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Review

High-performance chelation ion chromatography A new dimension in the separation and determination of trace metals

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Abstract

A review of so-called high-performance chelation ion chromatography (HPCIC) is presented. The principles of this separation technique are based on exploitation of the chelation effect of stationary phases in the presence of comparatively reduced electrostatic ion-exchange interactions. The common ways to suppress ion-exchange, including increasing the ionic strength and pH of the eluent and the column temperature, are discussed. Unlike the majority of low-efficiency chelation exchangers that are used as preconcentration columns for batch separations, HPCIC can be used for high-efficiency analytical separations of trace metals. The range of applications of chelating exchangers used for the separation and determination of alkaline-earth and transition metal ions by HPCIC as well as their types and properties are considered. The main features of HPCIC are the much greater flexibility in selectivity control compared to ion-exchange and the relative insensitivity to high ionic strengths. These properties make it a more preferable technique for the trace analysis of complex samples, such as seawater, saturated brines, etc. © 1997 Elsevier Science B.V.

Keywords: Reviews; Chelation ion chromatography; Metal cations

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1. Introduction

The beginning of ion-exchange as a major separation technique is generally credited with the use of phenol formaldehyde resins by Adams and Holmes in 1935 [1] but, perhaps, the separation of the lanthanides on polystyrene-based resins by Kettele and Boyd fifty years ago in 1947 [2] was the first notable milestone in the development of ion-exchange as a significant force in separation science. From our perspective today, retention times of several thousand minutes seem very slow, but, compared with the incredibly tedious chemical batch techniques of the period, they were a spectacular breakthrough in time-saving separation methods. Twenty years or so later, ion-exchange separation methods were available for all elements that formed relatively stable ionic species in solution. However, apart from a few important examples, ion-exchange appeared to be used more as a batch separation or sample treatment technique to isolate the species of interest, rather than as an analytical method for quantitative determinations. It was not until the 1970s, when the renaissance in liquid chromatography involving high-efficiency substrates took hold, that the ion-exchange technique became a viable analytical determination method using sophisticated instrumentation with on-line detection. This important step occurred with the introduction of ion chromatography (IC) by Small et al. in 1975 [3], involving high-efficiency latex agglomerated pellicular anion-exchange resins for separation, combined with suppressed conductivity detection. Although the term IC now incorporates other sorption mechanisms for ion separations, such as ion-exclusion and ion-pairing, the majority of methods use anion- or cation-exchange substrates. The first major impact of IC was of course in the area of trace anion determinations, as atomic spectrometric methods were not suitable for non-metal species. Nevertheless, in spite of the strong competition from atomic spectrometric techniques, the IC of metal cations is now well established, as the relative cheapness, ease of automation and on-line capability is particularly attractive in a wide variety of routine trace analytical situations.

Interestingly, the elution methods used for the separation of metal ions with 2+ and 3+ charges have not changed much from the methods developed for the separation of the lanthanides five decades ago. A relatively weak metal-complexing agent is a major component of the eluent, usually an organic carboxylic acid, which effectively reduces the positive charge on the metal cations, improving the speed and efficiency of the separation. Major developments have mostly been in the enhancement of high-efficiency ion-exchange substrates, with the sulphonate group still being the most popular of the cation-exchange groups. This approach gives very little scope for changing the selectivity of metal separations in terms of retention order. The lack of selectivity control limits the versatility of IC methods, particularly if there is interest in trace metals eluting in the presence of massive amounts of other metals. Furthermore, the trend to lower capacity, high-performance cation-exchange substrates, makes separations vulnerable to big changes in ionic strength. Injection of a sample with a high concentration of alkali metal salts, for example, could swamp the column and seriously degrade or destroy the separation of the metal cations. Both of these limitations of selectivity and capacity seriously restrict the use of IC for trace metal determinations in a number of areas. However, there is a solution to these problems, that is, to use a metal chelating ion-exchange substrate rather than a simple ion-exchange substrate. The chelating ion-exchange technique is well known, but primarily has been used for preconcentration and batch separation of metal cations in complex matrices. What has been little exploited so far is the development of high-performance chelating ion-exchange substrates. These

high-performance substrates can be used in analytical separation columns in an IC system just like ion-exchange columns, but have the added advantages of selectivity control and insensitivity to changes in ionic strength. We consider that chelation exchange adds a new important dimension to the exploitation of IC for metal ion determinations whose full potential has yet to be realized. This review outlines the development of chelation ion chromatography as a distinct sorption mechanism and describes the latest developments in the fabrication properties and uses of high-performance substrates for trace metal separation and determination. To distinguish the use of high-efficiency chelating ion-exchange columns for analytical determinations from the more general use of low efficiency batch separation columns, we have called this technique ''high-performance chelation ion chromatography, HPCIC''.

2. The chelation exchange sorption mechanism

The ion-exchange process mentioned in Section 1 was described as ''simple ion-exchange''. It is appreciated that the thermodynamic interactions in the substrate are quite complex, involving dehydration, rehydration, etc., but the basic process is essentially competitive ionic attraction for the ionic site. Harjula and Lehto [4] suggested a precise definition of ion-exchange as ''Ion-exchange is the equivalent exchange of ions between two or 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*''. (The italics are especially outlined by us). This statement defines what is referred to in this review as ''simple ion-exchange''. When considering chelation exchange involving metal cations only, it is generally accepted that a *coordinate bond* is formed between the metal cation and the group on the surface during the exchange process. Hence, this could be considered as a different type of sorption mechanism where the separation is controlled by the thermodynamics and kinetics of metal complex formation and dissociation. Chelation exchange invariably involves an ion-exchange process in conjunction with the formation of a coordinate bond, so, strictly, should be called ''chelation ion-exchange''. However, to use the terms ''simple ion-exchange'' and ''chelation ion-exchange'' is rather cumbersome and, from now on, will be referred to as ''ion-exchange'' and ''chelation exchange'', respectively.

Accepting the above definitions of the sorption mechanisms, some important differences between ion-exchange and chelation exchange become apparent. With ion-exchange, the alkali metal ions, although having a weaker affinity for the ion-exchange site than the polyvalent ion, will have a major competitive effect as the concentration of the alkali metal increases. Competition from the hydrogen ions will be even weaker, not becoming significant until the pH is quite low. The exception to this will be if the ion-exchange site is a weak acid anion, when the hydrogen ion competition will be much stronger. With chelation exchange, the alkali metals form such extremely weak coordinate bonds that they can be neglected in comparison to the polyvalent metal ion coordinate bond. Consequently, the effect of alkali metal concentration will be small. On the other hand, the hydrogen ion concentration will have a major effect on the formation of the polyvalent metal complex. This is because most chelating ligands are conjugate bases of weak acid groups and, accordingly, will have a very strong affinity for hydrogen ions. The pH, therefore, will be a dominant factor in the separation of metal ions by chelation as it will determine the values of the conditional stability constants of the metal complexes on the surface of the substrate. Another important difference concerns the selectivity relating to retention order. For metal cations of the same charge, except the alkali metals, the retention order for chelation exchange is, in general, the reverse of simple cation exchange. This can be seen in Fig. 1, which shows a comparison of the change in distribution coefficients and stability constants for the lanthanide series. It should be noted that the increase in stability of the lanthanide–iminodiacetic acid complexes from La(III) to Lu(III) covers about two orders of magnitude in terms of K_1, whereas the decrease in distribution coefficients (K_d) obtained for sulphonated polystyrene–divinylbenzene resin is about three orders of magnitude. That is the main reason for the use of gradient elution techniques in the separation of lanthanides by ion-exchange. More subtle changes in retention order between selected metals can also be obtained by

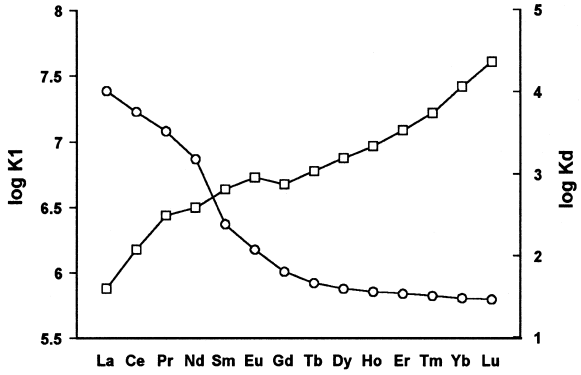


Fig. 1. Formation constants for chelates of lanthanides and Y^{3+} ions with iminodiacetic acid (from ref. [70]) and corresponding distribution coefficients for the cation exchanger Dowex 1-X4 in a nitric acid–methanol mixture (calculated from the data of Table LC 91 in ref. [75]).

careful choice of ligating atom in the chelating group. Steric effects involving bulky complexing groups can also further modify the retention order for certain metal species. Two more factors add to the complexity of chelating ion-exchange and, if clearly understood, can give further flexibility to the control of metal ion separations. The first factor is the use of complexing or chelating agents in the eluent. In ion-exchange, the use of complexing agents in the eluent, as mentioned earlier, is mainly for the control of the charge on the metal ions. For chelating ion-exchange, the presence of a complexing agent in the mobile phase will result in competition between the chelating group on the surface of the substrate and the chelating group in the mobile phase. When considering the large range of chelating agents available, the number of possible combinations is truly very large and this factor must produce the biggest scope of all the parameters controlling separation. The second factor, giving rise to more complex interactions, is the presence of more than one sorption mechanism on the substrate. Pure sorption mechanisms are very rare in chromatography and chelation exchange is no exception. The vast majority of chelating compounds contain weak acid groups whose conjugate bases form coordinate bonds with the metal cations. These groups, of course, can act as ion-exchange sites in their own right and are commonly known as weak acid cation exchangers. Furthermore, nitrogen-containing ligands will become protonated at low pH values, giving the possibility of anion exchange if the metal species in the mobile phase is in the form of an anionic complex. Mixed mode separations are therefore possible, where some metals will be influenced by both simple and chelation exchange. Nevertheless, the careful choice of pH and ionic strength can dictate which is the more dominant sorption mechanism. For example, the use of high concentrations of alkali metal salts in the eluent will suppress ion-exchange effects. On the other hand, a decrease in pH will suppress chelation exchange by lowering the conditional stability constants. These key parameters controlling separation in chelating ion-exchange will be discussed later in Section 4, together with specific examples. Before this, the types of chelating groups and phases available will be described.

3. Properties of chelation-exchange substrates

It is perhaps surprising, considering its much later development, that chelation exchange was recognized and reported at about the same time as the introduction of ion-exchange on polystyrene resins. Erlenmeyer and Dahn observed in 1939 [5] that mixtures of various cations could be chromatographed on a column of powdered 8-hydroxyquinoline. Subsequently, in a review by Meinhard [6], it was suggested that the potential problem of 8-hydroxyquinoline being displaced from the column could be overcome by ''fixing'' the complexing agent irreversibly on another solid, such as silica. Thus, the development of covalently bonded and impregnated chelating substrates was foreseen at an early stage, if not exploited until much later. The earliest covalently bonded commercially available chelation-exchange substrate was, in fact, polystyrene-based. This was Chelex 100 (or Dowex A1), a low cross-linked polystyrene substrate containing bonded iminodiacetic acid groups (IDA). The first notable analytical achievement with this resin was the determination of trace metals in seawater by Riley and Taylor in 1968 [7]. They used the Chelex 100 resin for the batch preconcentration of trace metals followed by atomic spectrophotometric detection after acid elution. This work showed the clear

potential of chelating surfaces for the analysis of notoriously difficult matrices, such as sea water, viz., selective adsorption of transition metals in the presence of massive amounts of other metals and insensitivity to high salt concentrations. Riley and Taylor's pioneering work led to a large number of studies involving Chelex 100, as described in a review by Alfassi and Wai [8]. A considerable number of chelation-exchange substrates other than Chelex 100 have now been developed and studied using both covalent bonding and impregnation methods. Most essentially follow the same approach where separate isolation and/or preconcentration columns are used followed by a variety of analytical determination methods, either off-line or on-line [14]. These methods, however, are outside the scope of this review as analytical high-performance separations are not involved. However, for those interested in the extent and range of the chelating sorbents available, there are a number of review articles available, three of which are listed in the bibliography [9–11].

It should be pointed out at this stage that a technique called chelation ion chromatography (CIC) was described in a paper by Siriraks et al. [12]. The CIC method involved the linking of a commercially available low efficiency chelation-exchange column with a high-performance cation-exchange column. The addition of switching valves, a preconcentration pump and another isolation column made up a self-contained ion chromatography system for the preconcentration and determination of trace metals in seawater. This rather complex three-column system has since been developed and modified by several other workers, as reviewed in a paper by Mou et al. [13]. The chelation-exchange column is used as a preconcentration column only and is not involved in the analytical separation of individual metal ions. As such, CIC also does not come under the scope of this review and is mentioned to avoid confusion with HPCIC, which only requires a single column for preconcentration and separation. However, there are some publications with CIC in the title that do actually involve analytical separations on chelating ion-exchange columns. This confusion is unavoidable as new techniques tend to develop in an empirical way. It is therefore suggested that the term HPCIC is used in future publications involving analytical separations on chelation-exchange substrates.

3.1. Types and properties of chelating ion-exchange groups

The quality of chelating ion exchangers was the main restriction on the development of HPCIC in the past. As mentioned above, a wide variety of chelating solid phases have been described for preconcentration of trace metals from diluted solutions and from different samples. However, until recently, only a few applications were being extended to high-performance chelation ion chromatographic separations. There are several reasons for this, one of which is the correct choice of chelating functionality, a critical factor for the efficient functioning of the separation system. This is because the kinetics of chelation exchange were found to be slower than those of ion-exchange, so much so for some chelating groups that efficient separations are not possible. Some of the more frequently used chelating ligands are the carbamates, β-diketones, diamines, iminodiacetate and amino acids, various azo- and triphenylmethane dyes, and 8-hydroxyquinolinol. Table 1 Table 2 Table 3 Fig. 2 give the key structural, substrate and separation details for those studies involving high-efficiency metal separations.

The homogeneity of the bonded functional groups also has an important effect on peak shape. One of the most popular chelating ion-exchangers for high-performance separations of metals is 8-hydroxyquinoline bonded to silica gel or to controlled pore glass. However, the attachment of this reagent to the backbone is usually a multistep synthetic procedure [37–40], providing at least two extra resulting functional groups that have been shown to be active in the retention of metal ions [17]. A reduction in the efficiency of separation was observed due to this multisite nature [40]. The use of a mixture of *ortho*- and *para*-aminophenyltrimethoxysilane for the preparation of 2-pyridinecarboxaldehyde phenylhydrazone chelating groups (Table 1, No. 8) could also be responsible for the existence of multisite interactions and extra broadening of chromatographic peaks [24,25].

The capacity of the chelating ion-exchanger and especially connected with it, the distribution of

Table 1
Silica based chelating exchangers used for the separation of metal ions

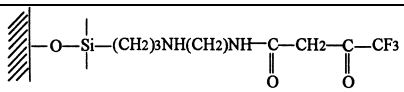
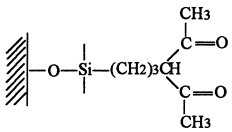
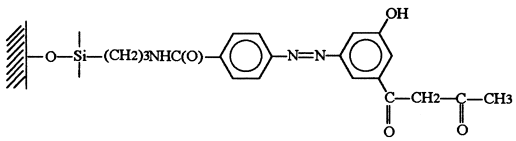
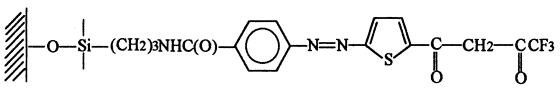
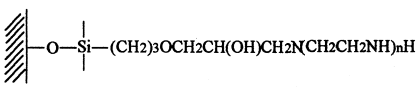
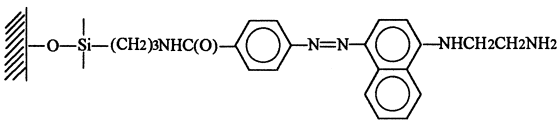
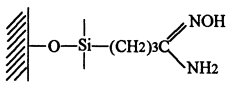
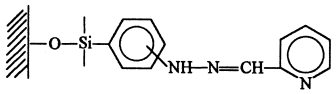
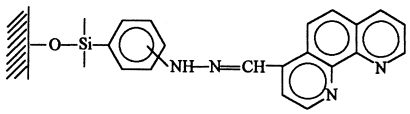
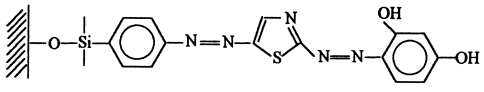
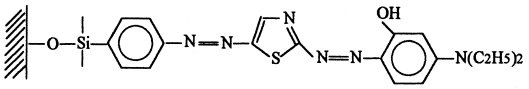
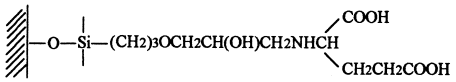
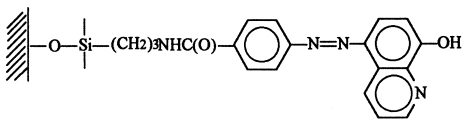
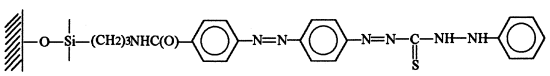
Number	Structure of bonded groups	Matrix (type, pore size, surface area, capacity)	Column and particle size	Separation	Reference
1		Baker silica gel 7	150×2 mm <40 μm	Cu(II), Zn(II), Ni(II)	[15]
2		Nucleosil Si 100-5, 10 nm, 350 m^2/g 885 μmol ligand/g	135×4.6 mm 5 μm	Co(II), Cd(II), Cu(II)	[16]
3		Adsorbosil-LC 7 nm, 480 m^2/g, 47 μmol metal/g	250×4 mm, 10 μm	Mn(II), Cd(II), Zn(II), Co(II), Ni(II), Pb(II)	[17]
4		Adsorbosil-LC, 7 nm, 480 m^2/g	250×4 mm 10 μm	Mn(II), Cd(II), Zn(II), Co(II), Ni(II), Pb(II)	[17]
5		Silochrom S-120, 40 nm, 120 m^2/g Silasorb Si300, 10 nm, 300 m^2/g 280 μmol ligand/g	300×10 mm 100–160 μm 250×4.6 mm 10 μm	Mn(II), Cd(II), Zn(II), Ni(II), Cu(II) Mg(II), Cd(II), Zn(II), Co(II)	[18] [19]
6		Adsorbosil-LC, 7 nm, 480 m^2/g 20 μmol metal/g	250×4 mm, 10 μm	Mn(II), Cd(II), Zn(II), Cu(II), Fe(II), Pb(II)	[17]
7		Silasorb Si600, 6 nm, 570 m^2/g 560 μmol ligand/g	250×4 mm, 5 μm	Cr(III), Cu(II), Fe(III), U(VI), Ca(II), Cd(II), Mn(II), Pb(II)	[20]
			62×2 mm	Ni(II), Cu(II), Fe(III), Mo(VI), Cr(III), U(VI), W(VI)	[21]
			100×3 mm 50×3 mm	Zn(II), Co(II), Ni(II), Cu(II), Hg(II), Fe(III)	[22,23]
8		Pierce CPG, 24 nm, 130 m^2/g, 25 μmol metal/g	250×4.6 mm 37–74 μm	Fe(II), Co(II), Ni(II), Cu(II)	[24]
		Chromosorb LC-6 12 nm, 400 m^2/g, 18 μmol ligand/g	250×4.6 mm 5 μm	Mn(II), Fe(II), Cd(II), Zn(II), Co(II), Pb(II), Cu(II)	[25]
9		Chromosorb LC-6 12 nm, 400 m^2/g, 39 μmol metal/g	250×4.6 mm 5 μm	Mn(II), Pb(II), Fe(II), Cd(II), Zn(II), Co(II)	[26]
10		Silasorb Si600, 6 nm, 570 m^2/g, 30 μmole metal/g	250×4.6 mm 10 μm	Mn(II), Cd(II), Pb(II), Zn(II), Co(II), Cu(II)	[27]

Table 1 (*continued*)

Number	Structure of bonded groups	Matrix (type, pore size, surface area, capacity)	Column and particle size	Separation	Reference
11		Silasorb Si600, 6 nm, 570 m^2/g	250×4.6 mm 10 μm	Mn(II), Cd(II), Pb(II), Zn(II), Co(II), Cu(II)	[27]
12		Silasorb Si300, 10 nm, 300 m^2/g 38 μmole metal/g	100×4.6 mm	Mg(II), Ca(II), Mn(II), Co(II), Cd(II), Zn(ii), Pb(II), Cu(II)	[28]
13		Silasorb Si300, 10 nm, 300 m^2/g 130 μmol ligand/g	250×4 mm, 6 μm	Rare earth elements	[29]
				Mn(II), Co(II), Cd(II), Zn(II), Ni(II), U(VI), Pb(II)	[30]
			150×3 mm	Na, K, Mg, Ca, Ba	[31]
				Fe(II), Co(II), Zn(II), Cd(II), Pb(II)	[23]
			250×3 mm	Co(II), Zn(II), Cd(II), Pb(II), Cu(II)	[32]
				Mg, Ca, Mn(II), Ba, Be, Co, Cd, Zn	[33,34]
		Nucleosil 300-7, 30 nm	100×4.6 mm 7 μm	Na, K, Mg, Ca, Sr, Ba, Mn(II), Fe(II), Co(II), Cd(II), Zn(II), Cu(II)	[35,53]
14	Iminodiacetic acid Si100, Serva	Si100, 10 nm, 300 m^2/g	250×4 mm 5 μm	Mg, Fe(II), Co(II), Cd(II), Zn(II), Pb(II), U(VI), Cu(II)	[36]
15		Porasil B, 15 nm, 180 m^2/g, 54 μmol/g	250×4 mm 37–74 μm	Mn(II), Cd(II), Pb(II), Zn(II), Co(II), Ni(II), La(III), Gd(III), Yb(III)	[37]
		Polygosil 60-10, 6 nm, 500 m^2/g, 10, 27, 46, 156 μmole/g	250×4 mm 10 μm	Mn(II), Tl(I), Zn(II), Ni(II), Co(II), Cd(II), Pb(II)	[17,38,39]
		Adsorbosil-LC 7 nm, 480 m^2/g, 40, 190 μmole/g	250×4 mm 10 μm	Mn(II), Cd(II), Pb(II), Zn(II)	[40]
16		Adsorbosil-LC, 7 nm, 480 m^2/g, 35 μmol metal/g	250×4 mm 10 μm	Mn(II), Cd(II), Zn(II), Cu(II), Fe(II), Pb(II)	[17]

bonded groups at the surface, is also a crucial factor for the improvement of efficiency and selectivity of the separation. Jezorek et al. [38] investigated a series of silica-based sorbents with different surface concentrations of bonded 8-hydroxyquinoline (from 10 to 156 μmole/g) and found that phases with 20–50 μmole/g capacity appear to be the most effective in terms of resolution and speed of separation. Jonas et al. [46] also studied the kinetics of sorption of copper(II) by resins with bonded 8-hydroxyquinoline with varying capacities. As expected, the high-capacity resins take longer to reach equilibrium. The exchange rates measured for resins of different capacities also indicate that the lower the capacity the more favourable the exchange rate. It should be noted that surface-modified substrates demonstrate the best efficiency and peak shape in chelation ion chromatography, so special synthetic schemes for the surface derivatization of substrates should be used if possible. The best distribution of chelating groups providing homogeneous sorption sites can be achieved by impregnation of appropriate substrates, widely used by Jones and co-workers (Table 3 Fig. 2) [54–61]. This approach also allows a wide range of chelating dyestuffs to be studied without the need for synthetic reactions. The dyestuffs shown in Fig. 2 can be divided into three broad classes of chelating functionality, depending

Table 2
Polymer-based chelating ion-exchangers used for the chromatographic separation of metal ions

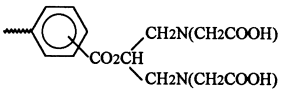
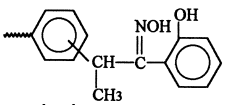
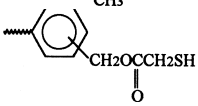
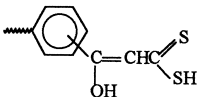
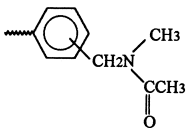
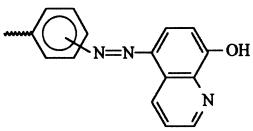
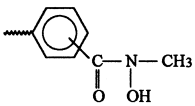
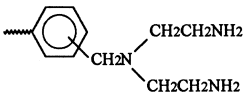
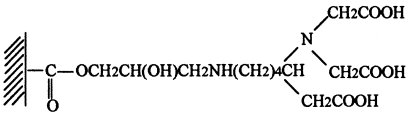
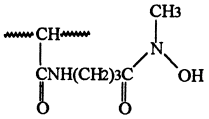
Number	Functional groups	Type of resin and capacity	Column and particle size	Separated ions	Reference
1		ST-DVB, XAD-4	28×6 mm, 45–75 μm	Cu(II), U(VI), Th(IV), Zr(IV)	[41]
2		ST-DVB, XAD-4		Cu(II), Mo(VI)	[42]
3		ST-DVB, XAD-4	100×2 mm, <75 μm	Au(III), Ag(I), Hg(II), Bi(III), Cd(II), Cu(II), Pb(II), Sn(II), Sb(III), As(V)	[43]
4		ST-DVB	50×6 mm, 70–140 μm	Ag(I), Au(III), Cd(II), Cu(II), Fe(III), Hg(II), Ni(II), Pt(IV), Zn(II)	[44]
5		ST-DVB, XAD-4		Au(III), Pt, Th(IV), Zr(IV)	[45]
6		ST–4% DVB, BN-4, gel type, 0.042–1.33 mequiv./g	250×3 mm, 7–10 μm	Mn(II), Fe(II), Co(II), Zn(II), Ni(II), Cu(II)	[46]
7		Spheron Oxine 1000, hydroxyethylmethacrylate gel, 57 ng Pd(II)/g	15×5 mm, 40–63 μm	Pd(II), Cu(II)	[47]
8		ST–DVB, XAD-4		Ti(IV), Zr(IV), Mo(VI), Fe(III), Th(IV), etc.	[48]
9		ST–2% DVB	45–75 μm	Zn(II), Ni(II), Cu(II)	[49]
10	PBE-94, Pharmacia, oligoethyleneimines	Polysaccharide, 32 μmole/ml pH unit	300×100 mm, 65–132 μm	Cd(II), Fe(II), Ni(II), Cu(II), Co(II)	[50]
11		Cross-linked glycidyl–methacrylate gel, 27 μmol Cu(II)/g	150×4.6 mm, 10 μm	Rare earth elements	[51]
12	TSK-Gel Chelate 5 PW, iminodiacetic acid	Hydrophilic polymer, 24 μmol Cu(II)/ml	75×7.5 mm, 10 μm	Mg, Ca, Sr, Ba, Mg, Mn(II), Co(II), Cd(II), Zn(II), Ni(II), U(VI), Pb(II)	[30,52]
13		8% cross-linked copolymer acrylonitrile–DVB, 0.15 nmol La/g	250×3 mm, 44–63 μm	Er(III), Eu(III), Sm(III), La(III)	[64]

Table 3
High-performance separation of metal ions on a chromatographic column (100×4.6 mm) packed with polystyrene and other substrates impregnated with chelating dyes

Number	Chelating dye	Pore diameter, specific surface area	Capacity, (μmole Zn/g)	Particle size, μm	Eluent	Separated metal ions	Reference
1	Xylenol Orange	10 nm, 414 m^2/g	10	8	1 *M* KNO_3, pH 7.7	Ba, Sr, Ca, Mg	[54,55]
					1 *M* KNO_3, pH 2.5	Cd, Pb	[54]
					Three step pH gradient from 10 to 6.5, from 6.5 to 3.5 and from 3.5 to 0.5 in 1 *M* KNO_3	Ba, Sr, Mg, Ca Mn(II), Cd, Zn, Ni, Cu	[56,57]
2	Methyl Thymol Blue	12 nm, 470 m^2/g	13	8.8	1 *M* KNO_3, pH 7.9	Ba, Sr, Ca, Mg	[58,59]
3	Phthalein Purple	12 nm, 470 m^2/g	22	8.8	1 *M* KNO_3, pH 3.7	Mn, Zn, Cd, Pb	[60]
					1 *M* KNO_3, pH 9.8	Ba, Sr, Ca	[59]
		Graphitised carbon				Mg, Ca	[65]
4	Glycine Cresol Red	12 nm, 470 m^2/g,	7	8.8	1 *M* KNO_3, pH 9.0	Ba, Sr, Ca, Mg	[60]
5	Chrome Azurol S	12 nm, 470 m^2/g	3	8.8	Two step pH gradient from 2.2 to 1.0 in 1 *M* KNO_3	Al, Ga, In, Fe(III)	[60]
					1 *M* KNO_3, pH 5.3	Mn(II), Zn, Cd	[60]
		10 nm, 414 m^2/g		8	1 *M* KNO_3, pH 4	Cd, Pb	[54]
					Two step pH gradient from 7.0 to 4.0 and from pH 4 to 1.5 in 1 *M* KNO_3	Mg, Cd, Pb, Cu	[54]
6	Calmagite	12 nm, 470 m^2/g	5	8.8	1 *M* KNO_3, pH 5.2	Mn(II), Cd, Zn, Pb	[60]
7	2-(3-Sulphobenzoyl) pyridine-2-pyridylhydrazone	12 nm, 470 m^2/g	1	8.8	1 *M* KNO_3, pH 3.6	Mn(II), Pb, Cd, Zn	[60]
8	PAR	Bimodal resin, 1000 m^2/g	140 (Cu)	25	0.5 *M* KNO_3, pH 10, with 0.05 *M* lactic acid	Ba, Sr, Ca, Mg	[61]
9	*N*-Dodecyl iminodiacetic acid	Develosil ODS-5	No data	5	Tartrate buffer, pH 5.5	Ba, Sr, Ca, Mg	[62]

on the ligating atoms involved. These are O,N (Nos. 1, 2, 3, 5 and 7), O,O (No. 4) and N,N (No. 6). Differences in retention order are observed, for example, O,O chelators give a reversed order for zinc and cadmium compared to O,N-containing ones and N,N chelators give a reversed order for lead and zinc compared to O,N- or O,O-containing ones. Very large differences in selectivity coefficients are also observed between different compound types with the same type of ligating atoms, such as the O,N chelators, phthalein purple and PAR (Nos. 5 and 8). However, it is interesting to note that closely related dyestuffs containing the same chelating group, such as Xylenol Orange and Methylthymol Blue, both of which contain iminodiacetic acid groups linked to a triphenylmethane nucleus, can produce surprisingly large differences in selectivity coefficients for certain metals. These aspects are discussed in more detail in reference [60].

The possibility of multistep interactions as well as secondary acid–base- and conformation equilibria are also possible for chelating ion-exchangers having polydentate functional groups. The practice and experience of chelation ion chromatography has supported this hypothesis and two- and three dentate ligands, such as 8-hydroxyquinoline and IDA seem to provide the better separation as a rule. Ligands of higher denticity are likely to have much slower kinetics of dissociation because of the number of bonds involved with the metal. The chelating groups should also have a broad spectrum of chelating action and have no special selectivity to one or two separate metals. The stability of complexes formed at the surface is defined by the composition and pH of the eluent. This can be described in terms of the conditional stability constants. Conditional stability constants decrease with pH and it appears that relatively low conditional constants are necessary for short retention times and reasonably narrow peak shapes. This can be explained by the fact that the kinetics of metal complex dissociation increase with decreasing pH. The actual value of the conditional constant at a particular pH will of course depend upon the value of the metal complex formation

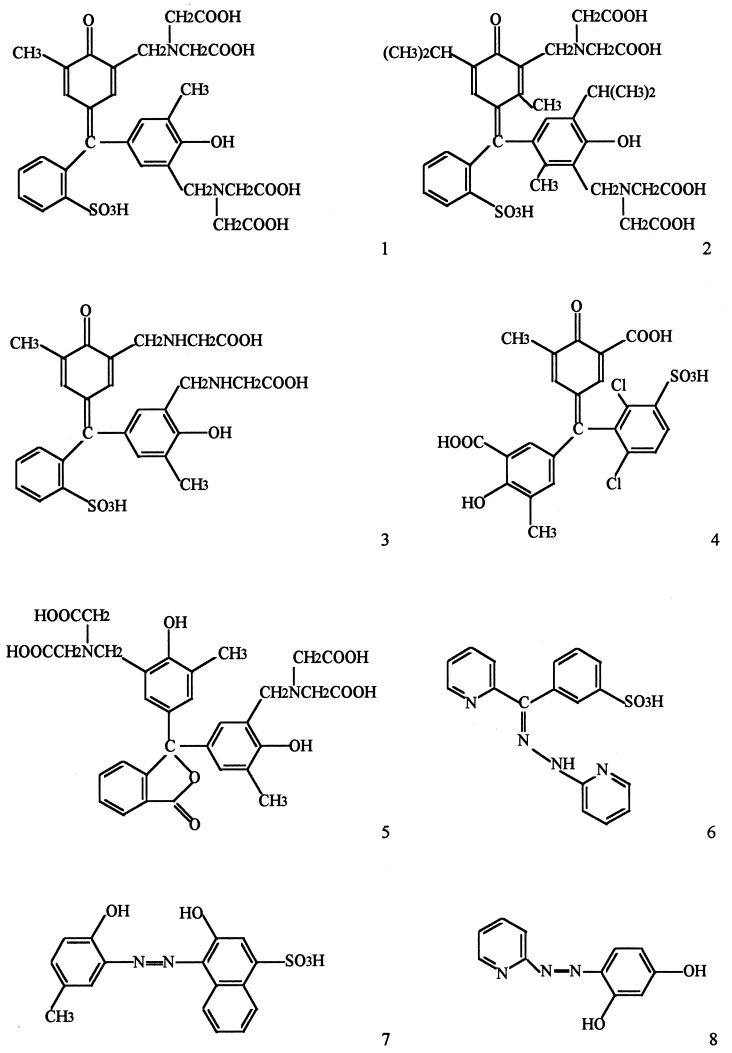


Fig. 2. Chemical structures of (1) Xylenol Orange, (2) Methyl Thymol Blue, (3) Glycine Cresol Red, (4) Chrome Azurol S, (5) Phthalein Purple, (6) 2-(3-sulphobenzoyl)pyridine 2-pyridylhydrazone, (7) Calmagite and (8) 4-(2-pyridylazo)resorcinol (PAR).

constant. Thus, lower pH values will be required for metal complexes with very high formation constants. At the moment, it is not clear what strength of chelating group gives the most efficient separations; i.e. strong chelates at low pH or weak chelates at higher pH. More investigations need to be carried out in this area before any conclusions can be made.

Apart from covalently bound and permanent impregnation techniques of forming a chelating surface, there is a third approach that, as yet, has been little studied. This is dynamic modification of the substrate where the chelating compound is added to the mobile phase and the substrate initially has no chelating surface. An equilibrium becomes established between a sorbed monolayer of chelating compound on the substrate and the chelating compound in the mobile phase. This is dynamic modification, because if the eluent is changed to one without the chelating compound, the sorbed layer would eventually be leached off. The separation of metal ions appears to be mainly controlled by the chelating groups on the substrate's surface, as the

dynamically sorbed layer could be considered much higher in concentration than the amount in the mobile phase. Paull et al. [65] developed a dynamically coated porous glassy carbon column using *o*-cresolphthalein complexone in the mobile phase. There are two interesting features in this work. The first is that the concentration of the chelating compound in the sorbed layer can be controlled by changing mobile phase conditions such as solvent polarity. Secondly, since the chelating compound chosen was also a colorimetric reagent for the metals of interest, it acted as the detection system as well. Perhaps less clear cut was the work reported by Elchuck et al. [66] using mandelic acid in the mobile phase. It clearly was not the authors' intention to form a chelating surface, but the indications from the order of retention of the metals and the response to pH changes points to a sorbed layer of mandelic acid. However, more work will be required to definitely establish if dynamic modification is occurring.

3.2. Matrix effects

Chelating ligands can be immobilized by simple adsorption forces onto a variety of substrates such as silica [62,63] and styrene–divinylbenzene resin [54–61], or anion-exchangers [74], but the bulk of the chelating phases studied to date involve the covalent attachment of ligand either directly, or via an organic spacer, to the solid matrix. The two most frequently used substrates are styrene–divinylbenzene copolymer and silica gel, although ligands have been bound to a number of other synthetic polymers and materials such as polymethacrylate and graphitized carbon (Tables 1 and 2). Although silica-bonded chelating agents exhibit favourable kinetics and high efficiency [29,30], they are unstable at high pH values, so the use of a silica backbone for the attachment of chelating ligands working in alkaline solution is not possible. Organic polymers on the other hand are more resistant to conditions of high pH. These resins are also suitable for impregnation with chelating dyestuffs, which is the alternative to covalent bonding (Table 3). The impregnated substrates, once conditioned, are very stable and produce very little if any ''bleed''. However, the capacity will decrease if the pore size is greater than 10 nm. To achieve a high-efficiency for both covalently bound and impregnated resins, it is necessary to use a monodisperse fine particle of mechanically stable material. There are two types of poly(styrene–divinylbenzene) copolymer matrices that are most widely used. The macroporous beads are more rigid and do not swell in organic solvents. Until now, most chromatographers have worked with gel-type polystyrene-based substrates with relatively low degrees of cross-linking (not higher than 12%). Recently, higher cross-linked polystyrene resins (degree of cross-linking of more than 50%) have become available from Hamilton, Polymer Laboratories and Purolite. The very high surface areas of some of these resins show potential for increasing the capacity of chelating surfaces. A macronet hypercross-linked resin from Purolite has been investigated for impregnation by chelating dyes [61] and the results do show an increased capacity compared to lower cross-linked resins. Unfortunately, resin was only obtainable in large particle size, but it was found to be relatively easy to crush down to 10 μm because of the very rigid highly cross-linked structure. Although reasonable separations were achieved, efficiencies should be much higher when spherical small particles are obtainable.

4. Elution parameters and influence of chelation and ion-exchange on the retention and separation of metal ions

The majority of chelating ion-exchangers have charged or ionizable groups, so both ion-exchange and chelation mechanisms can occur, depending on the elution parameters. The retention of metal ions in a chromatographic system based on ion-exchange and chelation-exchange will be affected by a number of factors, such as the concentration of complexing agent, pH of the eluent, ionic strength, column temperature, concentration of organic solvent and others. Varying these elution parameters will produce differing effects on the chelation and ion-exchange ability of chelating ion-exchangers and can change the mechanism of separation and selectivity. The presence of strong complexing eluents usually requires low pH values where ion-exchange is the dominant separation mechanism, so little or no

chelation exchange is produced. It would therefore be useful to consider the effects of the above-mentioned elution parameters only for the situation where non- or weakly complexing eluents are involved.

4.1. Influence of temperature on the retention of metal cations and on the selectivity of separation

Changing the temperature to modify separations is not widely used in traditional ion chromatography. However, increasing the temperature of a chromatographic column can improve the selectivity of separation and increases the efficiency. The dependence of retention ($\ln k'$) on the column's temperature can be expressed by the following equation [67]:

$$\ln k' = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \varphi \quad (1)$$

where k' = capacity factor, ΔH° = enthalpy of sorption, ΔS° = entropy and φ = phase ratio. In accordance with Eq. (1), a plot of $\ln k'$ against the reciprocal of the absolute temperature should be linear and, for negative values of ΔH° (exothermic process), the retention of ion will decrease with increasing column temperature and vice versa. According to data obtained by Fortier and Fritz [67] for ''pure'' ion-exchange, the ΔH° values for alkaline earth and transition metal ions do not exceed 3 kJ mol^{-1}. Thus, where only ion-exchange is involved, the temperature effect on retention time is relatively small. At the moment, not many studies involving temperature changes have been carried out on chelating ion-exchangers. However, one very recent investigation concerning a glutamate-based chelating exchanger showed that ΔH° values were much higher for chelation than for ion-exchange [28]. Other, more empirical studies also show that temperature has a significant effect on retention in chelation exchange [63,68]. Considering other thermodynamic factors, it is possible that the entropy change (ΔS) in chelation reactions may have a more important bearing on temperature effects than ΔH. The so-called ''chelate effect'' is usually put forward as an explanation for the much higher stability constants found for complexes containing polydentate ligands compared to monodentate ligands [69]. The ''chelate effect'' concerns a large positive change in entropy on formation of a chelate complex, which means that the change of Gibbs free energy (ΔG) with temperature will be negative. Thus, as the temperature increases the resulting (ΔG) will become even more negative and so the equilibrium constant will become bigger. If this is the more dominant thermodynamic process, then retention times will always increase with temperature.

$$-\Delta G = -\Delta H + \Delta ST \quad (2)$$

The increase in column temperature can therefore be a useful parameter for controlling separations using chelating substrates. It was successfully used for the HPCIC separation of transition- and rare earth metal ions [28,29]. It should be noted that in one study involving the presence of a relatively strong complexing agent in the eluent, the increase in the column temperature of the eluent did not produce a significant change in the selectivity of separation [52]. This could be explained by the fact that strong chelation effects both in solution and on the surface of the substrate cancelled each other out. However, an improvement of peak shape was observed in all cases due to better kinetics of chelation and a decrease in viscosity of the eluent. Clearly, more studies need to be carried out as temperature is likely to be an important parameter in controlling retention and selectivity in chelation exchange.

4.2. Influence of organic solvent

The small addition of organic solvents to the eluent changes its dielectric constant and can influence secondary equilibria in the chromatographic system, such as solvation of metal ions, conformation mobility of attached chelating ligands and solubility of metal complexes in the mobile phase if organic complexing reagents are present in the eluent. There have been few studies in this area. However, one study on the retention of metal cations on an amino acid-type chelating ion-exchanger in the presence of 5–20% (v/v) acetonitrile or isopropanol at different temperatures did not show a significant change in the retention mechanism or in the selectivity of separation for most of the cations in question, except copper (II) [28]. Nevertheless, an improvement in peak shapes for some metal ions was

observed. More studies are required, but only on bonded phases, as impregnated resins will lose their coating in the presence of significant amounts of organic solvents.

4.3. Ionic strength

The ionic strength of the eluent greatly affects the retention of ions in the case of ion-exchange chromatography. However, if a charged functional group is able to form a surface complex with metal ions, the effect of ionic strength will be much less. An apparent large change in retention time with ionic strength, which is occasionally observed on chelating substrates, is probably due to the occurrence of significant ion exchange. This could be the case when the capacity of chelating groups is high or highly charged metal ions such as the lanthanides are being separated. Nevertheless, chelation exchange is not totally insensitive to ionic strength. The concentration of the background electrolyte must have an effect on the activities of the ions and, therefore, on the stability constants. In homogeneous solution, stability constants at first decrease with increasing ionic strength and increase again as the ionic strength approaches three [70]. Interestingly, the stability constants at an ionic strength (I) of three are not very different from an I equal to zero. However, the effect of ionic strength on the stability of metal complexes on the surface of chelating substrates will not necessarily be the same as in homogeneous solution. The change in retention time with increased salt concentration on chelating surfaces will be expected to be more complex, particularly if residual ion-exchange is present. The increased complexity of ionic strength effects on chelating ion-exchange resins has been illustrated by Saldadze and Kopylova-Valova [10], who compared three chelating functional groups with different charges. For the carboxylic acid chelating resin, KB-4 (Reakhim, Moscow, Russian Federation), having negatively charged functional groups, the total sorption or distribution coefficients (K_d) of metal ions decreased with increasing ionic strength. For the anion-exchange chelating resin, AN-31 (Reakhim), having positively charged polyethylenepolyamine functional groups, the increase in the ionic strength of the solution lead to an increase in the K_d. Finally, for the ampholyte chelating resin, ANKB-2 (copolymer of 2,5-methylvinylpyridine and divinylbenzene, also from Reakhim), having picolinic acid functional groups, the ionic strength of the solution does not play a significant role. Fig. 3 demonstrates these three different effects of ionic strength on the capacity of the corresponding resins for copper (II). These curves clearly show that there is no major decease in capacity at very high ionic strengths. The relatively large change in the K_d curves for copper with the KB-4 and AN-31 resins at lower ionic strengths is almost certainly due to ion-exchange effects. Put more simply, an increase in ionic strength will increase the ratio of chelation-to-ion-exchange and, at very high salt concentrations, ion-exchange will become very small or insignificant. It is this relatively insensitive response to high ionic strength that is the main property exploited for the preconcentration and batch separation of trace metals from complex matrices using Chelex 100 and the many other chelating substrates developed since [71]. Although the variation in the ratio of ion exchange-to-chelation exchange is not particularly important for these batch isolation procedures, it is very important for HPCIC. Studies on a chelating substrate involving glutamate groups bonded to silica gel clearly show the variation in selectivity that can be obtained by controlling the proportion of chelating to ion exchange [28]. Workers studying im-

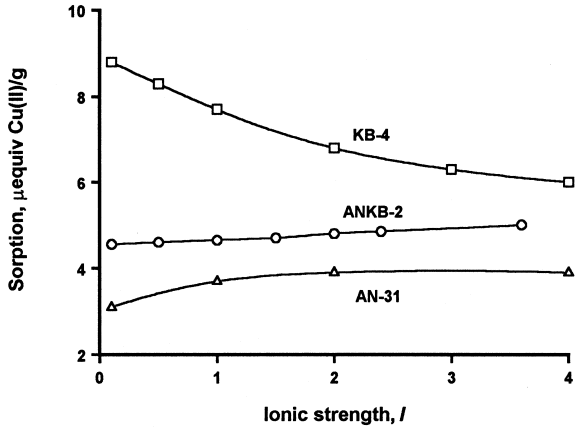


Fig. 3. Influence of ionic strength on the sorption of copper(II) by the chelating cation-exchanger, KB-4, the chelating ampholyte, ANKB-2, and the chelating anion-exchanger, AN-31. Derived from data contained in ref. [10].

pregnated dyestuffs, on the other hand, prefer to ''swamp'' ion-exchange effects using a high ionic strength eluent, producing essentially ''pure'' chelating ion exchange (Table 3). This is mainly because most of the chelating dyestuffs investigated contain the sulphonate group, which acts as a strong acid cation exchanger. If not suppressed, sulphonate groups will cause elution problems at low pH, as they will not be easily neutralized by protonation.

The most effective way to increase the ionic strength of the eluent is by adding common electrolytes. These salts should be reasonably pure and should have a good solubility in aqueous solution, low complexing ability towards analytes and should not produce any non-soluble products in the column. Alkali metals and ammonium salts, such as nitrates, chlorides and perchlorates are suitable for this purpose [56]. The optimum ionic strength of the eluent for use in HPCIC, where chelating ion exchange is required mainly, is between 0.5 and 1.0 *M*. It corresponds to a concentration of 0.5–1.0 *M* of a salt formed by monovalent cation and monovalent anion in the eluent. A further increase in the salt concentration may significantly increase the viscosity of the eluent and lead to a decrease in the efficiency of separation [29]. However, an increase in the column's temperature can minimize the negative effect of increased viscosity of the eluent.

4.4. Effect of eluent pH

Undoubtedly, pH is the most important of the elution parameters controlling the separation of metal ions in chelation exchange. In the absence of hydrolysis and complexed forms of metal ions other than the stable hydrated form, increased retention of the metal cations is usually observed on increasing the pH of the eluent. The dissociation of acid groups on the immobilised chelating ligand produces a sharp increase in the conditional stability constants of surface metal complexes. The dissociation of weak acid groups will also cause an increase in the ion-exchange capacity, depending on the values of the acid dissociation constant, K_a. This is especially so for the widely used classes of chelating exchangers, such as bonded iminodiacetic acid, amino acids, aminopolycarboxylic acids and immobilised phthalein complexones. Normally, for a typical isocratic separation, the pH is chosen to give capacity factors that do not exceed ten to fifteen. Taking into consideration that the conditional stability constants need to be fairly low to achieve this, it can be supposed that both chelation and ion-exchange impact on the retention of metal ions. The proportion of these factors controlling the retention of metals by the chelating cation-exchanger will be defined by the ratio of the K_a values of the functional groups and the conditional stability constants of the corresponding complexes.

The presence of a relatively weak complexing agent in the eluent, such as tartaric or malonic acid, would be expected to have the opposite effect and reduce the capacity factors as the pH is raised. This is because the complexing acid in the eluent would reduce the positive charge on the metal ions and also compete against the chelating group on the surface [30]. However, if the chelating exchanger is based on a strong complexing ligand, as is normally the case, then as the pH is raised higher and higher, the chelating-exchange process will eventually become the dominant mechanism. Depending on the exact choice of chelating exchanger and complexing acid in the eluent, a plot of retention time against pH would be expected to first show a decrease, reach a minimum and then start to increase again, as can be seen in Fig. 4 ([76]). The lack of a minimum for copper and nickel could be explained by the fact that these metals are still strongly complexed by the picolinic acid eluent, even in the low pH range, thus minimising ion-exchange effects.

A more straightforward effect of an increase in pH on retention will take place when any ion-exchange sorption is ''swamped'' or ''suppressed'' by using a high ionic strength eluent, as discussed in the previous section. A steady increase in retention will occur over the whole pH range. The presence of a complexing acid in the eluent will still have an influence, but only to slow the rate of increase of retention, i.e. the slope of log k' against pH will be lower [72]. For chelating-anion exchangers, representing a majority of all known chelating sorbents (among them 8-hydroxyquinoline, polyamines, amidoxime, hydrazones, etc.), the increase in the pH of the eluent means that retention occurs only through chelation on the surface. The retention and

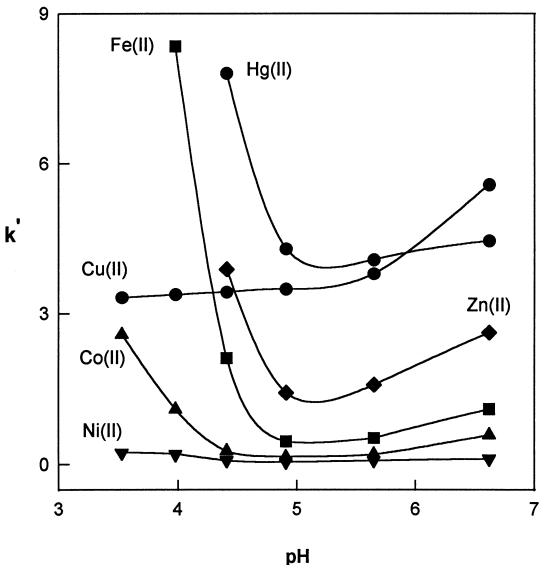


Fig. 4. Influence of the pH of the eluent on the retention of some transition metal ions. Column, iminodiacetic acid bonded to silica (250×4 mm I.D., particle size 6 μm); eluent, 0.01 *M* picolinic acid; flow-rate, 1 ml/min. From ref. [76].

selectivity of separation is defined solely by the values of the stability constants.

One aspect tends to be overlooked for certain substrates and that is the secondary equilibria connected with ionisation of some groups at the surface of the matrix of the chelating exchanger (e.g. silanol groups at the silica surface). This possibility should be taken into consideration when evaluating the separation of metal ions.

5. Typical separation modes

The previous section emphasises the increased complexity of the separation process when chelation exchange is involved. This is particularly so if a mixed mode, i.e. both simple and chelation exchange, is present. In our opinion, some publications that indicate that only an ion-exchange process is operating actually have a significant degree of chelation exchange taking place. The converse is also true in cases where a new chelating substrate is being studied, but the system is operated at a pH where mainly ion exchange takes place. Each new substrate will need to be characterised over a wide pH range, with different groups of metals, to fully evaluate the separation performance. Both isocratic and gradient elution can be used, but each will have its limitations. The following examples serve to illustrate the main types of separations that can be achieved for different sets of elution parameters.

5.1. Isocratic separations

Normally, isocratic separations on chelating substrates are limited to a relatively small number of metal cations. The main reason for this is that the conditional stability constants, and, therefore, the capacity factors, show large differences between individual metal cations. The capacity ''window'' is usually restricted to a maximum k' of between ten and fifteen, depending on the efficiency of the column. Another reason for the restriction to small groups of metal ions is that a significant decrease in plate number occurs with retention time. It is considered that this effect is due to the slower kinetics of metal complex dissociation as the conditional stability constants increase. Both of the above considerations apply to the situation where chelating exchange is the dominant sorption process. If a significant proportion of ion-exchange is present, this will allow a greater number of metals to be separated isocratically due to the more favourable kinetics.

The actual group of metals separated isocratically will depend on the pH, ionic strength and concentration of complexing agent, if present, in the eluent. For O,O and O,N chelating ligands, covering the majority of studies, the metals most frequently encountered fall naturally into four main groups; the alkaline earth metals at high pH, the dipositive transition and heavy metals at intermediate pH, the tripositive metal ions at low pH, and U(VI) and Th(IV) at very low pH values. This does not cover all of the metals of course and there will be some overlap between the groups and possibly different orders of elution, depending on the substrate chosen and the eluent composition. The chosen combination will also decide the actual pH required for the separation of a particular group of metal ions. In fact, the disparity in elution conditions between different base substrates with the same chelating group can be surprisingly large. A particularly striking example is the difference between silica gel with bonded IDA

groups and polystyrene resin impregnated with a dyestuff containing IDA groups [30]. The silica gel substrate shows apparently much stronger stability constants than the impregnated resin and, therefore, requires a much lower pH for the same kind of separation. The comparison is shown in Fig. 5, where the difference in pH for the two separations is about 1.5–2.0. The only conclusion that can be drawn is that the different bonding and steric environments of the two substrates affect the basicity of the IDA groups and, hence, produce differing stability constants. The silica gel substrate shows a higher efficiency due its hydrophilic nature and smaller particle size. However, the difference in efficiency is not quite as large as it looks, as the silica gel column is two and a half times longer than the polystyrene column. The better efficiency obtained on hydrophilic-based substrates seems generally to be true, as studies on a polymethacrylate-based resin also gave better separations compared to those on polystyrene [30,52,54,55].

The best example of a complex isocratic separation is for the lanthanides and yttrium and is shown in Fig. 6 (ref [29]). It fully exploits the better efficiency of the silica gel bonded IDA substrate and the temperature and ionic strength was optimised to achieve the best resolution. The fact that so many metal ions can be separated isocratically is a rather special case, the reason being the relative closeness of the stability constants of the lanthanides compared to other groups of metals. Ion-exchange sorption was strongly suppressed for this separation, but there was evidence that a small proportion was still present, as it is very difficult to completely eliminate ionic attraction involving +3 ions. Up to now, the lanthanides have always been separated on ion-exchange columns and require gradient elution to avoid extremely long retention times. As far as we are aware, this is the first example of an isocratic separation of the lanthanides since the first published separation 50 years ago. A similar study on a hydrophilic polymer-based chelating exchanger by Inoue et al. [51] also gave very good separations, but a small gradient programme was still required.

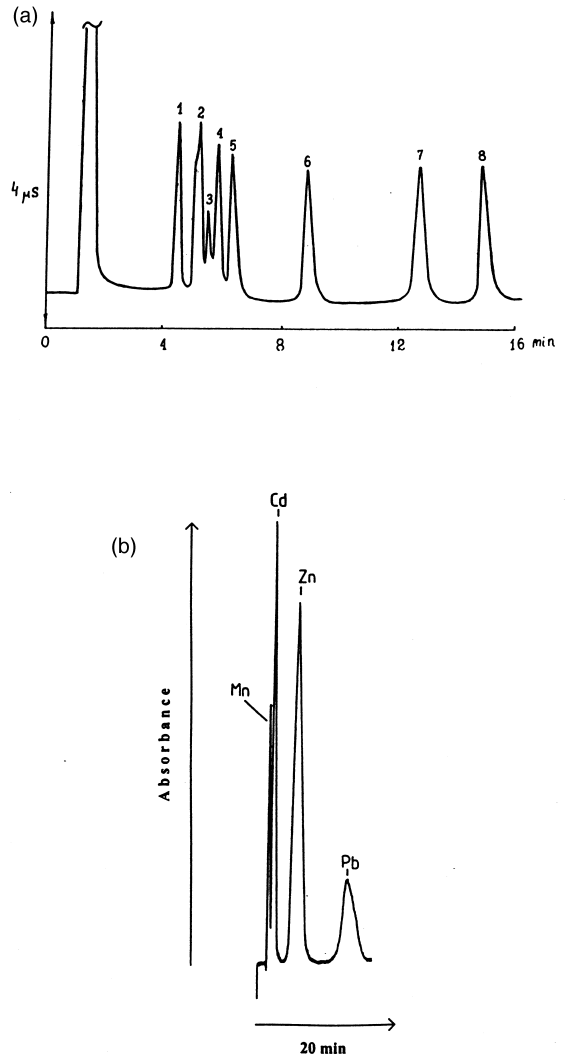


Fig. 5. Separation of alkaline earth, transition and heavy metals under isocratic conditions using non-complexing eluent. (a) Column, iminodiacetic acid bonded to silica (250×3 mm I.D.); eluent 7 m*M* nitric acid; flow-rate, 1 ml/min; indirect conductivity detection. Peaks: (1) Mg, (2) Ca, Sr (3) Mn, (4) Ba, (5) Be, (6) Co, (7) Cd and (8) Zn. (From ref. [34]). (b) Chromatogram showing the separation of Mn(II), Cd(II), Zn(II) and Pb(II) at pH 3.7 in 1 *M* KNO_3 using a Phthalein Purple-impregnated column. Spectrophotometric detection was at 540 nm after post-column reaction with PAR. From ref. [60].

5.2. Gradient elution

The special property of retaining a wide range of metal ions at high pH in the presence of high ionic strength eluents is a very important property that emphasizes the fundamental difference between chelating- and ion exchange. The pH can be raised to

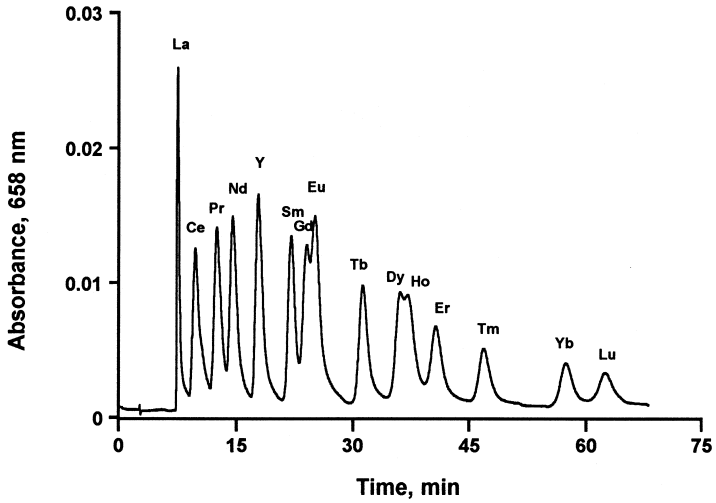


Fig. 6. Isocratic separation of a standard mixture of fourteen lanthanides and yttrium. Column, iminodiacetic acid bonded to silica; eluent, 0.5 *M* KNO_3 in $1.6 \cdot 10^{-2}$ *M* HNO_3; flow-rate, 1.0 ml/min; column temperature, 65°C, sample volume, 20 μl; sample concentration, 4 mg/l of each metal in 0.2% HNO_3. Detection was at 658 nm after post-column reaction with Arsenazo III. (From ref. [29]).

incorporate a particular group of metals and then the pH can be dropped to carry out a gradient separation. Thus, a greater number of metals can be determined in one run. The efficiency of separation is also considerably improved, as the later peaks ''sharpen'' in the falling pH gradient. Another very important operation can also be carried out with a gradient. If the pH is raised higher than is necessary for the gradient separation, so that the metals of interest are strongly retained, then preconcentration will occur. After preconcentration, where large volumes of sample can be treated, if necessary, a reduction in the pH will produce a gradient separation in the normal way. Thus, both preconcentration and separation can be carried out on a single column. Although a pH gradient is normally used, this can be combined with a change of ionic strength and/or complexing agent in the eluent to control the selectivity of separation. Two examples serve to illustrate the aforementioned factors (Fig. 7). In the first example (Fig. 7a), a multistep pH gradient is used to separate nine metal species on a chelating dye-impregnated resin [56]. The ionic strength is kept high and constant throughout and little if any ion-exchange sorption is present. The separation efficiency is much better for the later-eluting peaks due to the compression effect of the gradient (cf. Fig. 5b). In the second example (Fig. 7b), a pH step gradient is used as before, but this time, a more complex gradient programme was required [73]. The study involved the design of a gradient programme for the determination of trace metals in sea water after preconcentration. However, there was a problem with the elution order of cadmium and zinc. With a simple step pH gradient, large amounts of zinc masked the small, later-eluting, cadmium peak. Therefore, the gradient needed to be modified to reverse the retention order. This was achieved by reducing the ionic strength after the first step and using different complexing acids in the subsequent steps. Undoubtedly, significant ion-exchange was involved in the later steps. This last example shows the exploitation of all the main elution parameters, except temperature and organic solvent, to achieve the desired separation, i.e., pH, ionic strength and the type of complexing acid in the mobile phase. This example shows that as long as the principles of chelation separation are appreciated, a great deal of versatility in selectivity control can be obtained.

5.3. Internal pH gradients

The ability of chelating ion exchangers to bind transition metal ions is strongly dependent on the pH

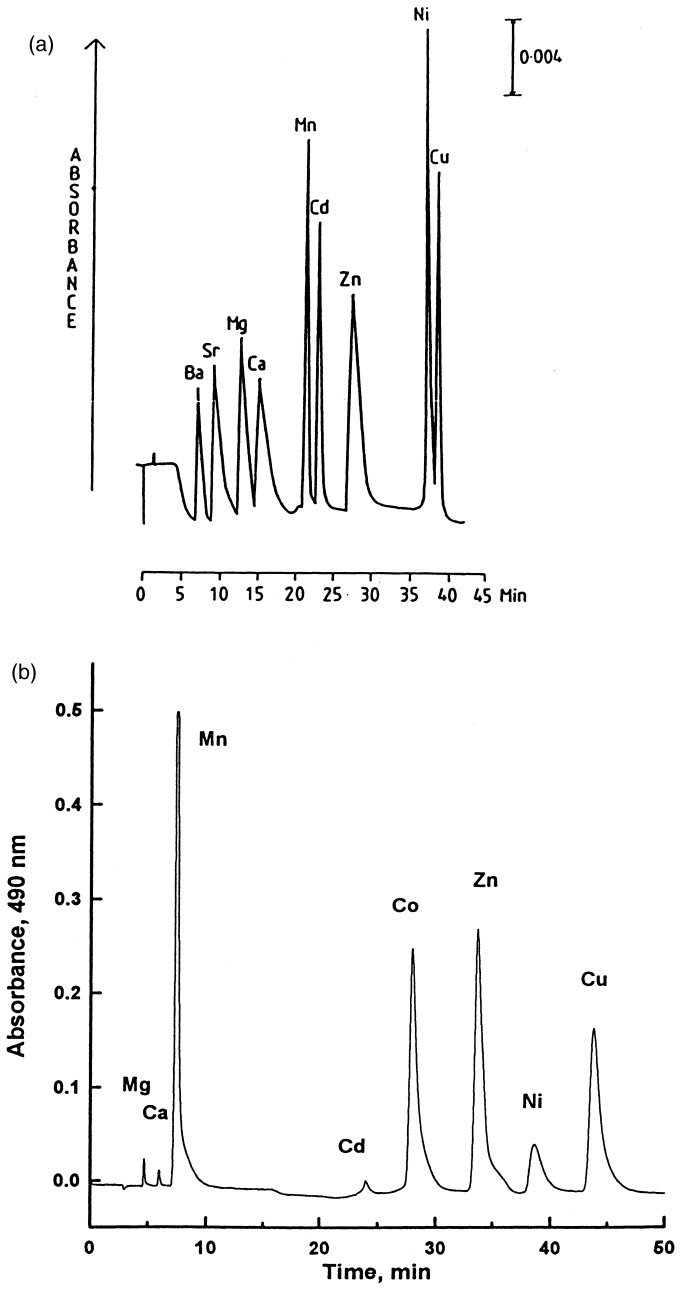


Fig. 7. Gradient separation of transition metal ions. (a) Chromatogram showing the separation of nine metals using a three-step gradient. The column (100×4.6 mm) was packed with a Xylenol Orange-coated PLRP-S dye. Sample conditions were direct injection of 100 μl of 1 *M* KNO_3 containing 0.05 *M* lactic acid, adjusted to pH 10, spiked with the following concentrations of metal ions: 5 mg/l Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+} and Cu^{2+}, 10 mg/l Cd^{2+} and Zn^{2+} and 20 mg/l Ba^{2+} and Sr^{2+}. (From ref. [56]). (b) Chromatogram of metals in estuarine water. Column, iminodiacetic acid bonded to silica (250×4 mm I.D., particle size 6 μm). Three step gradient: 0–10 min, 0.5 *M* KCl–$5 \cdot 10^{-4}$ *M* HNO_3; 10–30 min, 0.08 *M* tartaric acid and 30–50 min, 0.01 *M* picolinic acid; flow-rate, 0.8 ml/min; detection, 490 nm, 0.2 a.u.f.s.; post-column reaction with PAR–NH_4OH–HNO_3 reagent; Sample volume, 1 ml. (From ref. [73]).

of the eluent, so the use of an external pH gradient, as has been shown above, is the most useful means for separating a complex mixture of metal ions [51,54,56,57,60]. A different and novel way of producing a gradient was proposed by Nesterenko and Ivanov [50], involving the formation of an internal pH gradient in a chromatographic column that was packed with a chelating weak anion exchanger. The sorbent was bonded with an oligoethyleneimine functional group, which has a variety of primary and secondary amino groups with different pK_a values. Because of this, these weak ion-exchange groups will produce a constant buffer capacity over a wide range of pH values. These groups also have chelating properties and, under neutral pH conditions, can produce quantitative sorption and preconcentration of trace metals. After a preconcentration step on the column, which was initially equilibrated with an appropriate buffer at a predetermined higher pH, a linear pH gradient can be formed by flushing the column with a polybuffer ampholyte adjusted to the lower limit of the required pH range. Due to the buffering effect of the column and the polybuffer eluent, a linear pH gradient forms, moving at a much more slower rate than that of the mobile phase. A very efficient separation was obtained, based on the consecutive dissociation of surface metal complexes and elution in accordance with their stability constants (Fig. 8; [19]). The main features of this technique are closely related to the chromatofocusing of proteins, producing a high degree of resolution, column loading ability and selectivity of separation. A complete separation of a mixture of Cu(II), Co(II) and Ni(II) on a column packed with a polysaccharide-based weak anion exchanger, PBE-94 (Table 2, No.10), was obtained [50]. A more efficient separation was achieved on silica with a covalently attached tetraethylenepentamine group as a polybuffer ion-exchanger (Table 1, No. 5) [18,19]. The absence of a theory related to the formation of internal pH gradients involving a mixture of simple components, the possibility of complexation of the polyampholyte with separated metal ions and the difficulty of establishing the relative effect of ion-exchange interactions between metal ions in complexed form and protonated amino groups are the main factors that have restricted the development of this method. Nevertheless, this approach shows a particular potential for very high resolution separations of selected groups of metal ions, which may be difficult to separate by external pH gradients.

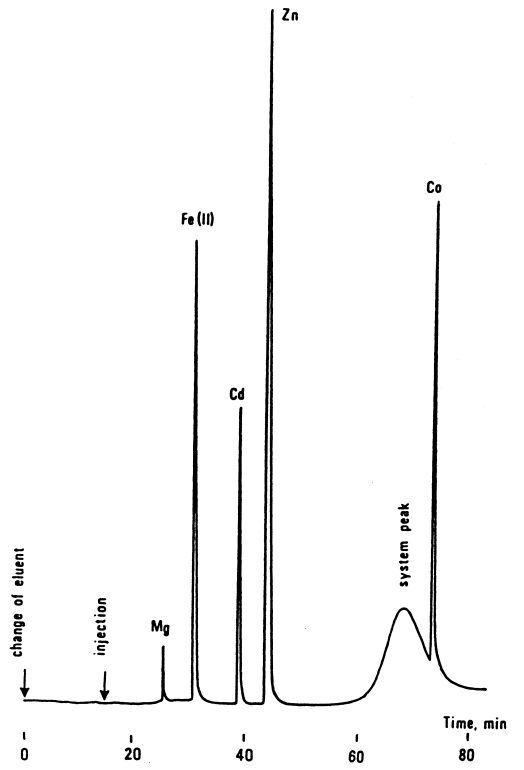


Fig. 8. Chromatogram of a model mixture of metals using an isoconductive pH gradient. Column, 250×4.6 mm I.D. tetraethylenepentamine bonded to silica, particle size 10 μm; capacity, 0.28 mmol/g. Starting buffer, 0.004 *M* histidine (pH 7.6); eluent, Polybuffer 74 (pH 3.4) diluted 1:50 with deionized water; flow-rate, 1 ml/min; spectrophotometric detection was at 540 nm after post-column reaction with PAR. (From ref. [19]).

6. Applications

Since the development of high-performance chelation substrates is relatively new, the number of applications is not large. However, the applications so far, shown in Table 4, clearly indicate the potential of HPCIC for the determination of trace metals in complex samples. Determinations in very high ionic strength samples of alkali metal salts are relatively straightforward, as only trace metals are

Table 4
Application of HPCIC to the determination of metal ions in complex samples

Sample	Chelating ion-exchanger	Determined metals	Concentration found (mg/l)	Reference
Offshore oil-well brines	Table 3, 2	Ba in the presence of $2\cdot10^3$ fold Ca, Sr and $1.6\cdot10^4$ fold Mg	1 Ba 8 Sr	[58]
Mineral water	Table 3, 2	Ba	0.09–0.84	[59]
	Table 1, 13	Mg, Ca	18.2–179 Mg, 11.1–572 Ca	[31]
Milk powder	Table 3, 3	Sr in the presence of 4×10^3 fold Ca	0.15	[59]
KCl and NaCl brines	Table 3, 1	Mg, Ca, Sr, Ba, Mn, Zn, Ni, Cu	0.5 Mg, Mn; 1 Cu, Zn; 2 Ca, Ni, Ba, 4 Sr	[56]
Coastal sea water, ref. material CASS-2	Table 3, 1	Mn, Zn, Pb, Ni, Cu	In agreement with certified data	[56,57]
Coastal sea water	[a]	Mg, Ca		[62]
	Table 1, 7 and 1, 13	Co, Zn, Ni, Cu, Mn, Ca, Cd, Pb	Around 0–0.0005	[23]
Estuarine water	Table 3, 1	Mn, Zn, Pb, Ni, Cu	0.147–0.357 Zn, 0.0009–0.0019 Pb, 0.0024–0.0037 Ni, 0.0034–0.0041 Cu	[56,57]
Sea water	Table 2, 12	Mg, Ca	1170–1330 Mg, 385–436 Ca	[52]
	Table 3, 5	Al	0.006	[60]
	Table 3, 3	Mg, Ca		[65]
	Table 2, 13	Rare earth metals	0.0025	[64]
Laboratory chemicals	Table 3, 1	Mn, Zn, Pb, Ni, Cu		[56]
Fresh water, ref. material IAEA/W-4	Table 3, 2	Ba, Sr	In agreement with certified data	[59]
Tomato leaves, National Bureau of Standards ref. material	Table 1, 8	Mn, Fe, Zn	In agreement with certified data	[25]
Vitamin tablets, NBS ref. material	Table 1, 8	Mn, Fe, Zn, Cu	In agreement with certified data	[25]

[a] Octadecylsilica coated with N-dodecyl iminodiacetic acid was used.

present as impurities. Alkaline earth metals, transition- and selected heavy metals can be determined in up to 5 *M* concentrations [56,74]. Sea water continues to be of major interest. Although the ionic strength is lower, it is still relatively high and has the added complication of large concentrations of magnesium and calcium. As regards the determination of magnesium and calcium themselves, because of their high levels, they can be determined isocratically on diluted samples [56,57,65]. For trace metals in seawater, preconcentration is almost certainly needed and gradient conditions need to take into account the potential competitive effect of magnesium and calcium. Exploiting the large differences in stability constants on IDA-based chelates, a starting pH can be chosen where the trace metals are preconcentrated, while allowing magnesium and calcium to go straight through unadsorbed (Fig. 9, [57]**)**. Using a weaker chelating group, aluminium can be preconcentrated at a relatively low pH, while most of the common transition and heavy metals will be unretained, except iron(III) [60]. Other examples show another important aspect of chelating-exchange separations, where retention order can be adapted to allow the determination of trace metals in the presence of massive amounts of other, closely eluting, metals. Traces of barium and strontium are very difficult to determine in the presence of large

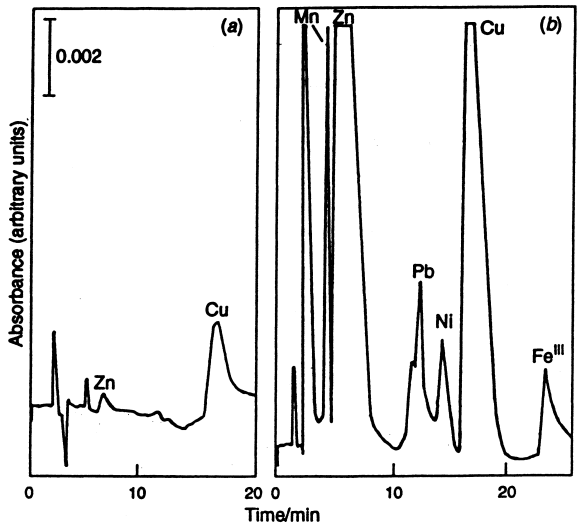


Fig. 9. Chromatogram showing the preconcentration and separation of transition metals in Carnon estuarine water. (a) Procedural blank, (b) sample. Column, 100×4.6 mm, Xylenol Orange-coated polystyrene–divinylbenzene resin PLRPS, 10 μm. Preconcentration was at pH 6.0 followed by a step gradient of HNO_3 in the presence 0.5 *M* KNO_3 and 0.05 *M* lactic acid; photometric detection was at 490 nm after post-column reaction with PAR. The preconcentration volume of seawater was 6 ml. (From ref. [57]).

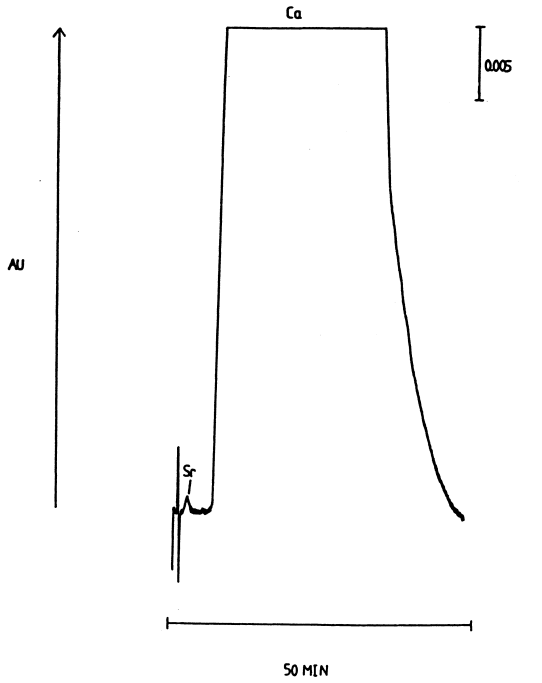


Fig. 10. Chromatogram showing the separation of strontium from calcium in milk powder digest on a Phthalein Purple-impregnated column (100×4.6 mm I.D.). Sample, 100 mm^3 injection. Eluent, 1 *M* KNO_3 with 0.05 *M* lactic acid (pH 10.2). Post-column reagent, $2\cdot10^{-4}$ *M* ZnEDTA, $1.2\cdot10^{-4}$ *M* PAR and 2 *M* NH_3. (From ref. [59]).

amounts of magnesium and calcium by simple cation exchange, as they would be swamped by the tails of the eluting magnesium and calcium peaks. However, there is no problem using chelating exchange, as barium and strontium elute first [59]. Strontium in milk powder is a particularly good example of this (Fig. 10) and there is no reason why the strontium cannot be isolated by fraction collection for isotope studies. The determination of selected transition metals in tomato leaves and vitamin tablets showed good agreement with certified values.

A reasonable question can be raised concerning the availability of commercial chromatographic packings and columns for HPCIC. Most of the chelating substrates considered in this review are laboratory-made. The single exception is the newly developed Dionex CS12A column that is packed with a mixed function carboxylic/phosphonic acid cation exchanger [77]. Although chelation exchange is not mentioned in the published study on this resin, the effect of an increased proportion of phosphonic acid groups on the retention order of manganese clearly indicates that chelation is taking place. However, there are several other high-performance commercially available columns containing chelating groups that are sold for different purposes, usually for use in other modes of liquid chromatography such as ligand-exchange chromatography, chromatofocusing of biological macromolecules and affinity chromatography. IDA and 8-hydroxyquinoline functionalised substrates for high-performance chromatographic separations are available from Serva (Heidelberg, Germany) and TosoHaas (Stuttgart, Germany). Different amino acid functionalised substrates frequently used for the separation of enantiomers are produced by many companies (Serva, Daicel, Sumitomo, etc.). The Mono P column (Pharmacia, Uppsala, Sweden), packed with polyamine functionalised silica, is widely used for high-performance chromatofocusing. Some of these columns may be suitable for HPCIC. The TosoHaas column, Chelate5PW, for example, has been studied, but the

lifetime was found to be quite short under the conditions used for metal separations [54].

7. Conclusions

Traditionally, chromatographers have used chelating or complexing reagents only as additives in the eluent to increase the speed and selectivity of the separation of metal ions in ion chromatography. The multiple attempts to achieve efficient and selective separation using immobilised chelating ligands has encountered the problem of obtaining a ''pure'' chelating effect. A new look, based on the correct realisation of the chelating- and ion-exchange properties of substrates through optimisation of ionic strength, temperature and pH of the eluent, as presented in this review, allows one not only to solve this problem, but also to achieve some unique selectivities in the preconcentration and separation of trace metal ions and to perform difficult analyses of samples having complex matrices.

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