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Review

Recent developments in the high-performance chelation ion chromatography of trace metals

There have been a number of significant developments in the high-performance chelation ion chromatography (HPCIC) of trace metals in recent years. This review focuses on these developments, while giving important information on the fundamental parameters controlling the chelation sorption mechanism, including type of chelating group, stability constants, kinetics, and column temperature. The discussion pays particular attention to the types and properties of efficient chelating stationary phases which have been fabricated for certain groups of metals. The review also describes a number of major improvements in postcolumn reaction detection including the use of the latest reagents and noise reduction strategies to improve sensitivity and reduce LOD. In the final section, an indication of the applicability of HPCIC to a range of complex sample types is given with some key examples and chromatograms using the latest high-efficiency chelating phases.

Keywords: Chelation ion chromatography / Postcolumn reactions / Trace metals

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1 Introduction

The formation of complexes was discovered by the German scientist Alfred Werner in 1893 [1], and Gilbert Morgan and Harry Drew in 1920 [2] introduced chelation as a special type of complexation, assuming the process of the formation of complexes having cyclic structures with two or more bonds between binding sites. The important rule was formulated later by Lev Chugaev who found that chelates containing five to six member rings are usually the more stable when considering the different structures of complexes [3]. Since this discovery, the selectivity of many analytical chemistry procedures using organic reagents was significantly improved, including different separation techniques of LC.

Chelation-in-a-sorbent based chromatographic techniques embrace ligand-exchange chromatography developed by Helfferich in 1961 [4], immobilized metal ion affinity chromatography introduced by Porath in 1975 [5], and silver ion or argentation chromatography introduced by Morris in 1962 [6]. These techniques are now widely used for the separation of polar organic molecules such as amines, amino alcohols and amino acids, phenols, carbohydrates, lipids, unsaturated fatty acids, peptides, proteins, *etc*. The chelation-related sorbate – sorbent specific interactions provide unique separation selectivities, promoting intensive developments in this area.

Surprisingly, the first chelating resin containing dipicrylamine groups was originally proposed in 1940 by Skogseid [7] for the selective isolation of K^+. A more universal aminocarboxylic prototype chelating resin for the preconcentration of heavy metal ions was prepared and used by Gregor *et al.* later in 1950 [6], but the related high-performance chromatographic technique for the column separation of metal ions did not appear until the works of Pohlandt and Fritz [8] in the late 1970s . Many of the early separations encountered problems of poor efficiency, difficulties of quantitative elution of some metal ions, even with gradient elution, and sensitive online

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Abbreviations: APA, aminophosphonic acid; **APA-silica,** aminophosphonic acid-bonded silica; **5-Br-PADAP,** 2-[(5-bromo-2-pyridyl)-azo]-5-diethylaminophenol; **CAS,** Chromazurole S; **CPC,** cetylpyridinium chloride; **HPCIC,** high-performance chelation ion chromatography; **8-HQS,** 8-hydroxyquinoline sulfonic acid; **IC,** ion chromatography; **IDA,** iminodiacetic acid; **IDA-silica,** iminodiacetic acid-bonded silica; **Nitro-PAPS,** 2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol; ***o*-CPC,** *o*-cresolphthalein complexone; **PAR,** 4-(2-pyridyl)azoresorcinol; **PBDMA,** poly(butadienemaleic) acid; **PCR,** postcolumn reaction; **PCV,** pyrocatechol violet; **PS-DVB,** poly(styrene-divinylbenzene); **Triton X-100,** polyoxyethylene-*t*-octylphenol; **TTMAPP,** *meso*-tetrakis(4-*N*-trimethylaminophenyl)porphine

Table 1. Commercially available weak acid cation-exchangers for IC of metal ions

Cation-exchanger	Functional groups	D_p (μm)	S (m^2/g)	d_{pore} (nm)	Ion-exchange capacity (μequiv/g)
Silica based					
Shodex IC YF-421/YK-421	PBDMA	5		12.5/2	–/1800
LiChrosil IC CA	PBDMA	5			20 – 200
Universal Cation	PBDMA	7			20 – 200
Universal Cation HR	PBDMA	3			20 – 200
Waters IC-Pak C M/D	PBDMA	5			60[a] (3.9 × 150)
Super Sep C 1-2	PBDMA	5	350	10	
Metrosep Cation 1-2 IC	PBDMA	7	350	10	122[a] (4.0 × 125)
Metrosep C 1	–COOH	5			123[a] (4.6 × 125)
Metrosep C 2	–COOH	7			117[a] (4.0 × 150)
Deltabond UCX	PBDMA	5	200	12	
Waters IC Pak C M/D	PBDMA	5			60
Nucleosil-5- 100-PBDMA	PBDMA	5	350	10	–
IonPac SCS 1	PBDMA	4.5	300	12	318[a] (4.0 × 250)
Methacrylate based					
TSKgel Super IC-Cation		5			1.0[b]
TSKgel IC-Cation I/II HR	–COOH	5			
TSKgel Super IC-A/C	–COOH	3 – 4			0.2[b]
TSKgel OApak	–COOH	5			0.1[b]
PS-DVB based					
Hamilton PRP-X 800	Poly(itaconic acid)	5.7		Macro	3700
IonPaC CS12	Acrylic – COOH	8.5	450	6	2800[a] (4.0 × 250)
IonPaC CS12	Acrylic – COOH, $-PO_3H_2$	5; 8.5	450	15	2800[a] (4.0 × 250)
IonPaC CS14	Acrylic – COOH	8	450		1300[a] (4.0 × 250)
IonPaC CS15	Acrylic – COOH, $-PO_3H_2$, crown ether	8.5	450	15	2800[a] (4.0 × 250)
IonPaC CS16	Acrylic –COOH	5	450		8400[a] (5.0 × 250)
IonPaC CS17	Maleic – COOH, EVB-DVB, 55%	7	450	15	1450[a] (4.0 × 250)
IonPaC CS18	Maleic – COOH, EVB-DVB, 55%	6	450		1450[a] (4.0 × 250)
Poly(vinyl alcohol) based					
Shodex IC YS-50 6D	–COOH	5			3000
Metrosep C3	–COOH	5			

[a] Ion-exchange capacity *per* column.
[b] Ion-exchange capacity in μequiv/mL.

detection was not available. Sometimes, chromatographers did not consider the possibility of the coexistence of both ion-exchange and chelation interactions for metal ions within the phase of the chelating ion-exchanger and failed to get the expected separation selectivity for them. So, high-performance chelation ion chromatography (HPCIC) got relatively late recognition as a result of the accumulation of experience involving preconcentration of trace metal ions on different chelating resins from complex solutions [7, 9], the advantages of using chelating reagents as a part of liquid stationary phases in extraction chromatography [10], precipitate forming reagents in precipitation chromatography [11] and, finally, improved chemistry of postcolumn reactions (PCRs) in flowing systems. The first relatively efficient separations of metal ions on chelating ion-exchangers with true complexation mechanism at the surface were obtained by Jones and Schwedt [12], who used poly(styrene-divinylbenzene) (PS-DVB) resins coated with hydrophobic chelating dyes. His group used high concentrations of KNO_3 in the eluents, allowing the study of substrate chelation properties on metal ion separation without interference from simple ion exchange processes. Since that time remarkable progress has been achieved [13].

It should be understood that the special chelating properties of the stationary phases in HPCIC allow the analysis of complex samples, which would have been impossible with classical ion exchangers, without the tedious time-consuming preconcentration and multicolumn switching techniques. The recent successes of HPCIC are mainly connected with the developments of new surface modified materials, providing better kinetics of interaction between metal ions and chelating groups and a better understanding of coordination chemistry at the surface [13 – 15]. It should be noted that a recent trend of cation chromatography is the reorientation away from the use of traditional sulfonic acid bear-

ing or strong cation-exchangers, which are noncomplexing, to the use of more selective weak cation-exchangers having functional groups of carboxylic or phosphonic acids (Table 1). These groups also exhibit the ability to chelate metal ions under certain separation conditions and can be used potentially in HPCIC [16 – 18].

The application of new construction materials and advanced technologies in chromatographic equipment has given rise to significant improvements in the sensitivity of PCR photometric detection. As a result of these advances, LODs of 1 μg/L or less for many metals are possible without preconcentration, which compares favorably with some of the most sensitive atomic spectroscopic techniques, such as inductively coupled plasmas. The main aim of this review is to summarize the recent achievements in the area of HPCIC for the last decade from the time of our last review [15].

2 Separation mechanism

Currently, ion exchange is associated with many different technologies and plays an extremely important role in different separation science methods like ion chromatography (IC). However, there is still some uncertainty concerning the right definition for this process. The most advanced term for ion-exchange was proposed by the International Workshop on Uniform and Reliable Nomenclature, Formulations and Experimentation for Ion Exchange in 1994 [19] which defined ion exchange as "equivalent exchange of ions between two and more ionized species located in different phases, at least one of which is an ion exchanger, without the formation of new types of chemical bonds".

There are many ways to achieve efficient separation of metal cations in IC including:

(i) Using cation-exchange columns with complexing or noncomplexing eluents, based on electrostatic sorbate – sorbent interactions; (ii) on-column formation of negatively charged complexes and their separation of on an anion-exchange column; (iii) using the ability of chelating ion-exchangers to form kinetically labile surface complexes and retain metal ions according to the stability of corresponding complexes.

The last approach (iii) is related to the subject of this review, which initially considers the factors affecting efficiency and selectivity in HPCIC.

2.1 Equilibria in the chelating ion-exchange column

For kinetic reasons that will be considered later in Section 2, the most efficient separations of metal ions on chelating-exchange columns may be achieved only when the corresponding exchangers have negatively charged or acidic functional groups. Correspondingly, both simple cation exchange due to electrostatic interactions and chelation at the surface can take place in dilute eluents or eluents of low ionic strength. The corresponding equlibria for a metal cation M^{y+} and chelating groups H_nR can be expressed by the following interactions:

$$M^{y+}_{aq} + H_nR = MR^{(n-y)+} + yH^+ \quad (1)$$

$$M^{y+}_{aq} + B_nR = MR^{(n-y)+} + yB^+_{aq} \quad (2)$$

$$nb^+_{aq} + H_nR = B_nR + nH^+ \quad (3)$$

$$M^{y+}_{aq} + R^{n-} = MR^{(n-y)+} \quad (4)$$

where M^{y+} is a metal cation interacting on both ion-exchange (1) and complexation (4) mechanisms with the chelating ion-exchanger. H^+ and B^+ are hydronium and alkali cations introduced in the eluent for the buffering or adjusting of ionic strength.

2.1.1 Distribution ratio under a mixed mode mechanism

Pure sorption mechanisms are very rare in chromatography and chelation ion-exchange is no exception. As the chelating groups are usually charged, so the chromatographic retention of alkaline-earth, transition and heavy metal ions on such a column in a noncomplexing eluent, occurs through the combination of repulsive and attractive electrostatic forces and coordination interactions between cations and chelating functional groups on the substrate. The distribution ratio D_M of a metal cation M^{y+} between the chelating ion-exchanger and the mobile phase can be expressed by

$$D_M = \frac{[MR^{(n-y)+}]^E + [MR^{(n-y)+}]^C}{[M^{y+}_{aq}]} \quad (5)$$

where $[MR^{(n-y)+}]^E$ and $[MR^{(n-(y)+}]^C$ are equilibrium concentrations of the cation retained by the stationary phase due to electrostatic interactions (conventional ion-exchange) and chelation ion-exchange, respectively. $[M^{y+}_{aq}]$ is the concentration of the cation in the mobile phase. The retention factor k can be expressed as

$$k = D_M\varphi \quad (6)$$

where φ is a characteristic constant for a given chromatographic column expressed as the ratio of the volumes of stationary V_R and mobile V_{aq} phases and

$$\varphi = \frac{V_R}{V_{aq}} \quad (7)$$

At constant pH of the eluent in the presence of excess of noncomplexing electrolyte or of ionic strength regulator, the equilibrium (Eq. 3) is shifted to the right and the ion-exchange process of cation M^{y+} and alkali metal cation B^+ as competing cation on the chelating ion-

exchanger can be expressed by Eq. (2). The following selectivity ratio is given by

$$K_M^B = \frac{\left[MR_R^{(n-y)^+}\right]\left[B_{aq}^+\right]}{[M_{aq}^{y+}][B_nR]} \quad (8)$$

As the resolution of chromatographic peaks, R_S depends on the coefficient $k/(k+1)$ from formula

$$R_S = 1/4\sqrt{N}\ \frac{k}{k+1}\ \frac{\alpha-1}{\alpha} \quad (9)$$

where N is the column efficiency and α the selectivity of separation, so a suitable chromatographic separation can be achieved at values of $k < 10-15$. Since the formation of complexes at the surface with more than one ligand is not possible due to thermodynamic and sterical restrictions, then in accordance with Eq. (4) $[M_R^{(n-y)+}]^C$ can be expressed as follows:

$$[M_R^{(n-y)+}]^C = \beta_1[M_{aq}^{y+}] \cdot [R^{n-}] \quad (10)$$

where $[R^{n-}]$ is the concentration of functional groups and β_1 is the stability constant of complex $MR^{(y-x)+}$ formed at the surface in accordance with Eq (4).

2.1.2 Selectivity ratio in noncomplexing eluents

Taking into account the two possible types of interactions in accordance with Eqs. (5), (8), and (10), the retention factor k of a metal cation M^{y+} can be expressed as

$$k = \left(K_M^B \frac{[B_nR]}{[B_{aq}^+]^y} + \beta_1[R^{n-}]\right)\varphi \quad (11)$$

The first member of the sum in brackets expresses the impact of conventional ion-exchange interactions on retention, and the second member expresses the impact of chelation on retention. Several conclusions can be formulated from Eq. (11).

There are important differences between the effects of ionic strength on electrostatic interactions and chelation. Electrostatic interactions are strongly suppressed or "swamped" by the addition of an electrolyte to the eluent. However, the effect of ionic strength on chelation is usually very small. For example, critical values of stability constant β_1 for complex of iminodiacetic acid (IDA) with Fe^{3+} are 13.7, 13.5, and 13.4 at ionic strength equal to 0.1, 0.5, and 1.0, respectively [20]. Thus, assuming little change in β_1 with high ionic strength (>0.1) the retention of a metal ion due to chelation should not depend upon the concentration of alkali metal cations $[B_{aq}^+]$, but solely on the concentration of functional groups in the chelating ion-exchanger. In practice, it means electrostatic interactions need to be suppressed for chelation to be the dominant sorption mechanism. Thus, in the case of domination by chelation

$$\beta_1[R^{n-}] \gg K_M^B \frac{[B_nR]}{[B_{aq}^+]} \quad (12)$$

at a high concentration level of B_{aq}^+ in the eluent, the retention of the metal cation M depends on its value of $\beta_1{}^M$ and the separation selectivity α is defined by the ratio of the corresponding stability constants of metal chelates formed at the surface.

$$\alpha = \frac{k_2}{k_1} = \frac{\beta_1^{M2}}{\beta_1^{M1}} \quad (13)$$

Strictly, it should be the ratio of conditional stability constants, but for a given ligand with no hydrolysis of the metal ions, the ratio is the same as the thermodynamic stability constants. The possible selectivity changes that can occur with an increase in the concentration of an indifferent (noncomplexing) electrolyte will be discussed in Section 2.2.2.

2.1.3 Secondary eqilibria in the eluent

The additional equilibria may affect the retention and the separation selectivity of metal ions in HPCIC. In a simplified form the retention under conditions of domination by HPCIC can be defined according to Eqs. (11) and (12) as

$$k = \beta_1[R]\,\varphi \quad (14)$$

or in bilogarithmic form as

$$\log k = \log\beta_1 + \log[R] + \log\varphi \quad (15)$$

In the case of secondary competing equilibrium in the eluent the concentration of free metal cation $[M_{aq}^{y+}]$ must be corrected by the application of the complex formation coefficient $\alpha_{M(L)}$ according to Weiss [21]

$$\alpha_{M(L)} = 1 + [L]\beta_{ML} + [L]^2\beta_{ML2} + \ldots \quad (16)$$

where [L] is the concentration of competing ligand in the eluent and β_{ML}, β_{ML2} are formation constants for complexes ML, ML_2, respectively. So, the retention of metal analytes in the HPCIC system in the presence of secondary equilibrium in the eluent will be expressed as

$$\log k = \log\beta_1 - \log\alpha_{M(L)} + \log[R] + \log\varphi \quad (17)$$

Regulation of the separation selectivity of metal ions on a chelating ion-exchange column is possible if the values of β_1 for the complex at the surface are comparable with $\alpha_{M(L)}$ or, for simplicity, with $[L]\beta_{ML}$. In other words, the separation selectivity may be changed in the presence of small concentration of strong complexing agents or of significantly higher concentration of weak complexing agents in the eluent.

In the case of an iminodiacetic acid-bonded silica (IDA-silica) column, the changes in separation selectivity were noted with additives of not only relatively strong complexing agents like oxalic acid [22, 23], dipicolinic acid [23–25], picolinic [15, 23], sulfosalycilic [25], and with tartaric, maleic, malonic, citric, and other carbonic acids [22, 24, 26] but also for relatively weak complexing agents for transition metals such as chloride [27, 28]. More information about the adjustment of separation selectivity for IDA functionalized resins with the addition of complexing reagents to the eluent can be found in recent review [25].

2.2 The main factors influencing separation in HPCIC

2.2.1 Temperature effects

A number of studies have been undertaken for the investigation of temperature effects concerning the separation of alkaline-earth metals [3, 16, 18, 29–33], Be^{2+} [34], transition and heavy metals [16, 18, 27, 33] lanthanides [17, 35], both under conventional IC and HPCIC modes. There are several effects of column temperature changes on chromatographic performance in HPCIC, which can be divided into two groups related to kinetic and thermodynamic properties.

The general thermodynamic effect of column temperature upon retention ($\ln k$) of metal cations in IC and HPCIC can be expressed by the van't Hoff equation

$$\ln k = \Delta H/RT + \Delta S/R + \ln \varphi \quad (18)$$

where ΔH and ΔS are sorption enthalpy and entropy, respectively, and φ is the phase volume ratio as expressed by Eq. (7). Obviously, there must be a difference between heats of adsorption of metal cations due to pure electrostatic interactions and due to the formation of surface complexes. In the case of conventional ion-exchange, the temperature effects are exothermic (negative values of ΔH) and heats of adsorption do not exceed 8–13 kJ/mol. In chromatographic systems with a dominant chelation mechanism, values of ΔH are usually much higher and both exothermic and endothermic effects can be observed. The entropy of metal cation – chelation group/groups interaction may also impact retention, especially in the case of multidentate ligands serving as chelating groups. Thus, the thermodynamic aspect of temperature effects could have a significant influence on separation selectivity.

The dramatic effect on separation selectivity of 14 rare earth elements (REE) on IDA-silica was demonstrated by Nesterenko and Jones [35]. Ytrium (III) has a steeper slope of the van't Hoff equation and can be placed in a gap between Nd(III) and Sm(III) (Fig. 1) that allows isocratic

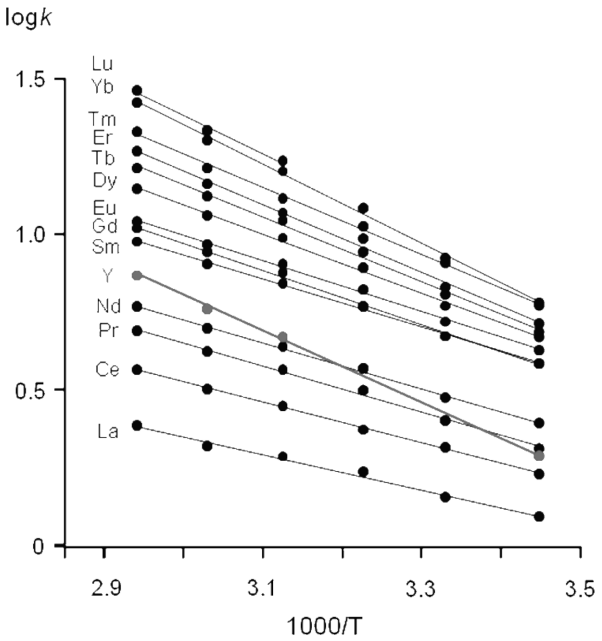


Figure 1. Effect of column temperature on the retention of REE on IDA-silica column. Eluent: 13.6 mM HNO_3–0.5 M KNO_3 (from ref. [35]).

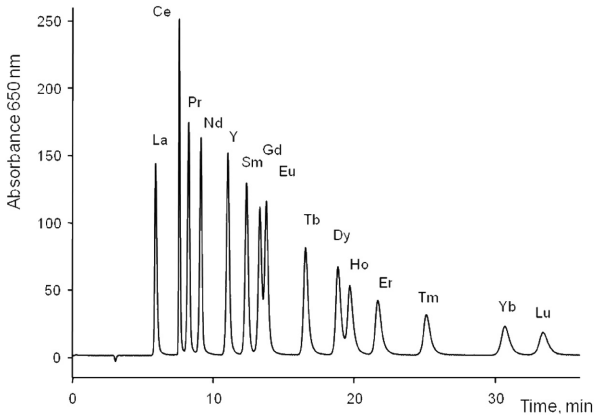


Figure 2. Isocratic separation of standard mixture of 14 lanthanides and Y(III) on 150 × 4.0 mm column, packed with 5 μm IDA-silica. Eluent: 25 mM HNO_3 with 0.75 M KNO_3; flow rate, 1.0 mL/min; column temperature, 75°C, sample volume, 20 μL, sample concentration of each metal was 4 mg/L in 0.2% HNO_3. Detection at 650 nm after PCR with Arsenazo III.

separation of a model mixture (Fig. 2). A reverse in elution order of the pair Mg^{2+}/Ca^{2+} on IDA-silica column under HPCIC conditions was noted by Paull and Bashir [27, 31] with a variation of column temperature that is connected with a partial change of retention mechanism. Similar trends were also noted for PRPX800 and Ionpac CS14 [31], IonPac CS12A, and Universal Cation [32] commercially available columns [16]. An interesting illustration of the dual mechanism for the retention of

alkaline-earth metal cations in diluted eluents was obtained by Kolpachnikova *et al.* [36]. They observed a convex type dependence of retention for these cations on IDA-silica with column temperature in 2 mM $HClO_4$, which was attributed to the shift from electrostatic interactions (exothermic process) dominating at low temperatures, to chelation (endothermic process) at high temperatures.

All investigations involving temperature effects on the retention of metal ions found that the slopes of van't Hoff plots for transition metal ions and lanthanides are usually higher [16, 18, 35] than for alkaline-earth metals under conditions of HPCIC. This is consistent with the assumption that there is a strong correlation between heats of adsorption and heats of complex formation.

There was always a special prejudice about the kinetics of chelation at the surface as a limiting efficiency factor. Usually, better kinetics of mass transfer ratio between mobile and stationary phases should take place at high temperatures due to changes in diffusion coefficients and to a reduction in viscosity of the eluent. Theoretically, it should improve the column efficiency. But it may not be so for HPCIC, where another factor, namely complexation at the surface of chelating ion-exchangers, makes such improvement doubtful. This is because the increased conformational mobility of chelating groups attached to the surface can lead to the formation of complexes with a higher denticity and thus a slower dissociation rate. The latter factor can in fact cause a decrease in separation efficiency.

At the moment, no evidence of a significant improvement of the column efficiency with variation of the column temperature has been obtained except results of Rey and Pohl [29], who observed a 32–41% increase in peak efficiencies with increase in the column temperature up to 50°C for alkaline earth metal cations separated on an IonPac CS12A column having mixed phosphonic-carboxylic functional groups. It should be noted that the authors separated these cations under noncomplexing conditions in 18 mM methane sulfonic acid. They also observed a 13–44% decrease in retention factors for the same experiment and associated this effect with changes in hydrated radii of the analytes and an increase in p*K* values for weak acidic functional groups. Shaw *et al.* [16, 17] studied the retention of lanthanides on the same column, but under suppressed electrostatic interactions and found changes to peak efficiency minimal in the temperature range between 25 and 40°C. Intensive investigations of temperature effects for the range between 27 and 60°C on the retention of alkaline-earth metal cations on an IonPac CS12A column have also been performed by Hatsis and Lucy [30], who confirmed the decrease in retention times of these cations with increase in the column temperature and noted improvements in peak efficiencies of 14–34% at 60°C as compared with those at 27°C. They also did not find any significant changes in peak asymmetry at different temperatures.

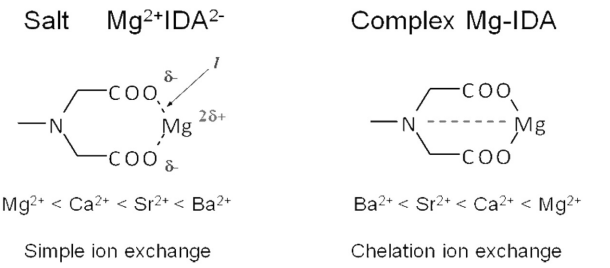


Figure 3. Comparison of ion-exchange and chelation ability of IDA-silica.

It should be noted that sometimes, an increase in the column temperature can cause a relatively sharp change in the retention of cations due to conformational changes in the bonded layer, as noted for poly(butadiene-maleic) acid (PBDMA) functionalized PS-DVB substrates [32].

2.2.2 Ionic strength

The regulation of ionic strength is the main tool to achieve a dominant chelation mechanism in mixed mode separations. For this purpose, electrostatic interaction can be suppressed by the creation of a high level concentration of simple electrolytes in the eluent. Usually, nitrates or perchlorates of alkali metals are the most suitable additives to the eluent, as they have no complexing ability and do not form precipitates with the separated metal cations. For these reasons, the use of alkali metal sulfates or chlorides needs more care.

The effect of ionic strength or concentration of an indifferent electrolyte is clear from Eq. (12). The dominant chelating mechanism can take place even in the absence of indifferent electrolyte additives to the eluent, if the stability constants for the surface complexes, β_1, are high enough for separated metal cations. For example, the retention order of the transition metal ions ($Mn^{2+} < Co^{2+}$ $Cd^{2+} < Zn^{2+} < Ni^{2+} < Pb^{2+} < Cu^{2+}$) for IDA-silica in dilute nitric acid eluent corresponds to the stability constant order of their iminodiacetates. This retention order remains unchanged after addition to the eluent of KNO_3 in concentrations up to 1 M [23, 27]. This means that the values of $\log\beta_1$ higher than 4.72 (for Mn^{2+} eluted first, [20]) ensure the domination of the chelation mechanism at the surface for the transition metal divalent cation. Similar regularities were observed for Ionpac CS12A column [16] and poly(itaconic) acid cation-exchange column PRP × 800 [18].

However, alkaline-earth metal cations are usually strongly retained on sulfonated cation-exchangers due to electrostatic interactions and therefore have relatively

high K_M^B values for weak cation-exchanger, but they form less stable complexes with IDA (1.67 Ba^{2+}, 2.23 Sr^{2+}, 2.60 Ca^{2+}, and 2.98 Mg^{2+}) [20]. This means that in dilute nitric acid the retention order on IDA-silica corresponds to electrostatic interactions or to conventional ion-exchange (Fig. 3). A good illustration of this was obtained by Haidekker and Huber [28], who used IDA-silica column for the separation of alkali and alkaline-earth metals. Nevertheless, after the addition of 1 M KNO_3 to the eluent, a reversed retention order confirms the domination of chelation over electrostatic forces [3]. The changes in the separation selectivity of alkaline-earth metal cations with variation of the concentration of an indifferent electrolyte were also noted for other chelating columns [18].

A similar behavior was found for the separation of lanthanides on IDA-silica [35]. The cation-exchange selectivity due to electrostatic interactions corresponds to the increase in K_M^B values from Lu^{3+} to La^{3+}. So, the corresponding retention order of lanthanides is usually observed on cation-exchange columns [37]. However, the formation constants for complexes of lanthanides with IDA increase in opposite order from a $\log\beta_1$ value of 5.38 for La^{3+} to 7.61 for Lu^{3+} [20]. Because lanthanide cations are triple charged, the strength of the electrostatic interaction with negatively charged IDA groups is of the same level as the chelation ability of IDA groups. So, no resolution of chromatographic peaks for the group of heavy lanthanides is observed in a dilute nitric acid-based eluent. However, the addition of an indifferent electrolyte to the eluent and an increase in the column temperature shifted the mechanism toward chelation and thus isocratic separation of all lanthanides and Y^{3+} was achieved (Fig. 2).

It can be seen therefore that an increase in the ionic strength of the eluent in HPCIC always promotes chelation as the major separation mechanism by suppressing electrostatic interactions. A strong correlation between the retention of metal cations on IDA-silica and the corresponding stability constants has been demonstrated at high ionic strength of the eluent and increased column temperature (Fig. 4). From a practical point of view, it expands the possibilities of this chromatographic mode to the analysis of complex samples, allowing further control of the separation selectivity. One small negative effect of using high concentrations of indifferent electrolytes in the eluent is that the increase in the viscosity of the eluent can lead to a slight decrease in column efficiency.

2.2.3 pH of the eluent

The majority of functional groups of chelating ion-exchangers include weak acidic or basic groups. This means that the conditional stability constants of their complexes with metals will depend on the pH of the eluent. However, at the same time, these groups will dissociate or become protonated with an increase or decrease in pH, thus also changing the electrostatic interactions with separated metal ions.

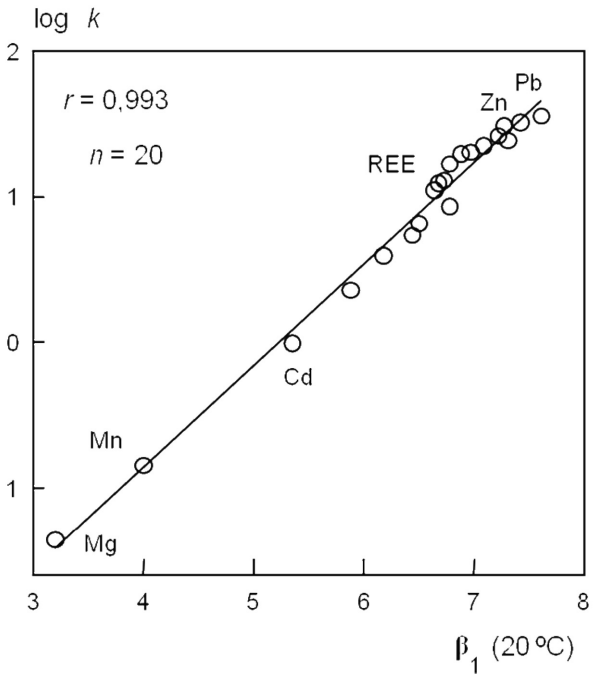


Figure 4. Correlation between retention of metal ions on IDA-silica and stability constants of corresponding iminodiacetates measured at 20°C. Eluent: 13.6 mM HNO_3–0.5 M KNO_3, 65°C.

If for the attached chelating groups R the dissociation constant is

$$K_{diss} = \frac{[H^+]^n[R^{n-}]}{[H_nR]} \quad (19)$$

So the final expression is

$$\log k = \log\beta_1 + \log[H_nR] + \log K_{diss} + n\,pH + \log\varphi \quad (20)$$

An analysis of the equation shows that the pH should not affect any separation selectivity for metal cations of equal charge under HPCIC mode if any secondary complexation with components of the eluent takes place. So, assuming no changes in retention mechanism takes place due to hydrolysis of some metal ions or other secondary equilibria, an increase in the eluent pH will lead to an increase in retention times of the metal cations. Usually, these dependences are linear using bilogarithmic axes, while the slopes can provide some information about the stoichiometry of interaction between metal ion and chelating group. Such dependences have been observed for poly(itaconic)acid functionalized resin [18], mixed carboxylic and phosphonic ion-exchanger [16], and IDA silica [3, 34].

As a rule, the more remarkable effects were noticed for impregnated chelating exchangers. The changes in protonation degree of adsorbed ligands causes a variation in their hydrophobicity leading to their partial desorption and hence to alterations in ion-exchange capacities [38, 39].

2.2.4 Organic solvent additives

Organic solvent additives to the eluent may improve the conformational mobility of chelating groups attached to hydrophobic matrices and change their accessibility for interaction with cations [40]. In the case of hydrophilic amino acid-type silica-based chelating ion-exchangers the addition of ACN, methanol, or 2-propanol did not produce any significant changes in separation selectivity but reduced the retention of alkaline-earth and transition metal cation by 10–15% [41]. Hatsis and Lucy [30] investigated the effect of addition of ACN on the separation of alkaline-earth metal cations on Ionpac CS12 column and observed a decrease in retention times of these cations. They also noted a small decrease in peak efficiencies without changes in peak asymmetry.

The less important effects for HPCIC could be connected with changes in hydrated ions radii and in dielectric constants of the eluent, but no experimental results confirming it have been obtained.

3 Stationary phases

The preparation and investigation of new chelating resins remains a big area in chemical analysis [9, 42, 43]. Usually chelating resins are used for selective isolation of one or groups of metals by adsorption or for separation by a low pressure LC. As a rule only a small concern about column efficiency and kinetics of adsorption takes place. In contrast, these parameters play a key role in HPCIC making the variety of suitable chelating ion-exchangers not too large.

3.1 Choice of the chelating ligand

There are three parameters which must be taken into consideration when choosing the chelating groups in HPCIC. Chelating groups should provide fast kinetics of complexation with metal ions, be selective to the group of separated metal ions but not to a single one and, finally, they should not form too strong complexes so allowing the use of relatively mild elution conditions for chromatographic separations. The faster kinetics of interaction with metal ions was noted for negatively charged chelating groups (Table 2). This is in accordance with the assumption about electrostatic attraction of ions from the eluent to the surface of chelating ion-exchanger as a first step of interaction followed by the formation of surface complexes. Thus, mostly amino- and iminoacids or polycarboxylic type ion-exchangers are used in HPCIC. Excellent separation selectivity was noticed for aminophosphonic acid-bonded silica (APA-silica) [44, 45]. Both covalent bonding and adsorption can be used for immobilization of chelating ligands.

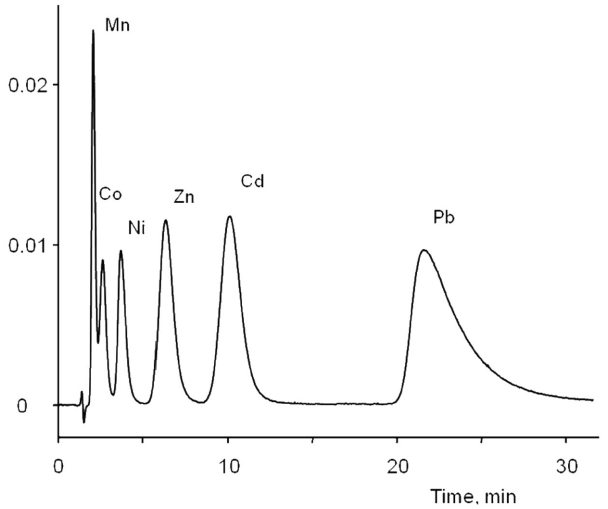


Figure 5. Isocratic separation of 0.1 mg/L Mn(II), 0.1 mg/L Co(II), 3 mg/L Ni(II), 0.2 mg/L Zn(II), 0.5 mg/L Cd(II), and 10 mg/L Pb(II) at pH 2.5 on a 15 × 4.0 mm column packed with 9 μm hypercrosslinked polystyrene particles dynamically modified with 0.5 mM dipicolinic acid. Eluent: 1 M KNO_3, 0.5 mM dipicolinic acid, pH 2.5. Sample volume 100 μL, detection at 520 nm with PAR/Borate PCR.

3.2 Impregnated and dynamically loaded substrates

Impregnation of PS-DVB resins is a time consuming but very simple procedure for the preparation of chelating ion-exchangers. Usually, this type of modification works quite well for the big group of dyes containing several aromatic rings in a molecule [13, 15, 46]. The bulk molecules of organic dyes such as aurin tricarboxylic acid [47] or quinaldic acid [48] are strongly retained at the surface of PS-DVB microspherical particles due to combination of hydrophobic and π–π interactions. The selectivity of adsorbed organic dyes remains unchanged after adsorption but separation efficiency is usually not too high because of the low conformational mobility of adsorbed molecules. However, the separation of six metal on the column impregnated with aurin tricarboxylic acid was obtained with gradient elution [47].

More efficient are PS-DVB substrates impregnated or dynamically loaded with relatively small molecules like picolinic, 4-chlordipicolinic, or dipicolinic acids [38, 48–50]. These substrates demonstrated good separation efficiency but were less stable, so the eluent must contain a

Table 2. Separation of metal ions on chelating ion-exchangers with covalently bonded functional groups

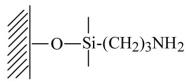
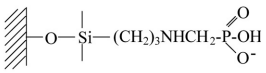
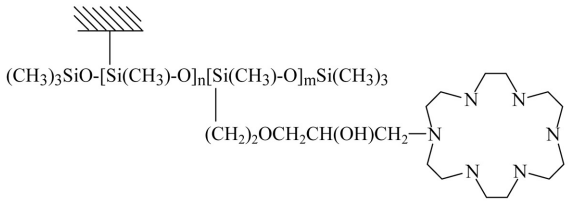
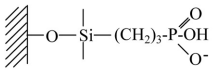
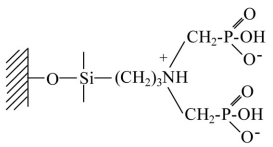
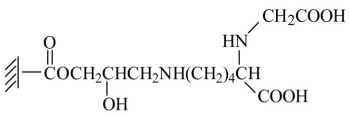
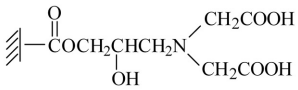
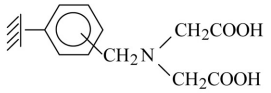
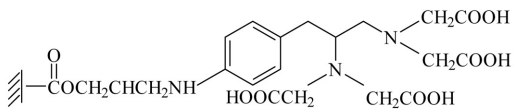
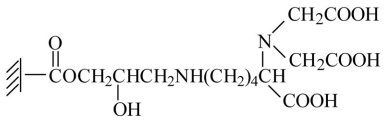
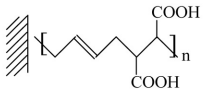
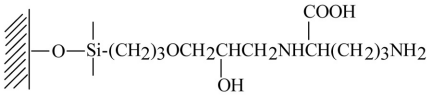
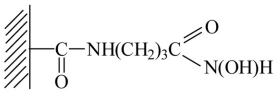
No.	Chelating substrate	Studied ions	Reference
1	 Separon SGX-NH_2	MeHg, PhHg, Hg(II), Cu(II)	[68]
2	 IDA-silica	Co, Cd, Fe Mg, Ca, Sr, Ba, Mg, Ca, Mn, Cd, Co, Zn, Pb Be	[28] [3] [27] [34]
3	 APA-silica	Ni, Zn, Cd, Mn, Al, Be, La, Lu Ni, Co, Zn, Fe, Cu, Cd, Pb Ba, Sr, Ca, Mg, Ni, Co, Zn, Cu, Pb, Cd, Mn	[80] [45] [44]
4		Mn(II), Cd(II), Zn(II), Cu(II), Pb(II), Co(II), Ni(II), Hg(II)	[81]
5		Lanthanides	[82]
6		Cu^{2+}	[83]
7		Lanthanides	[84]
8		Lanthanides	[84]
9		Lanthanides	[84, 85]
10		Lanthanides	[86]

Table 2. Continued

No.	Chelating substrate	Studied ions	Reference
11		Lanthanides	[85]
12	 PRPX800	Mg, Ca, Sr, Mn, Ba, Cd, Zn, Co, Pb, Cu	[18, 32]
13		Mn, Co, Cd, Zn	[53]
14	PolyCat A, poly(aspartic)acid functionalized silica	Mg, Ca, Sr, Ba	[32, 87, 88]
15	IonPac CS 12A mixed phosphonate-carboxylic acid groups	Lanthanides Fe, Mn, Zn, Co, Ni, Cd, Cu, Pb	[16, 17] [16]
16		Fe, Zn, Mn, Cd V(IV), Mo(VI), W(VI)	[89] [90]

small amount of these reagents to maintain a dynamically stable layer.

Dynamically loaded columns can also produce efficient separations of metal ions. For example, there is increasing interest in using very short columns. However, this could be difficult for some chelating substrates if the capacity is relatively low and there is a wide range of alpha values for the metals of interest. Studies by Cowan showed that hypercrosslinked polystyrene resins dynamically loaded with dipicolinic acid do have the characteristics which give good separations on very short columns. Figure 5 shows a six metal separation on a 15 mm length column packed with relatively coarse particles of 9 μm diameter.

The chelating reagents can be dynamically loaded not only onto a hydrophobic surface but also onto the surface of an anion-exchanger. Kocjan *et al.* [51] modified the surface of aminopropylsilica with calcon [1-(2-hydroxy-naphthylazo)-2-naphthol-4-sulfonic acid] and its analog calconcarboxylic acid [1-(2-hydroxy-3-carboxy-naphthylazo)-2-naphthol-4-sulfonic acid] [52] and used the prepared chelating sorbents for the separation of Ca(II), Zn(II), Co(II), Cu(II) and Mg(II), Zn(II), Cu(II), Fe(III), respectively.

3.3 Covalently bound ligands

Silica is usually used for the attachment of chelating groups as acidic conditions are mainly used for the separation of transition metal ions to avoid hydrolysis. Also, the columns packed with silica-based chelating ion-exchangers are more efficient. The use of chelating ion-exchangers based on 3 μm silica particles is published. The other novelty is associated with the use of monolithic type chelating ion-exchange columns for the separation of metal ions [33, 53].

The very low hydrolytic stability of methacrylate polymers at pH <1 – 2 restricts their application for HPCIC, where acidic cleaning of the columns from strongly retained polyvalent metal ions is sometimes required.

Potentially, microdispersed sintered nanodiamonds may be used as a chelating ion-exchanger due to excellent mechanical properties, hydrolytic stability, and the possibility of modification of the surface with different functional groups. The original detonation nanodiamonds have carboxylic groups at the surface and reveal some chelating properties similar to carboxylic cation-exchangers [54]. However, the column efficiency needs to be improved.

4 Detection

4.1 Background

Detection was always considered to be one of the weak points in the rapid development of HPLC methods in the 1970s. The range of sensitive and selective detectors available was rather limited compared to those enjoyed by GC. The UV-Vis spectrophotometric detector soon became the most common one used in HPLC. Although historically, absorbance measurements were considered to be only of medium sensitivity, instrument manufacturers made great efforts to reduce electronic noise and drift to very low levels. Now, even relatively low-cost UV-Vis spectrophotometers have short-term noise levels of around ±0.00001 absorbance units. Nevertheless, the UV-Vis detector would only give sensitive detection for compounds with high molar extinction coefficients. There were still many groups of compounds with little or no absorbance in the range of 200 – 800 nm. One such group, of interest in this review here, are metal ions separated by IC techniques. An alternative detection method based on conductivity, originally developed for anions, gives high sensitivity, but is only suitable for the alkali and some alkaline earth metal ions. As this article is mainly concerned with transition and the so-called heavy metal ions, conductivity detection will not be dealt with here.

4.2 PCRs for the detection of metal ions

One approach which produces high sensitivity detection for non- or low-absorbing analytes is to use PCRs. With this approach a reagent is mixed with the column effluent at a T junction and reacts with the eluted analytes in a short coil to form a species with a much higher molar extinction coefficient. One well-known PCR example, developed in the late 1940s, is the detection of amino acids after separation on anion exchange columns using ninhydrin [55].

For the PCR detection of metal ions by IC, a colorimetric reaction is also the one most commonly employed. The reagent is usually a chelating dyestuff, producing an intensely absorbing metal complex at a different wavelength to that of the dyestuff. Although many hundreds of colorimetric reagents were developed, particularly in the 1950s and 1960s, for the determination of trace metals, only a small number were suitable for use as PCR reagents (Table 3). This is because the reagent and the metal complexes had to be water-soluble and to be fast reacting. The colorimetric reagent also needed to react with a wide range of metals and produce complexes with very high molar absorptivities, with a wavelength of maximum absorptivity well removed from that of the free reagent. 4-(2-Pyridyl)azoresorcinol (PAR) fulfilled all these criteria and after pioneering work by Fritz and Story [56] in the 1970s soon became established as the one of the best and most commonly used PCR reagents. PAR is particularly suited to the detection of most of the first row transition elements and Cd and Pb. The addition of ZnEDTA to the PAR reagent can improve the sensitivity for Mg, Ca, Sr, and Ba due to a displacement reaction between ZnEDTA and alkaline earth metal ions. The sensitivity to metals such as Pb is also improved [57 – 59]. However, the use of ZnEDTA is not used very often as sensitive detection of alkaline earth metals is not normally required for many sample types, as those from environmental and biological sources contain relatively large amounts of Ca and Mg.

It is claimed that PAR can be used for the detection of 44 metals. However, for the higher charged or hydrolysing metal ions such as the lanthanides and actinides better sensitivity is obtained using other reagents, which can be used in acid conditions, reducing the risk of precipitation due to hydrolysis. Arsenazo I and Arsenazo III give good sensitivity for U (VI), Th and the lanthanides, though Arsenazo III is the one preferred by most workers [60, 61]. Pyrocatechol violet (PCV) and Chromazurol S (CAS) have also been used [38]. A number of reviews have compared the different PCRs [62].

Although colorimetric PCRs are by far the most commonly used for trace metal detection, some fluorometric reactions have been used with success. They are more selective and studies have been mainly restricted to the alkaline earths and Al, Ga, and In. Trace Al determinations have attracted a lot of attention with fluorometric PCR, 8-hydroxyquinoline sulfonic acid (8-HQS) being particularly sensitive [63].

4.2.1 New reagents for PCR

A priori, the molar extinction coefficient defines the sensitivity of photometric detection with formation of complexes of metals with corresponding organic reagents. The frequently used PAR gives molar absorptivities of about 7×10^4 L · mol^{-1} · cm^{-1}. New reagents, which are derivatives of PAR, like 2-[(5-bromo-2-pyridyl)-azo]-5-diethylaminophenol (5-Br-PADAP) and 2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol (Nitro-PAPS) have higher molar absorptivities, approximately 8×10^4 and 1.2×10^5 L · mol^{-1} · cm^{-1}, respectively and could be potentially more suitable in future. They have been used in PCR detection [64, 65] and a significant improvement in sensitivity of detection was noted as shown in Fig. 6, but the relatively low solubility of 5-Br-PADAP and high cost of Nitro-PAPS still restrict the wide use of these reagents. Potentially, even more sensitive reagents could be the porphyrins, *e.g.*, *meso*-tetrakis(4-*N*-trimethylaminophenyl)porphine (TTMAPP), which gives molar extinction coefficients for the copper complex of 4.8×10^5 L · mol^{-1} · cm^{-1} [65]; however, the first attempt of their

Table 3. PCRs for the detection of separated metal ions

No.	PCR reagent	Reaction conditions	Detection	Detected ions and LODs[a] (μg/L)	Reference
1	PAR	1.0×10^{-4} M PAR, 0.125 M $Na_2B_4O_7$, 0.2 M NaOH, pH 10.5	Vis, 520 nm	Transition, heavy metals and lanthanides	[39]
2	PAR-ZnEDTA	1.2×10^{-4} M PAR, 0.2 mM Zn-EDTA, 2 M NH_4OH	Vis, 520 nm	Alakaline earth metals	[39]
3	5-Br-PADAP	3.0×10^{-4} M 5-Br-PADAP, 0.95% w/v Triton X-100, 66 mM glycine, 67 mM NaOH, and 0.12 M NaCl	Vis, 565 nm; 676 μL	1.5 Cu(II), 5.0 Ni(II), 0.5 Zn(II), 5 Co(II), 5 Cd(II), 3 Mn(II), 20 Hg(II), and 200 Pb(II) with 115 μL loop	[64, 70]
4	Xylenol orange	5.0×10^{-5} M xylenol orange, 4 mM CTAB, 2 M NH_4OH, 4 M NH_4Cl, pH 8.7, 40% methanol	Vis, 618 nm	3 Ce(III), 4 Nd(III), 30 Er(III), 60 Lu(III)	[66]
5	Nitro-PAPS	6.0×10^{-5} M Nitro-PAPS, 0.15 M CHES, 0.1 M NaOH, pH 9.4	Vis, 574 nm, 588 μL	4.8 Pb(II), 1.9 Cd(II), 1.5 Mn(II)	[65]
6	Arsenazo III	1.5×10^{-4} M Arsenazo III, 1 M HNO_3	Vis, 654 nm	U(VI), Th(IV), Bi(III), Hf(IV), Zr(IV)	[38, 39, 82]
7	PCV	1.04×10^{-4} M PCV, 2 M hexamine, pH 6.9 adj. HNO_3	Vis, 580 nm	Al(III)	[39]
		1.04×10^{-4} M PCV, 1.5 M hexamine	Vis, 585 nm	V(IV), V(V)	[38]
8	*o*-Cresolphthalein complexone (*o*-CPC)	4.0×10^{-4} M *o*-CPC, 0.25 M H_3BO_3, pH 10.5 adj. NaOH	Vis, 510 nm	Alkaline-earth metals	[18, 33]
9	Dithizone	4.0×10^{-5} M dithizone, 0.01 M cetyltrimethylammonium hydrogen sulfate (CTAHS), pH 2 adj. H_2SO_4	Vis, 500 nm; 28 μL	63 Cu(II), 40 Hg(II), 100 MeHg, 600 PhHg	[68]
10	Carboxylated or sulfonated dithizones	6.5×10^{-4} M dithizone, 0.5% w/v Triton X-100, 50 mM NaOH	Vis, 570 nm; 250 μL	4.0 Hg(II), 8.5 MeHg, 11.5 PhHg	[69]
11	Eriochrom black T	2.0×10^{-4} M Eriochrom black T, 4 mM CTAB, pH 8.7 adj. 1 M NaOH	Vis, direct at 512 nm; indirect at 650, 100 μL	30 – 70 for indirect and 80 – 170 for direct detection from La(III) to Lu(III)	[67]
12	TTMAPP	Four reaction coils: R_1, 6.0×10^{-6} M EDTA in 0.1 M CH_3COOH, 0.2 M NaAc, 0.12 M NaOH; R_2, 6.0×10^{-6} M Cu(II), 4.4×10^{-6} M TTMAPP in 0.1 M HNO_3; R_3, 0.5% ascorbic acid in 25 mM acetic acid, 0.8 NaOAc; R_4, 1.9 M HNO_3	Vis, 433 nm, 100 μL	10 for lanthanides	[91]
13	CAS	2.6×10^{-4} M CAS, 2% w/v Triton X-100, 50 mM MES, pH 6.0	Vis, 590 nm; 250 μL	3 Be(II)	[34, 80]
14	1,5-Diphenyl carbon hydrazide	2 mM 1,5 Diphenyl carbon hydrazide	Vis, 520 nm; 100 μL	0.5 Cr(VI)	[92]
15	8-HQS	2 mM 8-HQS, 0.01 M NaAc/HAc buffer, pH 4.1	Fluorescence λ_{ex} 360 nm, λ_{em} 512 nm	1.0 Al(III)	[92]
16	Lumogallion	4.0×10^{-5} M lumogallion, 0.2 M NaAc/HAc buffer, pH 5.2	Fluorescence λ_{ex} 500 nm, λ_{em} 590 nm	1.0 Al(III)	[93]
17	Luminol	3.4×10^{-4} M luminol, 0.1 M H_3BO_3, 0.1 M H_2O_2, pH 11.5 adj. NaOH	Chemiluminescence, 50 μL	0.002 Cr(III) and Cr(VI)	[94]
18	Chlorphosphonazo III	6.24×10^{-5} Chlorophosphonazo III, 0.02 M HNO_3	Vis, 660 nm; 50 μL	Lanthanides	[84, 86]

[a] In case of few references the lowest LOD value is presented.

application for the PCR did not provide any significant improvements in the sensitivity of detection (see Table 2, row 14). A big problem with using larger organic molecules, such as the porphyrins, with higher molar absorptivities, is that the reactions with the metals become significantly slower, requiring longer reaction coils and/or higher reaction temperatures.

4.2.2 Effect of surfactants

The addition of surfactants to PCR reagent mixture may have few positive effects including (i) solubilization of organic reagent; (ii) formation of micelles which can enhance the analytical response of photometric/fluorescent reaction; (iii) modifying of surface of reaction coil and other parts of reactor to diminish possible interaction of formed complexes with surface and hence possi-

ble peak distortion. Gautier *et al.* investigated the effect of additives of cationic surfactants (cetylpyridinium chloride (CPC) and CTAB, CMC for both 0.9 mM), anionic surfactant (SDS, CMC 8.1 mM), and nonionic surfactant polyoxyethylene-*t*-octylphenol (Triton X-100) with CMC 0.2 mM on a PCR reaction of separated transition metal ions with Xylenol Orange [66] and Eriochrom Black T [67]. They noted almost three times absorbance increase for PCR reaction of Xylenol Orange with lanthanides in the presence of cationic surfactants CPC at pH 8.7 and attributed this effect to the formation of ternary complex with a stoichiometric ration of lanthanide-Xylenol Orange-CPC = 1:2:4. Some spectral shift for absorbance maximum from 604 to 618 nm was also noticed due to changes of microenvironment of complex due to electrostatic interactions between micelles of cationic surfactant and anionic dye and due to hydrophobic interactions between alkyl chains of surfactant and organic dye. In the case of Eriochrom Black T [67], no changes in sensitivity were observed with additives of anionic surfactant SDS and nonionic Triton X-100, but decrease in the sensitivity of detection was noticed with the addition of cationic surfactants such as CPC and CTAB. The authors also noted some positive effects of presence of surfactants in PCR mixture such as improvement with baseline drift and decrease of background noise of PCR.

Usually the additive of 0.5–2.0% w/v of nonionic surfactant Triton X-100 was used for the solubilization of such organic reagents as Dithizone derivatives [68, 69], CAS [34], and 5-Br-PADAP [70] in PCR.

4.2.3 Methods for improving S/N ratios

4.2.3.1 The detection limit

The LOD is a very important parameter in any quantitative trace analytical method. It not only gives vital information on the lower LOQ, which is generally accepted to be ten times the LOD, but also can be used to compare different instrumental techniques, so that a decision can be made on the choice of method for a particular sample analysis. The most common way of obtaining an LOD, which is usually defined in chromatography as an analyte signal twice the peak-to-peak noise level of a continuously monitored baseline, relies on S/N calculations. Since most modern instrumental techniques require conversion of detector responses to electrical signals, noise is normally revealed as random voltage or current fluctuations in the baseline before and after an analyte passes through the detector. The analyte response above the background noise is considered as the signal. As the analyte concentration is decreased the analyte signal will eventually merge into the background noise and be "lost". The lowest concentration at which one can assert with a certain degree of confidence that a particular analyte is present in the sample, *i.e.*, a "definite" response

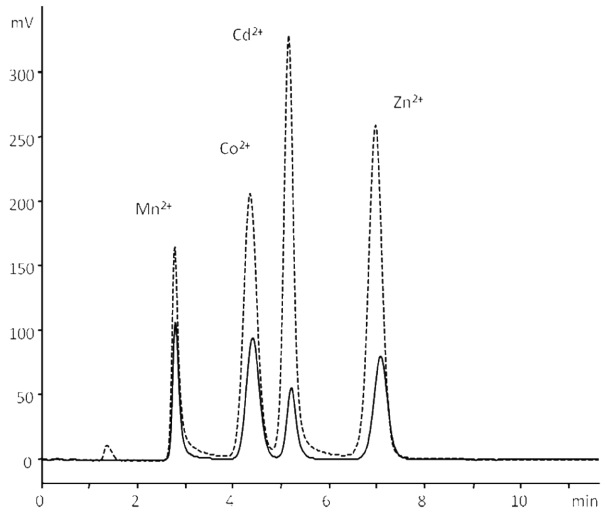


Figure 6. Comparison of Nitro-PAPS and PAR PCR sensitivity. Column: IDA-silica, 150 × 4.0 mm, 5 μm. Eluent: 8 mM HNO_3. PAR/NH_3 at pH 10. NPAPS with borate buffer pH 9. Solid line, PAR; dashed line, NPAPs.

above the noise, is the LOD. Rather than use a statistical approach, the common method in chromatography is to assess the average peak-to-peak baseline noise just before and after the analyte signal. The LOD is then defined as twice, or sometimes more conservatively as three times this figure. Although a purist would prefer a more statistically exact method, the concept of the LOD being two or three times the baseline noise is widely accepted and used by chromatographers.

4.2.3.2 Characterization of baseline noise

There have been a number of attempts at defining the various types of background noise typically seen in chromatograms. The most common definition is in terms of the time scale of the noise, namely, short term, long term, and drift. The difference between each type is somewhat arbitrary, but serves to cover most situations.

Short term noise is generated by relatively fast, fairly even fluctuations in the baseline where the time between fluctuations is much shorter than the analyte peak base width.

Long term noise develops from slower random variations in the background, where the time between the major fluctuations is of the same order as the base width of the analyte peak. This type of noise is also referred to as baseline wandering. This is the most serious type as most noise reduction procedures would not be able to improve S/N without seriously distorting the signal.

Drift A slow unidirectional change in the baseline, which is more or less linear, though can be curved.

In calculating the average peak-to-peak background noise, linear drift, unless severe, is normally discounted as it does not directly impact on the LOD evaluation.

4.2.3.3 Noise reduction methods

Many analyses now require the determination of very low levels of components in a wide variety of samples. Therefore, instrument manufacturers have put a tremendous effort into reducing the noise and drift of spectrophotometric detectors, the ones most commonly used in LC. However, these low noise levels can be difficult to achieve in practice as external factors can increase the noise when the detectors are used in conjunction with other equipment in HPLC systems. Because of this a number of noise reduction methods have been developed to try and improve S/N ratios.

Pulse dampeners The construction of dampeners depends on the level of pressure at which pulsations take place. HPLC dampeners are incorporated in-line between the pump and the injector. Most commonly they involve a long coil or chamber with a slight elasticity in the walls. The energy of the pump pulses is absorbed and released out of phase with the frequency of the pump strokes, thus reducing or dampening the amplitude of the pulses. Because of the relatively slow frequency of most pump piston reciprocations, it is difficult to design a pulse dampener to completely suppress short-term pump pulses and they have little effect on pump variations which produce long-term noise. Low-pressure dampeners have a similar construction but from softer coil materials.

Electronic methods: Many detectors have built-in electronic filters, which can control the time constant or rise time of the signals at the output of the detector, before being sent to the computing integrator or recorder. The level of the time constant is chosen by the operator. Again it is only useful for suppressing fast or short-term noise. As the electronic suppression affects both the baseline noise and the peaks, the rise time has to be chosen with care otherwise serious peak distortion could result.

Software methods: Computer programs have been developed as part of integration software, which can suppress noise either during a chromatographic run, *i.e.*, in real time, or postrun. In real time this can be done by digital sampling of the noise followed by averaging to smooth out the baseline. Baseline noise suppression postrun can be achieved by using a number of special algorithms involving averaging or filtering protocols. The most common ones are, moving average, Savitsky Golay and Olympic processes. For example, Gettar and coworkers [66, 67] applied a Savitzky – Golay algorithm to a registered chromatogram and got a 5 – 10-fold decrease in noise. Also, a 6 – 32-fold enhancement of the S/N ratio was noted after a similar smoothing procedure on a chromatogram of the lanthanides detected by PCR reaction with Xylenol Orange [66]. For both real time and postrun methods the degree of noise suppression is under the control of the operator and like electronic suppression can seriously distort peaks if overused. For long-term noise, the degree of peak distortion in terms of broadening and reduction in height will offset any reduction in noise, so lower LODs will not be achieved.

4.2.3.4 Noise suppression using a multiple wavelength approach

Although the systems discussed above can suppress a significant amount of the short-term noise, they are virtually useless at suppressing long-term noise of the same order as the peak widths in the chromatogram. In future, even short-term pump noise will become increasingly difficult to suppress as faster and faster LC systems are developed. This is because peak widths early on in a chromatogram could become similar to the width of pulses from higher frequency piston pump cycles, which are gradually replacing the slower piston types.

The situation is made significantly worse when PCR detection is involved, where the eluents and/or reagents have different absorbances. Not only is the noise from the two pumps or solvent delivery systems additive, but also the noise is amplified by the absorbance differences. For example, two pumps producing a combined flow variation of 0.4% will give a peak-to-peak baseline noise of 0.4 mAU, when the absorbance difference between the eluent and PCR reagent is 100 mAU. This is about 40 times the electronic noise levels of typical high quality UV-Vis absorbance detectors. In some ways this is the best case scenario as in reality, even the best pumps get noisier with age, particularly in combination with slightly worn check valves. Furthermore, for PCR, the PCR reagent is usually delivered with a low cost pump or pneumatic-based solvent delivery system, which will produce larger flow variations than high quality HPLC pumps.

The increased pump noise seen in many systems using PCRs will obviously seriously worsen LOD. Since much of the noise will be long term and of the same order as peak widths, current noise reduction systems, as explained above, will not be of much help. A radical new approach to reducing pump noise has just been introduced by a new company (JPP Chromatography, UK) using a multiple wavelength procedure (www.jppchromatography.co.uk. 2007).

They have designed an electronic block called a Signal Extractor, containing specially programmed microprocessors specifically designed to extract a much purer analyte signal where noise is present, which may be due to pump variations, even when the variations are very large. It works in real time, processing the signals from a dual or multiple wavelength detector, removing the pump noise electronically, whilst leaving the analyte signal unchanged. Unlike present noise reduction methods, such as rise-time filters and statistical algorithms, men-

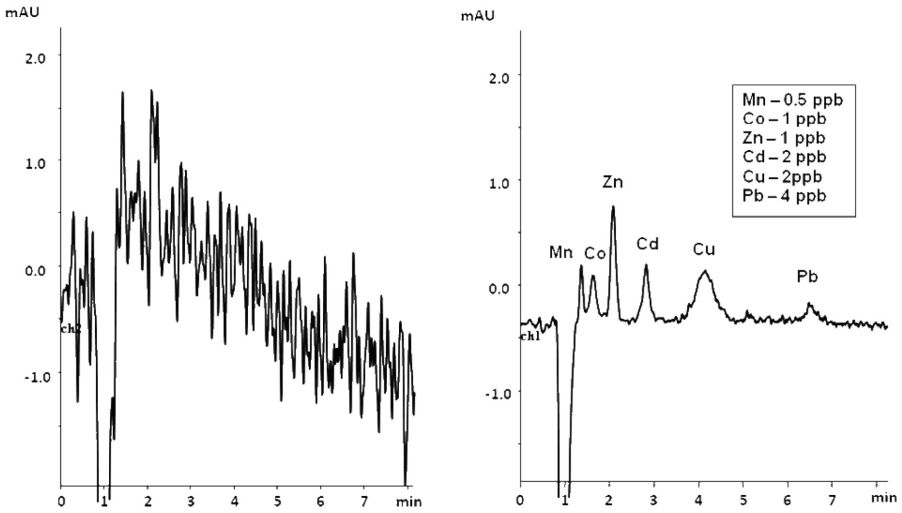


Figure 7. A six metal separation at very low concentrations on a 100 × 4.0 mm IDA-silica, 5 μm. Sample volume, 100 μL. Detection with PAR/NH_3 PCR reagent (left) without signal extraction, (right) with a JPP signal extraction system (www.jppchromatography.co.uk. 2007).

tioned above, the Signal Extractor has no effect on peak width or symmetry. It is claimed that the extractor can virtually eliminate pump noise whatever the frequency, including long-term drift or baseline wandering. The chromatogram in Fig. 7 is a dramatic example of the Signal Extractor considerably reducing short and long-term noise and drift for the HPCIC separation of six metals. As can be seen, LODs of 1 μg/L or less are achieved for five of the metals, rivaling or exceeding some of the most sensitive atomic spectroscopic methods.

5 Applicability of HPCIC

The determination of metal cations in complex samples by chromatography has attracted more and more attention over time [71, 72]. The presently established IC determination of trace metals has changed little since the 1980s. There have obviously been improvements in column design and stationary phase efficiency, but all essentially use simple cation or anion exchange, or a mixture of both, to achieve separation. This has always put a severe restriction on the range and complexity of samples that can be analyzed. There are two main reasons for this. First, although there is some control of selectivity with simple ion exchange it would be useful to have an even greater control of selectivity, where for example, it would be very useful to change the retention order to suit the range and concentrations of trace metals in different sample types. Second, simple ion exchange is very sensitive to the ionic strength of a sample. Even the presence of relatively low concentrations of alkali metal salts can seriously distort or even destroy a separation. The recent development of HPCIC has radically changed this rather limiting situation. The incorporation of chelating groups into high efficiency stationary phases opens up a whole new range of exciting possibilities for the analysis of complex and difficult samples. The use of mixed mode chelating exchange and ion exchange and/or the addition of complexing agents in the eluent allow a wide range of selectivities to choose from. When chelation on the substrate has the main influence on separation, which is true for most mobile/stationary phase combinations, the ionic strength has little or no effect on the separation, allowing even very concentrated salt solutions to be analyzed. The use of chelating columns is not new of course, but as mentioned in Section 3, those commonly available are of low efficiency and specifically designed for matrix isolation and preconcentration in rather complicated multicolumn setups. In contrast, HPCIC using high efficiency chelating substrates with rapid exchange kinetics will produce fast analytical separations.

In this final section, the intention is to show how HPCIC can be so much more versatile than established IC techniques based on simple ion exchange. A number of key examples, in addition to those already described in Table 4, will be described to illustrate how HPCIC can be used to analyze a range of difficult or complex sample types.

Stationary phases containing the IDA chelating group have been the most studied for metal separations. As stated previously Jones and coworkers were the first to show the potential of HPCIC using IDA substrates. They showed that the analysis of samples with high salt content, such as concentrated KCl and Na_2SO_4, was easily realized [73, 74]. They also found that one of the consequences of using IDA phases was that it could be too selective for some metals when using simple acidic eluents. Although retention times for some metals such as Mn, Fe(II), Cd, and Zn were relatively short, the retention times of Pb and Cu were much longer and Fe(III) was very

Table 4. Practical applications of HPCIC for the determination of trace metals

Application	Chelating ion-exchanger	Column (mm)	Eluent	Detection	Ions studied	Reference
Mg, Ca, Mn, Cd, Co, Zn, Ni, Cu in seawater	Table 2, 2	250 × 4.0	Gradient elution	PCR PAR, 550 nm	Mg, Ca, Mn, Cd, Co, Zn, Ni, Pb, Cu	[23]
Mg, Ca in NaCl eyewash saline solution and laboratory grade KCl		250 × 4.0	1 M KNO_3, pH 4.9	PCR *o*-CPC, 572 nm	Mg, Ba, Sr, Ca, Be	[3]
Mn, Cd, Co, Zn in freshwater NIST 1640 standard reference sample		250 × 4.0	0.035 M KCl, 0.065 M KNO_3, pH 2.5	PCR PAR, 550 nm	Mg, Ca, Mn, Cd, Co, Zn, Pb, Ni, Cu	[27]
Na, K in drinking water, river water, rain water		250 × 4.6	10 mM 18-crown-6 in HNO_3, pH 2.75	Indirect conductivity	Co, Cd, Fe, Li, Na, NH_4, Cs, K	[28]
Be in freshwater NIST 1640 standard reference sample, seawater		250 × 4.0	0.4 M KNO_3, pH 2.5	PCR, CAS, 590 nm	Na, Ca, Mg, Mn, Sr, Ba, Co, Pb, Cu, Ni, Zn, Al, Fe	[34]
Be in stream sediment	Table 2, 3	50 × 4.6	1 M KNO_3, 0.5 M HNO_3, 0.08 M ascorbic acid	PCR, CAS, 560 nm	Ni, Zn, Cu, Cd, Mn, Al, La, Lu	[80]
Cu and Zn in Oyster tissue NIST SRM 1566a, seawater	Table 2, 4	100 × 4.6	25 mM oxalate, 25 mM $NaNO_3$ at pH 4.2	PCR, PAR, 510 nm	Cu, Hg, Pb, Mn, Cd, Ni, Co, and Zn	[81]
Zn in industrial gypsum	PS-DVB dynamically coated with MTB, μ Mm	150 × 4.1	0.5 M KNO_3, 0.2 mM MTB, pH 1.2	Vis 600 nm	Mg, Mn, Zn, Cd, Pb	[95, 96]
U in saline lake water		150 × 4.1	0.5 M KNO_3, pH 1.2	PCR, Arsenazo III, 600 nm	Ca, Mg, U, Cu	[97]
Pb, Cd, Cu in rice flour	PS-DVB dynamically coated with 4-chloro-dipicolinic acid, μ Mm	300 × 4.6	1 M KNO_3, 0.25 mM chlorodipicolinic acid, pH 1.5	PCR PAR, 520 nm	Mn, Co, Ni, Zn, Cu, Pb, Cd, Al, La, Lu, Fe, U	[39]
Pu in standard reference sample NIST 4251 Human Lung and NIST 4353 rocky flats soil	PS-DVB dynamically coated with dipicolinic acid, μ Mm	100 × 4.6	0.1 mM dipicolinic acid, 0.75 M HNO_3	ICP-MS	Th, Np, U, Pu, Am	[98]
U in stream sediment, mineral water, seawater		100 × 4.6	1 M KNO_3, 0.5 M HNO_3, 0.1 mM dipicolinic acid	PCR, Arsenazo III, 654 nm or PCV, 585 nm	Fe, Th, V, Bi, U, Hf, Zr	[50]
Mg, Ca in seawater, saline lake, mine process sample	Porous graphitic carbon dynamically coated with *o*-CPC	100 × 4.6	45 – 58% MeOH, 0.4 mM *o*-CPC, pH 10.0 – 10.5	Vis, 575 nm	Mg, Ca, Sr	[99]
Sr in an Antarctic saline lake water	PS-DVB dynamically coated with *o*-CPC	150 × 4.1	0.5 M KNO_3, 0.2 mM *o*-CPC, 20 mM borate buffer, pH 9.5	Vis, 575 nm	Ba, Sr, Ca, Mg,	[100]

strongly retained [75]. This was solved using step gradients with decreasing pH. Later work by Jones and Nesterenko analyzing seawater [13, 15] on IDA-silica showed the potential of using complexing agents such as tartaric acid in the eluent, rather than simple acid solutions. However, gradients were still used, which added complexity to the analysis and high blanks were a problem, caused by the re-equilibration of the column before the next gradient run. It was not until the highly efficient small particle size IDA-silica phases were fabricated in the very latest work that the true potential of using complexing agents in the eluent became clear, removing the need for complicated gradients, retaining the simplicity of isocratic elution. It was found that two or even three complexing agents were required to obtain fast, efficient separations. Figure 8 shows the effect of picolinic acid addition on copper retention using an eluent already containing oxalic acid. Figure 7 (already discussed in the detection section) shows an eluent optimized for six metals, where low LODs are obtained because of the short retention times combined with a special electronic noise reduction system.

In contrast to IDA-silica, not many studies have been carried out on APA-silica as it is relatively new. Nevertheless, the work done so far indicates great potential for this chelating phase. Unlike IDA, the aminophosphonic acid (APA) chelating group gives more evenly spaced retention times, so only simple nitric acid eluents have been used so far. Figure 9 shows a chromatogram of 11 metal species on high efficiency silica substrate. Cu^{2+}

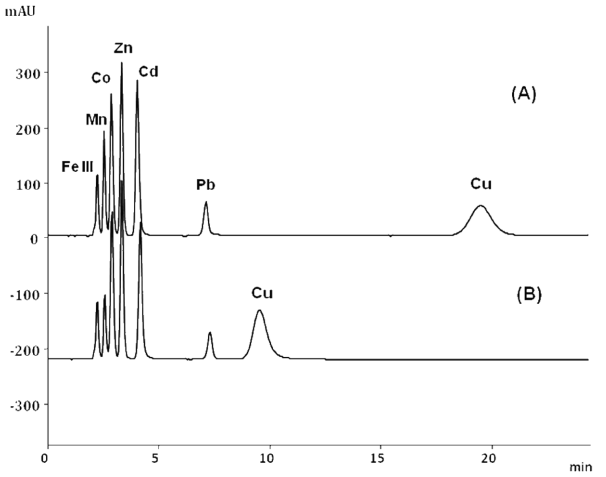


Figure 8. The effect of picolinic acid additives on retention of copper. (A) Separation with oxalate-based eluent. (B) As (A) but with a small amount of picolinic acid added. Column: IDA-silica, 150 × 4.0 mm, 5 μm. Detection with PAR/NH_3 PCR.

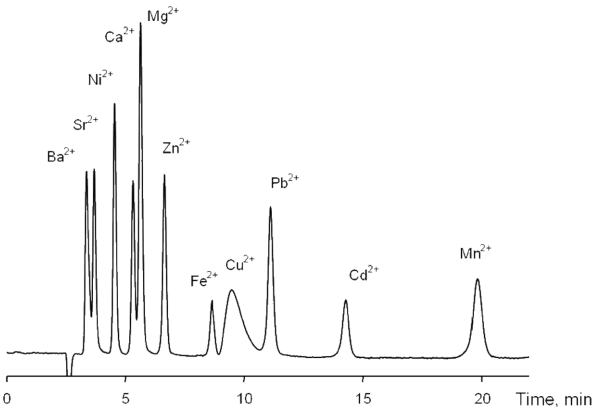


Figure 9. Separation of model mixture of alkaline-earth and transition metal ions. Column: APA-silica, 150 × 4.0 mm, 3 μm. Eluent: 50 mM HNO_3 – 0.8 M KNO_3, detection at 510 nm with PCR reaction with PAR-ZnEDTA.

tends to give a broader peak than the other metals, which is also observed on IDA.

5.1 Fine chemicals

HPCIC can be used to determine trace metals in alkali metal salts sold as fine chemicals (usually called analytical reagent grade). The samples need to be fairly concentrated to achieve low LODs. It is interesting to note that one of the most sensitive of trace metal techniques, ICP-MS, cannot tolerate even small salt concentrations and samples have to be considerably diluted, thus losing the advantage of the very low LODs obtainable by this technique. Figure 10 shows the chromatogram obtained from the analysis of 0.25 M KCl, where low levels of Zn, Cd, and Pb were detected. This is a good example of the considerable improvement obtained when using very high efficiency silica substrates under isocratic conditions compared with the earlier work by Challenger *et al.* [74, 76] using complex step gradients on relatively low efficiency chelating PS-DVB-based substrates.

5.2 Saturated brines

If there is one example which shows the special nature of HPCIC and its ability to analyze very concentrated samples, it is the determination of alkaline earth metals in saturated brine. Saturated brine (30% NaCl) is used on the very large scale to produce NaOH and Cl_2, key raw materials for the chemical industry. This is achieved by electrolysis and in the past the Castner/Kellner process was used with mercury pools in the electrolytic cells. Environmental concerns have led to the phasing out of the mercury system in favor of membrane technology. However, traces of alkaline earth metals, particularly Ca, Mg, and Sr, in the saturated brine can lead to "clogging up" of the membrane lowering its efficiency. Therefore, it is important to monitor the brine for these metals. This can be done by present IC methods, but they involve very complicated multiple column systems with a number of different eluents and switching valves [77]. With HPCIC, only one column is necessary as can be seen in Fig. 11. Using only a 20 μL injection, low μg/L levels of Ca, Mg, and Sr can be determined. In this case, the Ca level is relatively high and close to the Sr, but the Sr level can still be measured. As expected, APA-silica can also be used to analyze fine chemicals. An example is shown in Fig. 12 where 30% KNO_3 can be analyzed with no problem.

5.3 Iron speciation

There has always been considerable interest in determining traces of Fe(II) and Fe(III) in many sample types. One important example is in the power industry where the measurement of the two forms in boiler water can give important information about potential corrosion problems. IDA-silica is ideal for this analysis as the eluent can be optimized to give very short retention times. Figure 13 shows a chromatogram of Fe(II) and Fe(III) separations at two different concentration levels. The high sensitivity and low LODs are due to the short retention times.

5.4 Lanthanides

Nesterenko and Jones [35] were the first to show the isocratic separation of the lanthanides and Yttrium. Until then all lanthanide separations had to involve gradient

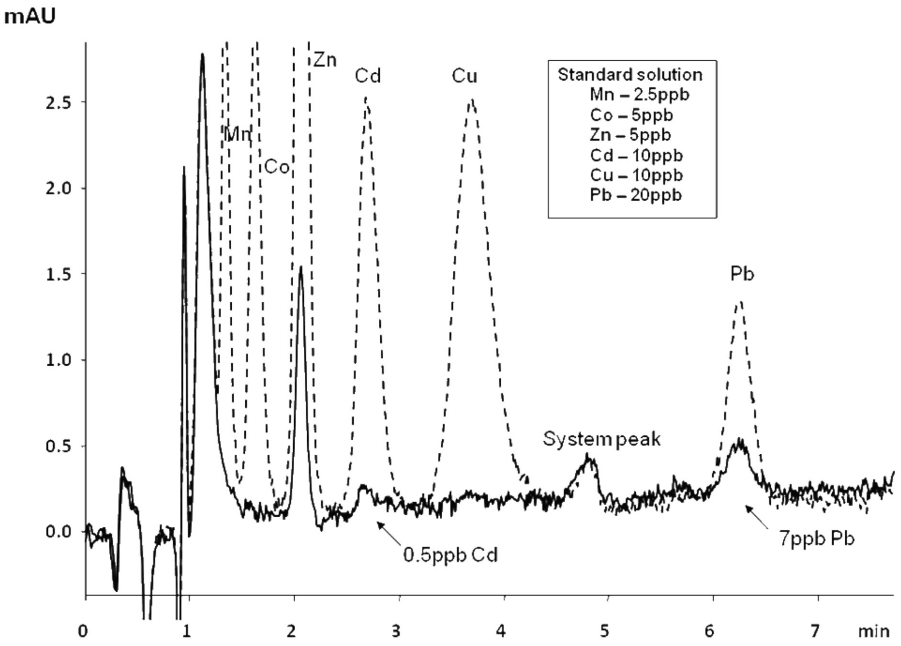


Figure 10. Determination of trace metals in 0.25 M KCl using a 100 × 4.0 mm IDA bonded 5 μm silica column. Hundred microliters injection of sample (solid line) and 100 μL injection of sample containing a standard addition of six metals (dashed line). Detection with PAR/NH_3 PCR.

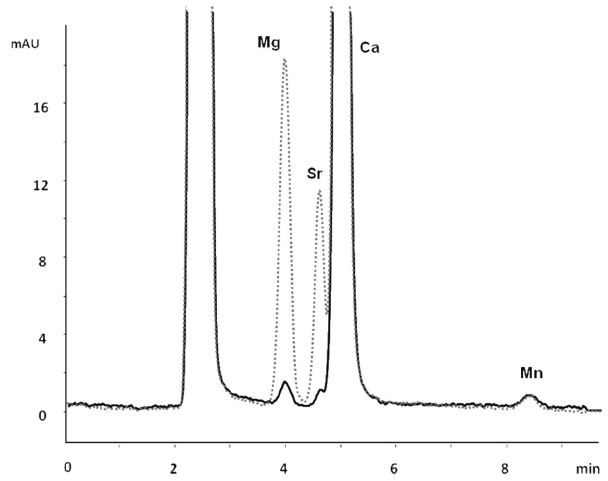


Figure 11. Determination of alkaline earth metals in 4 M NaCl brine using a 100 × 4.0 mm IDA-silica, 5 μm column. Eluent, 0.5 M KNO_3 containing very dilute nitric acid. Injection volume 20 μL of 30% NaCl (solid line) and 20 μL of 30% NaCl containing a standard addition of 200 μg/L Mg, 200 μg/L Ca, and 400 μg/L Sr (dashed line). Calculated concentrations in the 30% brine are 30 μg/L Mg, 35 μg/L Sr, and 1.3 mg/L Ca.

programs [66, 67, 78]. However, the analysis took nearly 60 min for completion. In their latest work using the very latest highly efficient silica substrates, the analysis time was dramatically reduced by nearly a half to just over 30 min (Fig. 2).

5.5 High valence metals

The high valence metals pose the biggest challenge for IC separation. Their tendency to hydrolyze even in strong acid makes it particularly difficult to find good eluent combinations. One way to solve this is to use strong chelating agents such as dipicolinic acid, dynamically loaded on polystyrene resins. This area was studied in some detail by Cowan [79], who developed a separation scheme for some of the most hydrolysable metals such as Bi(III) and Zr(IV). Figure 14 shows a chromatogram obtained during the analysis of a sediment sample for Bi(III), Zr(IV), and U(VI).

An even more challenging separation involves some of the actinides, particularly U and Pu. This is one example where the separation column is coupled to an ICP-MS to achieve very low LODs. U(VI) needs to be separated from Pu(IV) as the U forms UH in the plasma with the same mass as ^{239}Pu, so causing a major interference. Figure 15 shows the separation of U(VI) and Pu(IV) on a dynamically loaded column where the Pu is easily determined in lung tissue in the presence of large amounts of U.

6 Conclusion

Ion exchange chromatography has been the dominant method for the analytical separation of metals for over 60 years now, with little change in the main separation systems, except perhaps for a recent trend to use weak acid carboxylic acid-based substrates, rather than those with the strong acid sulfonate groups. Chelation on the surface of a substrate is a distinctly different sorption mechanism for metals and although this has been known almost as long as simple ion exchange, until relatively recently has been little studied for analytical separations. One possible reason was that the sorption

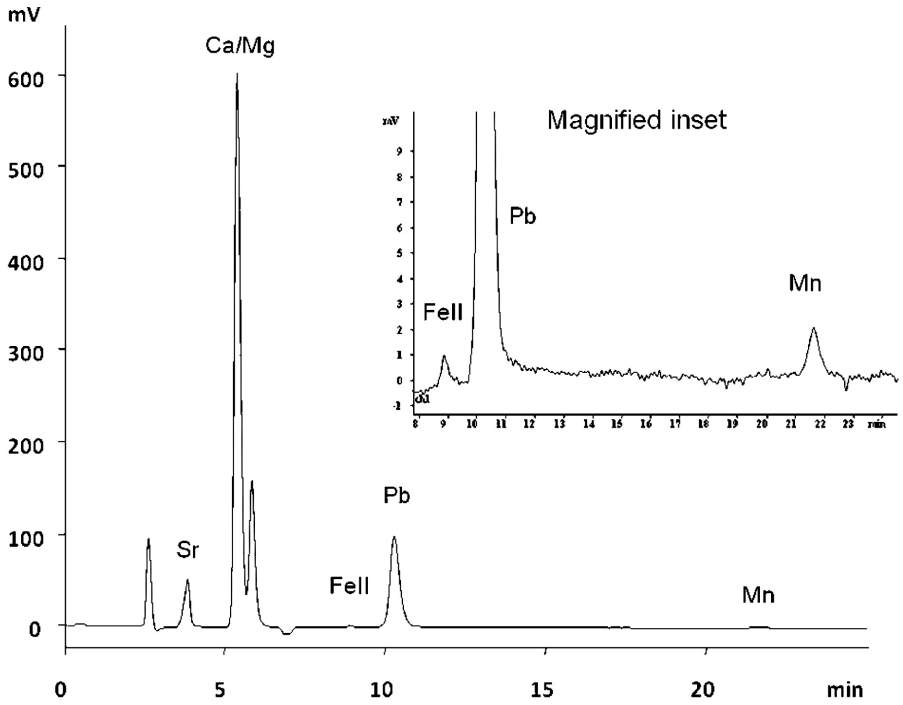


Figure 12. Determination of trace metals in 30% KNO_3. Determination of trace metals in 30% KNO_3. Column: APA-silica, 150 × 4.0 mm, 3 μm. For separation conditions see Fig. 9. Injection volume 100 μL. Calculated concentrations are Sr 250 μg/L, Ca 3.0 mg/L, Mg 420 μg/L, Fe(II) 50 μg/L, Pb 1.2 mg/L, and Mn 23 μg/L.

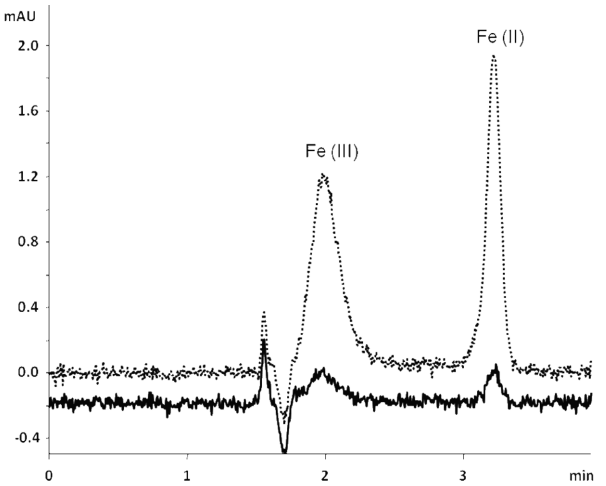


Figure 13. Separation of Fe(III)/Fe(II) at two different concentration levels using a 100 × 4.0 mm ID-silica, 5 μm column. Injection volume, 100 μL. 20 μg/L of each species (dashed line) and 2 μg/L of each species (solid line).

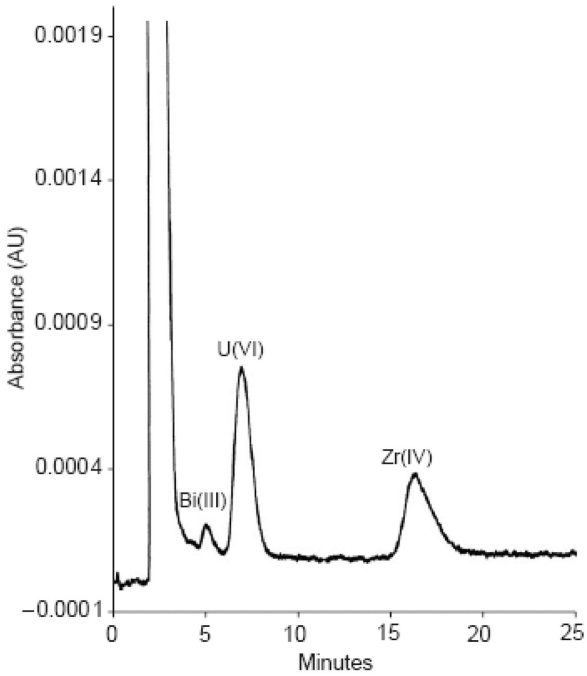


Figure 14. Isocratic separation of Bi(III), U(VI), and Zr(IV) in GBW07311 sediment sample at pH 0 column 150 × 4.6 mm, PS-DVB 5 μm resin dynamically modified 0.1 mM dipicolinic acid. Injection volume used, 500 μL; PCR detection at 654 nm with Arsenazo III.

mechanism was poorly understood, which when combined with preconceived ideas that the kinetics of chelation would be too slow to give high efficiency separations give little incentive for detailed study. This review shows that the situation regarding chelation sorption has changed dramatically in the last few years. The factors regarding not only the kinetics of mass transfer, but also the relationship with simple ion exchange are now much better understood and have led to the development of substrates with metal separation efficiencies close to those found with RP systems. The review also shows that a greater range of complex sample types is now accessible for analysis by HPCIC. For example, it

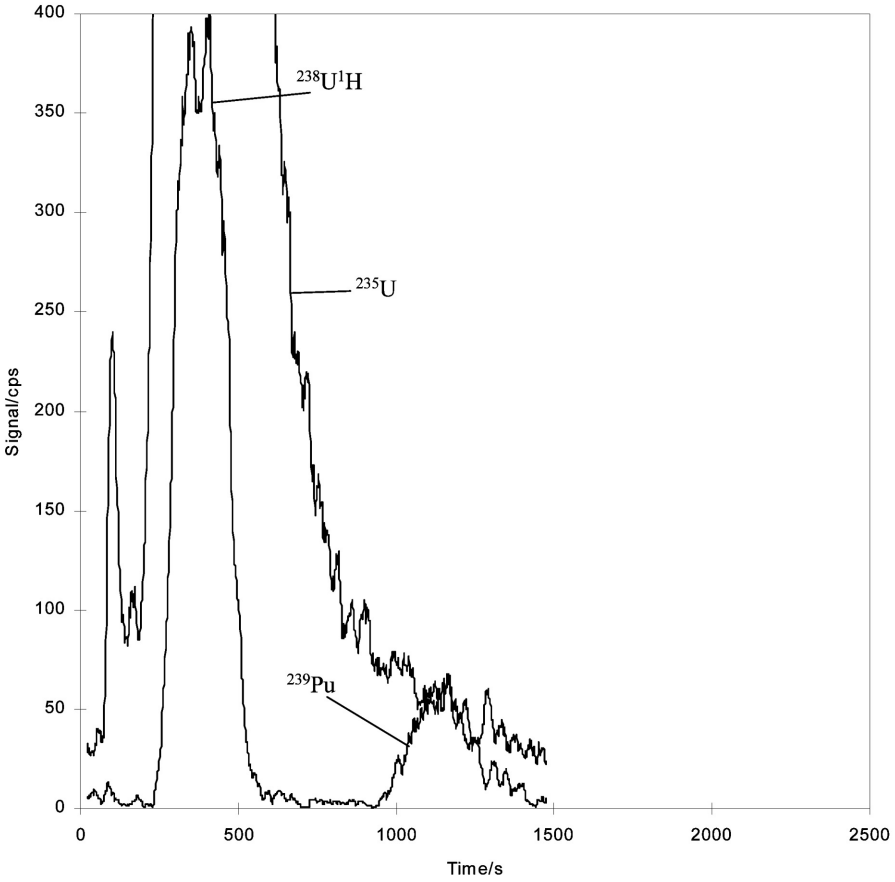


Figure 15. Separation of ^{239}Pu from uranium, and consequently the $^{238}U^{1}H$ interfering in NIST 4351 Human Lung standard reference material. Column 100 × 4.6 mm, dipicolinic acid-coated PS-DVB 5 μm resin. Eluent: 0.1 mM dipicolinic acid plus 0.75 M HNO_3 (from ref. [98]).

would be inconceivable a short time ago to try and attempt the direct analysis of saturated brines by IC, but HPCIC finds no problem with this kind of sample. New developments in PCR detection have also occurred and when combined with these HPCIC columns can give LOD for some metals rivalling those of the most sensitive AAS techniques. The characteristics of chelation columns are clearly different from those of simple ion exchange, but the price of learning the new techniques is small compared to the much greater versatility gained in analysis.

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