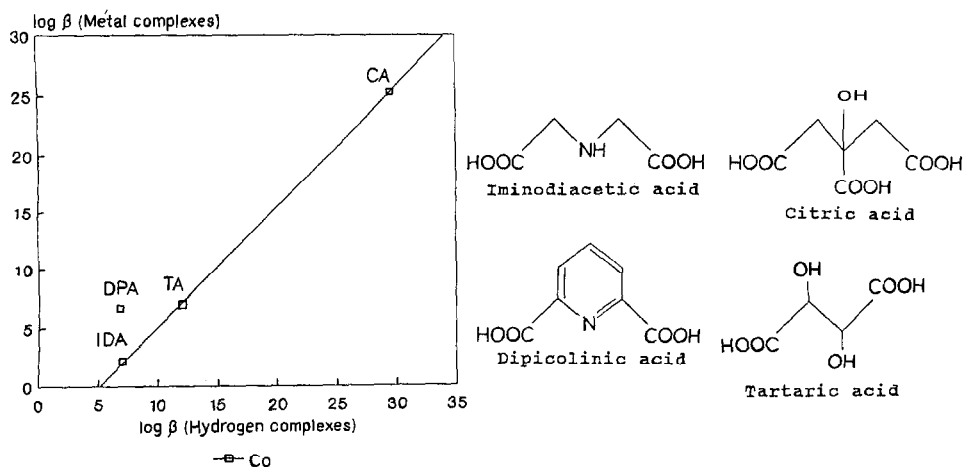


Fig. 4. Correlation between basicity and stability of  $Co^{2+}$  complexes. DPA = dipicolinic acid; TA = tartaric acid; IDA = iminodiacetic acid; CA = citric acid.

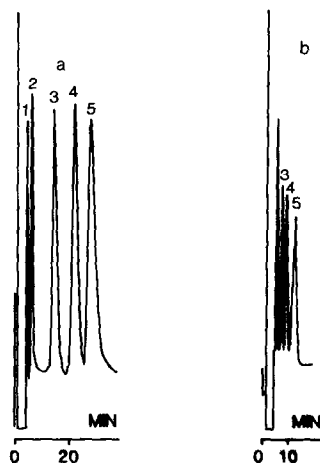
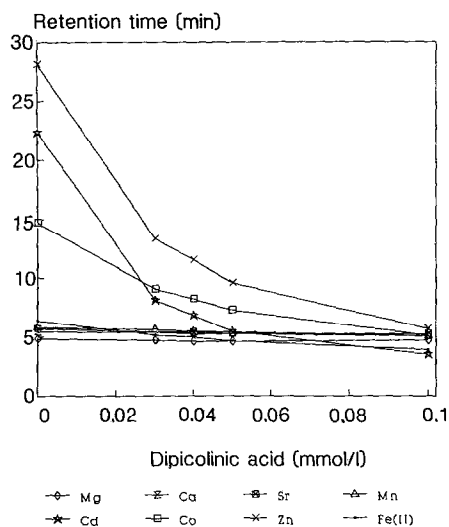


Fig. 5. Addition of DPA to 10.0 mmol/l citric acid. Stationary phase, IDA-silica; mobile phase, citric acid (10.0 mmol/l)-DPA (0-0.10 mmol/l); flow-rate, 1.0 ml/min; detection, conductivity; sample concentration, 5 ppm each.

Fig. 6. Separation of transition metals. Stationary phase, IDA-silica; mobile phase, (a) 10.0 mmol/l citric acid, (b) 10 mmol/l citric acid-0.04 mmol/l DPA; flow-rate, 1.0 ml/min; detection, conductivity. Sample: standard solution containing (1) 1 ppm  $Mg^{2+}$ ; (2) 3 ppm  $Fe^{2+}$ ; (3) 5 ppm  $Co^{2+}$ ; (4) 10 ppm  $Cd^{2+}$ ; (5) 10 ppm  $Zn^{2+}$ .

Nevertheless, the IDA molecule has a flexible structure, whereas the nitrogen atom of DPA is part of an aromatic system, which rigidly holds the two carboxylate groups in a position coplanar to the pyridine ring, thus favoring complex formation.

Normally the tendency of the ligand to bind a proton parallels its complexation ability. In Fig. 4 the stability constants of cobalt complexes are plotted against the protonation constants of the corresponding acids. If a double-logarithmic scale is employed, the values for IDA, tartaric acid and citric acid lie on a straight line, showing that the stability of metal complexes is in direct proportion to the affinity of the ligand for protons. The dipicolinic acid anion, on the other hand, shows a chelating ability higher than would be expected from its basicity. This is due to the steric effect discussed above. The uniquely high degree of complexation of DPA is especially advantageous in acidic solutions. In this investigation, the complexing ability of DPA in acidic solution was monitored. An eluent consisting of 10.0 mmol/l citric acid was modified by addition of up to 0.10 mmol/l DPA which caused a slight decrease in pH from 2.58 to 2.50. The retention times achieved with these eluents are depicted in Fig. 5. A concentration of 0.03 mmol/l DPA, added to the citric acid eluent, halves the retention times of transition metals, whereas the elution of alkaline earth metal ions and manganese is not significantly influenced by the DPA concentration. The small decrease in retention time observed for the latter is rather caused by the pH changes. This indicates that in the pH range investigated DPA acts as a strong complexing agent for transition metals but has little affinity for alkaline earth metal ions (Fig. 6). If the  $\log k'$  values are plotted against the logarithm of DPA concentration,

straight lines with slopes proportional to the stability constants of the metal–DPA complexes are obtained. Owing to the high affinity of the ligand for cadmium, the elution of this ion is speeded up most, and this causes the observed cross-over of  $\text{Cd}^{2+}$  and  $\text{Co}^{2+}$  (Fig. 5).

In order to investigate the equilibrium between complexation of DPA and iminodiacetic acid, further experiments were carried out in a simpler system. Hydrochloric acid was used instead of citric acid for pH adjustment to eliminate any complexation reactions apart from those involving DPA and IDA (Fig. 7). The retention of  $\text{Na}^+$  and  $\text{Mg}^{2+}$  showed a strong dependence on pH but was not significantly influenced by the amount of DPA in the eluent.

Transition metals, on the other hand, exhibit a strong dependence on both DPA concentration and pH. The order of affinity towards DPA is  $\text{Cd} < \text{Co} < \text{Zn} < \text{Cu}$ . At low DPA concentrations (Fig. 7, 0.04 mmol/l) even transition metals of high complex stability, such as  $\text{Cu}^{2+}$  or  $\text{Co}^{2+}$ , exhibit a retention behavior similar to that

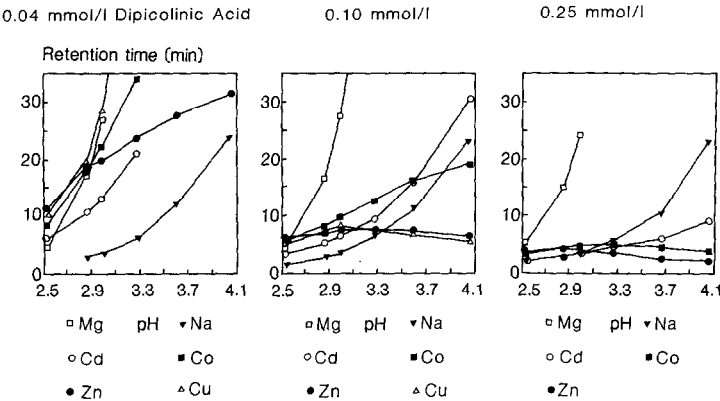


Fig. 7. Influence of pH of DPA eluents on the retention of alkali, alkaline earth and transition metals. Stationary phase, IDA–silica; mobile phase, dipicolinic acid (0.04, 0.10 and 0.25 mmol/l), pH adjusted with 1 M HCl; flow-rate, 1.0 ml/min; sample, 5 ppm of each ion.

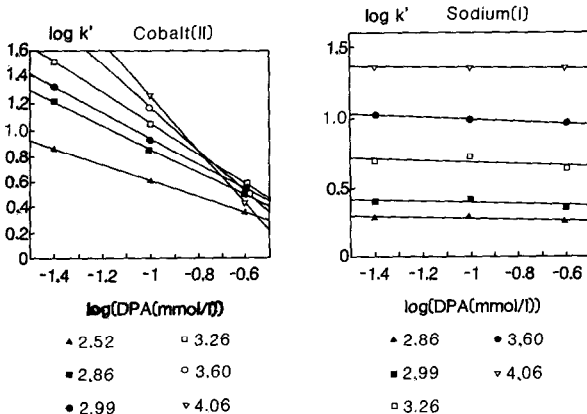


Fig. 8. Influence of complexing agent concentration and pH on the retention of metal ions. Chromatographic conditions as in Fig. 7.

of other bivalent ions, such as  $Mg^{2+}$ . Under these conditions elution is governed mainly by ion-exchange mechanisms. If higher levels of DPA are incorporated in the eluent, the graphs for metal ions with a high affinity towards DPA change their shape, indicating that complexation processes are favored. This causes lower retention times at higher pH (see  $Zn^{2+}$  and  $Co^{2+}$  in Fig. 7; 0.10 and 0.25 mmol/l DPA).

In Fig. 8 the  $\log k'$  for two representative ions ( $Co^{2+}$  and  $Na^+$ ) are plotted against the logarithm of the DPA concentration. The different lines of the graph correspond to different pH values.

$Co^{2+}$  shows the behavior typical of transition metals, with a linear relationship between  $\log k'$  and the logarithm of DPA concentration. The slopes of these regression curves increase with increasing pH, reflecting the increasing dissociation and complexing ability of the ligand.

Sodium is representative of ions with a low tendency to form chelates. The regression curves have a slope of zero, indicating that the amount of DPA present in the eluent has no influence on elution. One must bear in mind, however, that two complexation reactions take place under these conditions. The metal ions form chelates with either the IDA groups immobilized on the stationary phase or with DPA incorporated in the eluent. Changes in pH influence the complexing ability of both agents. By careful choice of the experimental conditions, either complexation by an eluent ion or complexation by the stationary phase can be favored.

Conditional stability constants<sup>9</sup> were calculated from the original stability constants to account for the loss of chelating properties due to protonation of the ligand. These conditional constants were plotted against pH. Fig. 9a shows the conditional stability constants of  $Mg^{2+}$ , a cation with a generally low tendency toward complex formation. The conditional constants for IDA are negative throughout the pH range studied, indicating that  $Mg^{2+}$  reacts with IDA only by ion exchange under acidic conditions. Tartaric acid and DPA show higher affinities to  $Mg^{2+}$ , but their complexation ability can also be neglected at  $pH < 3$ . pH alone is the determining factor in eluting metals of low complexation tendency.

$Co^{2+}$  (Fig. 9b) behaves differently. The conditional stability constants are positive, which means that all three of the acids are capable of forming cobalt chelates. The stability of cobalt-DPA complexes is high throughout the pH range studied, confirming the ability of this complexing agent to accelerate the elution of transition metal ions under acidic conditions.

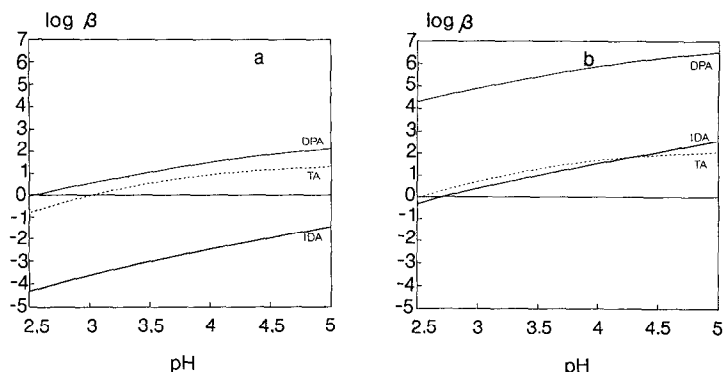


Fig. 9. Conditional stability constants of  $Mg^{2+}$  and  $Co^{2+}$  complexes with change in pH. IDA = iminodiacetic acid; TA = tartaric acid; DPA = dipicolinic acid.



Fig. 10. Separation of transition metals in sea water. Stationary phase, IDA-silica; mobile phase, 10 mmol/l tartaric acid (pH 2.54); flow-rate, 1.0 ml/min; detection, conductivity; integration, attenuation 8. Sample: sea water spiked with (1) 5 ppm  $\text{Co}^{2+}$ , (2) 5 ppm  $\text{Zn}^{2+}$  and (3) 10 ppm  $\text{Cd}^{2+}$ .

The affinity of the IDA stationary phase towards transition metal ions is a great advantage for the determination of trace metals in samples containing high levels of alkali and alkaline earth metal ions, such as sea water, which contains large amounts of  $\text{Na}^+$  and  $\text{Mg}^{2+}$ , among other ionic constituents. In order to investigate the influence of high concentrations of alkali metal ions on the determination of transition metals, solutions containing up to 10% of  $\text{Na}^{2+}$  and  $\text{Mg}^{2+}$  were chromatographed. These ions were well separated from the transition metal peaks and did not exert any significant influence on either retention times or signal strength. This means that transition metals can be determined in alkali and alkali earth metal-rich matrices. Fig. 10 shows a chromatogram of sea water spiked with transition metals in the range 5–10 ppm.

In subsequent work, these investigations at ambient temperatures will be extended to include the influence of higher temperatures on separations.

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