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Review

Liquid chromatography: a tool for the analysis of metal species

Corrado Sarzanini*

Department of Analytical Chemistry, University of Turin, Via P. Giuria 5, 10125 Turin, Italy

Abstract

An overview is presented of classic and more recent applications of liquid chromatography for the analysis of metal species. The different approaches involving ion-exchange, ion-pair, and chelation separation mechanisms are discussed as well as the new philosophy of simply removing interferents before specific detections of metal ions (alkali and alakaline earths, rare earths, heavy and transition metals). New more selective materials enabling difficult separations and studies on multimodal or hyphenated techniques for metal speciation (e.g. arsenic and chromium) are considered.

Keywords: Reviews; Speciation; Inorganic cations; Metals

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1. Introduction

Due to a strong environmental impact, trace metal ion determination and speciation have received particular attention in the last years. Metal speciation refers to the identification and quantitation of organometallic, chelated or free metal ion forms or its oxidation states in a particular sample. Liquid chromatography has become one of the main powerful analytical tools for these elements, and some reviews [1,2] with particular regard to ion chromatography [3] and complexation ion chromatography [4,5] summarize its potentialities.

Why high-performance liquid chromatography (HPLC) for metal analysis? The most widely used instruments (e.g. atomic absorption and inductively coupled plasma atomic emission spectrometers) used for metal determination suffer from both spectral and chemical interferences and, in some cases, are un-

*Tel.: +39-11-670-7628; fax: +39-11-670-7615.
E-mail address: sarzanini@silver.ch.unito.it (C. Sarzanini)

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suitable for direct trace analysis in complex matrices or for studies on metal speciation. HPLC has become in the last years the most flexible tool for separating different species and for removing matrix interferences, and may be coupled with different detectors enabling determinations of specific or group elements. Several devices including electrochemical detection (ED) systems and inductively coupled plasma (ICP) and graphite furnace atomic absorption (GFAA) spectrometers have been used as well as inductively coupled plasma mass spectrometry (ICP-MS) coupled with liquid chromatography [6–8].

The main approaches for metal determinations by HPLC are:

(i) ion chromatography (ion-exchange: cation, anion, ion-exclusion);

(ii) reversed-phase ion interaction chromatography;

(iii) chelation ion chromatography; and

(iv) multidimensional and multimode chromatography.

Multidimensional and multimode chromatography involve, respectively: (i) the use of two different mechanisms (e.g. ion-exchange coupled with ion-exclusion); (ii) the use of two or more columns, switching the total flux or a portion of eluate from one to the other. The multimode technique is not usually employed for liquid chromatography of metal ions, but in this field, there are some applications of gas–liquid chromatography. The multidimensional method, also called *heart cut column switching*, includes all techniques by which the direction of the flow of the mobile phase is changed by valves so that the effluent (or a portion of it) from the primary column is passed to a secondary column for a definite period of time.

Among the trace elements to be determined in environmental (e.g. natural waters, seawaters, sediments, soils), biotic and technical materials by the techniques previously mentioned, we will consider the groups selected later: (1) alkali and alkaline earths; (2) heavy and transition metals; and (3) rare earths.

Refractive index, spectrophotometric and electrochemical detectors were traditionally used in liquid chromatography, while ion chromatography (IC) introduced the use of suppressed eluent conductivity detection. The analysis of complex matrices (e.g. foods, new materials, pharmaceutical and environmental samples) and speciation studies, in the field of metal analysis, are fundamental topics and have stimulated studies for the improvement of detection sensitivity and selectivity. For instance spectrophotometric or electrochemical detection have been coupled with post-column reactions, but a new approach, *hyphenation*, concerning the coupling of *unconventional* detectors with LC, has become the emerging field of research.

2. Ion chromatography

Ion-exchange chromatography is basically a simple process: cation- or anion-exchange columns are used with eluents, which are dilute solutions of ions, to separate, respectively, cations or anions. The use of a conductometric detector allows two ion chromatographic modes: suppressed and non-suppressed. In the first mode the eluate, before detection, is driven through the suppressor unit, where the background conductivity is greatly reduced, so that the sensitivity with which sample ions can be detected is increased. Non-suppressed ion chromatography is performed without the use of a suppressor unit with ion-exchange resins of low exchange capacity and very dilute eluents, so that the background conductivity is quite low. Much of the practice of ion chromatography today is well-established science transformed into easily practised technology. The reliability of IC is often taken for granted and the technique has become a routine tool for process analysis and control, notably for trace analysis.

When IC was first introduced [9], column packing for cation determinations consisted of surface-sulfonated 25-μm beads of polystyrene (PS) cross-linked with 2% divinylbenzene (DVB), and they were used with packed bed suppressors. Due to the large differences in selectivity of divalent (alkaline earths) towards monovalent (alkali) cations, HCl was needed to elute the monovalent cations, while stronger divalent eluent components (e.g. *m*-phenylenediamine) were required for the elution of divalent cations. Due to the long column equilibration time between the two eluent system, it has proved difficult to provide simple isocratic elution to allow the separation of both classes of cations in a reasonable

period of time, so that two different columns were dedicated for the two different classes of cations. Nevertheless chromatograms were characterized by quite long total run times and by poor peak efficiency. The development of new methods of synthesis for ion chromatography (latex-coated columns, IonPac CS3; Dionex), together with the replacement of *m*-phenylenediamine by the zwitterion 2,3-diaminopropionic acid monochloride [10], made it possible to simultaneously analyze both the alkali and alkaline earth elements, in the presence of ammonium, in one column and to improve peak efficiencies, although analysis still required a long time and baseline resolution was not completely achieved. A different kind of column containing carboxylate functionalities, instead of the traditional sulfonic acid with a low selectivity for the hydronium ion, the so-called *Schomburg column*, has been introduced by Kolla et al. [11]. This stationary phase, based on a poly(butadiene–maleic acid) (PBMA) copolymer silica gel coated, coupled with eluents containing slightly acidic complexing agents (e.g. tartaric acid), was used to separate Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} in non-suppressed IC. The unique selectivity of the carboxylic groups, and the competition of the chelating agent in the eluent with the cation exchange sites for divalent cations only, provided good separation and short analysis times, the only limitation being the operative pH range imposed by the silica material. Poly-(butadiene-maleic acid)-coated silica columns with mineral acid eluents have also been used in a suppressor-based system by Nair and co-workers [12]. The performance of PBMA silica columns for separation of alkali, alkaline earths and heavy metal ions (Cu, Zn, Co, Fe(II)) has been studied in the presence of different organic complexing agents (α-hydroxyisobutyric, tartaric, citric, oxalic, pyridine-2,6-dicarboxylic acid and EDTA) and applied to determine cations in water, food, ore-cinder and sole brine samples [13].

Other ion-exchange columns containing sulphonic functionalities used in a *column switching mode* (IonPac Fast Cation I and II) and columns at decreased cation-exchange site density (IonPac CS10) were introduced for the simultaneous determination of alkali and alkaline earth elements. Considering the experimental data available for the separation of these cations, the best separations were achieved through the use of polymer-based columns containing carboxylic (IonPac CS12, CS14) [14,15] or carboxylic and phosphonic (IonPac CS12A) [16,17], or carboxylic/phosphonic/crown ether (IonPac CS15) [18] functionalities.

Since the development of new materials for mono- and divalent cations, expert systems for the planning of appropriate dilutions, suitable detector output ranges and standard additions, were developed for alkali and alkaline earth metals in mineral waters [19]. Moreover, applications of the optimized systems (CS12 and methane sulfonic eluents) for the determination of Na^+, K^+, Mg^{2+}, Ca^{2+} in terrestrial waters [20] and in melted snow samples from high-alpine sites [21] have been shown.

Satisfactory results for the determination of Na^+, K^+, Mg^{2+}, Ca^{2+} in a well water sample were also obtained using a IC Pak CM/D (Waters) column with an eluent containing citric and pyridine-2,6-dicarboxylic acids [22]. Tartaric and dipicolinic acids have also been used for mono- and divalent cations [23]. Simultaneous separation and determination of Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} in environmental samples were obtained by Ohta et al. [24] by coupling nitric acid and pyridine-2,6-dicarboxylic acid with a selective complexation of divalent cations.

A different kind of weak cation exchanger has been synthesized by copolymerization of vinyl groups covalently bound on silica surfaces with acrylic acid and used as stationary phase in presence of 18-crown-6 ether and acetonitrile in the eluent for the separation of alkali and alkaline earth elements [25].

In addition to cation- and anion-exchange chromatography, Tanaka et al. [26] have shown that it was possible to determine simultaneously the weakly ionized aliphatic carboxylic acids and mono- and divalent cations by an ion-exclusion–cation-exchange mechanism when an anion-exclusion column packed with polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form (TSKgel OA-PAK) was used with a strong acid eluent (H_2SO_4). More recently [27], they have developed an anion-exclusion method in non-suppressed IC of mono- and divalent cations, establishing a simple technique to be applied in acid rain, river, underground, lake

and forest soil waters. The simultaneous separation of Na^+, NH_4^+, K^+ and Mg^{2+}, Ca^{2+} has been obtained with an anion-exclusion chromatographic column packed with polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form (TSKgel OA-PAK-A 300×7.8 mm). An eluent containing 0.75 m*M* sulfuric acid, 2 m*M* tartaric acid, 7.5% methanol has allowed the separation of mono- and divalent cations in about 25 min.

As an alternative way to simultaneously determine mono- and divalent cations, a column switching (dual column) technique was reported [28]. Samples are eluted through two separate columns, each optimized for the chromatography of one group of cations. Separation is accomplished by switching the eluent flowpaths form one column to the other. This method has several advantages compared to gradient techniques (e.g. shorter run times, no equilibration period between runs). Nevertheless, this technique requires sophisticated instrumentation and sensitivity for divalent cations is poor. Column switching techniques between latex-based IonPac CG3 and CS10 columns have been used by Betti et al. [29] to determine alkali and alkaline-earth elements in sea water. Transition and heavy metals have been determined in sea water samples by multidimensional liquid chromatography [30]. The separation was achieved by coupling a dynamically coated sorbent column, a preconcentrator and a chelating column. The metal concentrations (Cu, Ni, Co, Mg, Ca, Sr, Fe and Pb, Zn, Mn, Cd) in the sea water sample were 5.0–50 μg/l.

As discussed in depth, samples containing alkali metals, alkaline earth cations and the ammonium ion are difficult to analyze. Environmental samples, at low levels of ammonium in matrices with a high concentration of sodium, are a typical case. This is mainly due to the similar selectivities of ammonium and sodium ions for the common stationary phases containing sulphonate or carboxylate cation-exchange functional groups. This problem has been solved by a column-switching technique which enables the determination of trace concentrations of the common inorganic cations (Li, Na, K, Mg, Ca) and ammonium in the presence of large concentrations of either sodium or ammonium [31].

It must be mentioned that a great number of papers, referring to column switching or multidimensional liquid chromatography, concern methods not actually using two chromatographic columns, but simply a short column (preconcentrator) coupled with an analytical column.

The column switching technique has also been used in IC to solve the column overloading problems encountered when difficult samples have to be analyzed. In this case, matrix elimination is effected mechanically [32] rather than chemically via sample pretreatment. The technique can use two four-way valves inserted before and after a pre-column (e.g. guard column, separator column, or an entirely different type of column from the separator column such as a reversed-phase). Configuring the valves in such a way that the bulk of the matrix is diverted to waste and only a heart-cut of the analyte of interest is transferred to the separator column, the system is effective in either eliminating or at least simplifying sample preparation [33].

The main practical problems in real sample analysis are related with sample handling and include sample collection, dissolution, clean-up, trace enrichment and, as previously described, matrix elimination. Sample clean-up can be performed off-line, prior to chromatographic analysis, or on-line, incorporated in the chromatographic hardware. Among the clean-up procedures, those using ion-exchange mechanisms can be used to reduce the alkalinity or the acidity of a sample [34] by using a high capacity cation-exchange resin in the H^+ form or a high capacity anion-exchange resin in the OH^- form, respectively. Alternatively, electrochemical devices can be used [35]. Similar procedures can be designed to suit different sample types, by varying the form of the resin used to achieve an alternative chemical modification of the sample. For example, cation-exchange resins in the Ag^+ form have extensively been used to precipitate chloride from drinking water samples [36,37]. Cation-exchange resins in the Ba^{2+} form have also been developed to remove sulfate from aqueous solutions [38]. The clean-up resins are often commercially available as disposable cartridges, offering a rapid and versatile sample pretreatment.

With regard to metal ions, ion-exchange is performed by using both cation and anion exchangers. Due to their charge density leading to high affinity for the separator column, in the cation-exchange technique the metal ions are normally reacted with an anion of a weak acid to reduce their charge

density in the eluent solution before entering the separation column. The separation occurs according to their respective affinities towards the active sites of the separating resin. Ligands are also required to avoid precipitation when an acidic eluent is not suitable for the columns selected. A low-capacity silica-based cation-exchange column was used for the separation of transition metals (Co, Cu, Fe) and coupled with post-column chemiluminescence detection to enhance sensitivity [39]. In this case attention has been paid to eluent composition not only to improve the separation, but also for its compatibility with the post-column reaction.

Ion chromatographic separation of metal ions based on anionic exchange offers the potential of different selectivity, reduced problems for metal ion hydrolysis and can be applied to complex sample matrices. Notwithstanding the fact that many organic acids (from mono-, di-, tricarboxylic acids to chelating agents such as α-hydroxyisobutyric acid, tartaric, citric, oxalic, pyridine-2,6-dicarboxylic acid, 1,2-diaminocyclohexanetetraacetic acid, diethylenetriaminopentaacetic acid) have been evaluated for the simultaneous ion-chromatography of anions, alkali, alkaline earth and heavy metals [40,41], ethylenediamminetetraacetic acid (EDTA) plays a fundamental role. EDTA has also been used to avoid metal ion interference arising from possible precipitation at the eluent pH (masking effect). Due to the negatively charged complexes formed by EDTA and divalent or trivalent metal ions, the simultaneous separation of anions from metal ions as well as speciation of metal ions is feasible. In these procedures complexes can be obtained, at proper pH values, in two ways: (i) before the chromatographic separation (pre-column complexation, complexes must be stable enough to avoid decomposition during separation or ligand must be added to the eluent); (ii) inside the chromatographic column itself. Some examples of applications of an EDTA eluent have been reported and experiments were also performed with binary eluent systems comprising EDTA, as complexing agent. For seawater samples [42], silica-based anion exchange analytical columns enhanced sensitivity and enabled detection limits of μg/l units for Mg^{2+} and Ca^{2+} to be reached with UV and conductivity detection.

Ion chromatography, as well as reversed-phase liquid chromatography, is increasingly used for the separation of lanthanide metals. As lanthanide ions have a 3+ charge, they exist in solution in a strongly hydrated state, and, in solution, they show similar properties. For this reason, classical ion-exchange techniques do not always provide satisfactory separation.

An approach to rare earth separation has been proposed by Strelow and Victor [43] who evaluated the distribution coefficients for yttrium and some relevant lanthanides between AG 50W-X4 resin and hydroxyethylenediaminetriacetate in monochloroacetate buffer solutions and the results have been applied to the quantitative separation of yttrium and neodimium from samarium and heavy lanthanides. In these conditions, the control of pH is essential because the peak positions are very sensitive to change in this parameter. Silica gel-based sulfonated form cation-exchange gel and sulfonated polystyrene gel columns were used with lactic and α-hydroxyisobutyric (HIBA) acids by Kawabata et al. [44]. Detection was performed by ICP-MS, solving the interferences due to the polyatomic ions by directly combining the ion chromatographic analyzer with the ICP-MS. The Dowex 50-X8 cationic exchange resin has been used by Fariñas et al. [45] as a tool to separate rare earth elements, as a group, in geological materials from the matrix elements, using increasing HCl concentrations (from 2 to 6 *M*). The determination of lanthanides is finally performed by ICP spectrometry.

The separation of each rare earth element can be performed either with a cation- or anion-exchange mechanism, according to the eluent composition and to the properties of the stationary phase. Cation exchange of lanthanides has been performed in pellicular, latex-agglomerated columns (IonPac CS3 [46] and CS10 [47]) in the presence of appropriate chelating agents such as HIBA. Lanthanides form complexes with HIBA that lower the affinity of the lanthanide for the cation-exchange resin. The elements are eluted according to the stability of the complexes formed, with Lu (the most stable complex with HIBA) eluting first and La (the weaker complex with HIBA) eluting later.

In the anion-exchange mechanism, lanthanides have been predominantly separated using a mixed bed column (IonPac CS5 [46,48–51] and more recently CS5A) containing both anion and cation-exchange sites. Different complexing agents (e.g.

lactic, oxalic, diglycolic acids) can be used in a gradient mode, as single components or in a mixture, to obtain a baseline resolution among each rare earth element. When such a mixed bed column is used, it can be shown that the retention mechanism (anion or cation exchange) is a function of the complexing agent concentration [51].

3. Chelation ion chromatography

Solid-phase extraction [52,53] is commonly used for matrix removal and preconcentration of analytes and different resins have been designed to selectively interact with metal ions according to different partitioning mechanisms (ion-exchange, chelation, reversed-phase via hydrophobic ligands specific for metal species).

Chelation ion chromatography (CIC) or, more correctly, high-performance chelation ion chromatography (HPCIC), is based on the use of high-performance substrates for trace metal separation and determination. Chelation chromatography involves both an ion-exchange process and the formation of coordinate bonds. Two main approaches can be followed to obtain proper stationary phases: (i) chemical bonding of the chelating group to the substrate; (ii) coating of a substrate with a ligand which is permanently trapped onto the substrate.

The different kinds of stationary phases, available on the market or *laboratory made*, have recently been reviewed [5] and they are silica- or polymer-based materials with chelating agents grafted onto the surface. Alternatively, chelating ligands can be immobilized by adsorption onto a styrene–divinylbenzene copolymer, silica gel or other synthetic polymers. For the separation of metal ions, these pre-coated columns use an aqueous mobile phase at a relatively high concentration of an inorganic salt, e.g. 0.5–1.0 *M* KNO_3. Voloschik et al. [54] used a silica gel-based sorbent with chemically bonded amidoxime functional groups for the selective determination of metals in waters (Mn, Cd, Co, Cu, Ni, Pb, Zn and Hg) with oxalic and dipicolinic acids as eluent. The amidoxime NH_2 groups are responsible both for the sorbent's anion exchange and for its complexing ability. Due to the weak affinity of this resin for Mg and Ca and for the strong affinity to Fe it was possible to eliminate their interference in the determination. The separation of mono- and divalent cations on a PBMA stationary phase coated on silica materials has been performed with different eluents, and a detailed study on this material [55] enabled the determination of some transition metals by conductivity detection.

More recently, a porous graphitic carbon reversed-phase column has been used with a mobile phase containing a selective metallochromic ligand for the separation of alkaline earth metals [12] and a polymeric reversed-phase column with a mobile phase containing Methylthymol Blue for the separation of Mn, Zn, Cd and Pb [56]. Since the ligand is a part of the eluent we can say that, in analogy with the dynamic ion-exchange, a dynamic chelating chromatography has been developed. For HPCIC the acting separation mechanisms, as a function of pH, ionic strength and organic modifier content of the eluent, are *chelation* and *ion exchange*.

For samples of high ionic strength, such as seawaters or concentrated brines, the selectivity of the chromatographic separation can be enhanced by chelating chemically bonded phases. Complexation reactions in the stationary phase, ion-exchange due to free or protonated chelating groups which act as ion-exchange sites, and, in some cases, complexation in the eluent, are responsible for the separation.

The most widely used resin to preconcentrate and to separate elements and groups of elements in seawater, applied for the first time almost three decades ago [57], is an iminodiacetate resin (i.e. Chelex-100). Since this resin was successfully used in batch analysis for the determination of many metals in complex matrices, it was packed in a column form and eluent forced through at 1–2 ml/min. Experimental results showed that only partial recoveries of some metal ions could be obtained under these conditions. Despite the attempts to improve metal retention on Chelex-100 resin, it was finally concluded that the low recovery of metal ions was due to a physical degradation of the resin under pressure because of the low degree of cross-linking of the polystyrene–divinylbenzene supporting polymer.

A more highly cross-linked macroporous PS–DVB resin containing the iminodiacetate functional group that allows operation at high pressure without

physical degradation (MetPac CC-1 column, Dionex) has been more recently developed [58].

The alternative approach to ligands chemically bound to silica or polymer phases refers to a technique, applied for a long time in metal ion preconcentration and matrix removal before spectroscopic determination, based on permanently loading the sorbent with the chelating agent [59–62]. Jones and co-workers widely investigated this field in order to improve detectability of both alkaline-earth and heavy metals in brine samples. Barium separation and determination at mg/l concentration in 1600 mg/l Ca samples with a neutral hydrophobic resin (PS–DVB) precedingly impregnated with Methylthymol Blue {3,3′-bis[*N*,*N*-di(carboxymethyl)aminomethyl]thymol - sulphonephthalein} coupled with an acid elution (lactic acid and KNO_3) and UV detection by post-column reaction (4-(2-pyridylazo)-resorcinol+ZnEDTA) [63] was successfully achieved showing a detection limit of 3 μg/l for Ba and Sr. As well as this, Sr and Ca separation was optimized in milk powder analysis with Phthalein Purple (*o*-cresolphthalein-3′,3′-bis-methyleneiminodiacetic acid) impregnated column [64]. Similar studies devoted to transition metal determination [65–67] resulted in an interesting procedure using a 10-μm particle size, 100-Å pore size PS–DVB resin impregnated with Xylenol Orange, stable from pH 0.5 to 11.5 [68]. The stationary phase enables Ca and Mg removal during the sample on-column preconcentration at pH 6 and the separation and determination of Zn, Pb, Ni and Cu with a step-gradient pH elution. The only drawback of the method is Cd–Mn coelution, but satisfactory results are obtained for a certified seawater sample.

Generally, small variations in chelating ability among dyes can result in different selectivity and, therefore, can be very useful allowing specific separations [69], as detailed by a recent paper considering dye impregnated chelating columns (10 chelating dyes, mainly based on triphenylmethane or azo-based dyes) for preconcentration and separation of alkaline earth, transition and heavy metals at trace levels [69]. Finally Haddad and co-workers developed *dynamic chelating chromatography* [56] by studying a procedure in which a polymeric reversed-phase was dynamically coated with the Methylthymol Blue (MTB) ligand. The new system, compared to MTB pre-coated chelating columns [70], allows the separation of transition and heavy metals. pH and MTB concentration are the key parameters for the retention and the method is suitable for samples of high ionic strength (1.0 *M* NaCl).

4. Reversed-phase liquid chromatography

This technique has been widely used for the separation of neutral or weakly charged metal complexes, but the more extensive applications are based on the ion pairing mechanism, so related procedures will be detailed hereafter.

Analysis for trace metals proceeds through formation of metal chelates and their further separation by reversed-phase liquid chromatography on C_{18} columns. Mobile phases employed are organic-based. Dithiocarbamates are the most frequently reported complexing agents due to the strong chelating ability of their sulphur atoms and their ability to form water-insoluble metal salts with all metals except sodium and other alkali and alkaline earth metals.

The behavior of diethyldithiocarbamate (DEDTC) complexes of Cu, Co, Cr, Ni and Hg with a variety of columns and mobile phases has been detailed by Dilli et al. [71]. In this case DEDTC complexes were formed off-column (60°C, 15 min), extracted into chloroform and finally dissolved in CH_3OH and injected for the separation onto a C_{18} column (μBondapak, Waters). The study pointed out that, for low concentrations of chelates, the ligand is required in the mobile phase in order to avoid their dissociation.

Azo dyes have also been considered suitable for the chromatographic separation of metal chelates on a reversed-phase RP-18 column. By introducing a tetraalkylammonium salt into the system, selectivity of the chromatographic determination was improved in the separation of V(V) at trace levels in natural waters [72]. Interferences of other metal ions (e.g. Fe, Al) were avoided throughout the determination.

Another ligand extensively used for the separation of metal ions by HPLC is 8-quinolinol (HQ). Metal ion complexation is the basis for chromatographic retention. Complexation usually occurs by heating the sample in presence of HQ. One or two steps of

extraction with an eluent-compatible solvent are required before injection of the complexes into the chromatographic system. As an example, the simultaneous determination of Mo(VI), V(V), Cu(II) and Fe(III) at the ppb level in sea water can be mentioned [73].

Reversed-phase liquid chromatography in aqueous media has been used for rare earth separation and determination. Saraswati et al. [74] used 4-(2-thiazolylazo)resorcinol as a chelating reagent in the reversed-phase HPLC separation of transition metals from the rare earth elements in low-alloy steels by increasing the concentration of the eluent (octane-1-sulphonate-tartaric acid). Several applications dealing with determinations of rare earth elements by dynamic ion-exchange in reversed-phase columns are present in current literature and, due to their affinity to the ion interaction mechanism, they will be discussed in a separate paragraph.

5. Ion interaction chromatography

Ion interaction chromatography (IIC), also known as soap chromatography, ion pair chromatography and dynamic ion-exchange chromatography, is a typical example of a chromatographic process based on secondary equilibria. Eluents containing an ion interaction reagent (IIR) (e.g. alkylammonium salts, alkyl sulphates or alkylsulphonates) are used and stationary phases (conventional RP or polymers) are dynamically modified into low-capacity ion exchangers.

Another approach to IIC is the use of common reversed-phase stationary phases permanently coated with suitable hydrophobic agents such as alkylsulphonates or alkylsulphates with a sufficiently long alkyl group. Recent studies on the chromatographic behaviour of metal ions in IIC when the stationary phase is modified with alkanesulphonate reagents (one to 10 carbon atoms in the alkyl chain) show that very good resolution is achieved, even if in the ion-interaction mode the number of theoretical plates of the column is lower than that obtained in the reversed-phase mode [75].

Elution of cations is mainly achieved by complexation with a ligand, so the conditional formation constants for the solutes are of prime importance. The main approaches involve metal complex formation by adding the ligand to the sample or in situ complexation by reaction of metal ions with the ligand added to the eluent. Ligands such as citric, tartaric, oxalic and α-hydroxyisobutyric acids are suitable, with each of this species showing increased complexation as the eluent pH is raised until ionization of the ligand is complete. Beyond this pH value, further increases of this parameter do not significantly alter retention times. Since pH can alter the stoichiometry of the complexes and their overall charge, resolution of chelates is greatly dependent on eluent acidity. Therefore, research in this field is devoted to evaluate the nature and concentration of proper ligands and IIR as well as organic and ionic strength modifiers and eluent pH.

The wide number of parameters involved and governing retention reflect the great versatility of the system to manipulate selectivity and to obtain good separations even when complicated matrices have to be separated. Nevertheless, the number of variables involved turns out to be a drawback when the effect of each mobile phase parameter has to be considered during the modeling of the ion interaction mechanism, which indeed has been controversial to interpret since the introduction of ion-interaction chromatography in the early 1980s.

Besides the previously cited traditional ligands, extensive work using nitroso-naphthol sulphonates has been made by Sirén and co-worker [76,77]. Azo ligands have also proven suitable for trace metal ion determinations. The chromatographic behaviour of 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Plasmocorinth B) and its metal ion complexes has been studied in ion-pairing reactions for metal preconcentration and separation by HPLC [78] in the presence of tetrabutylammonium ion. The separation of analytes, optimized with a flow-gradient elution, was successfully applied to river water samples, enabling analyte metals to be separated from alkaline and alkaline earth elements.

The suitability of a ligand for metal ion determinations in an ion-interaction chromatographic mechanism depends on the selectivity of the ligand for the metal cations considered. An approach to select the appropriate ligand has been followed by Sarzanini et al. [79]. In this approach, the determination of the

thermodynamic and conditional stability constants of Cu^{2+}, Ni^{2+}, Fe^{3+}, Al^{3+} with cyclo-tris-7-(1-azo-8-hydroxynaphthalene-3,6-disulfonic acid (Calcion or Calcichrome) allowed selection of the proper experimental conditions for further application in the IIC separation. In this case pH was a crucial parameter for controlling selectivity and thus avoiding interferences by certain metal ions.

Metal ions have also been separated as anionic chelates with pyridylazosulphoaminophenol derivatives [80,81]. The retention behaviour of these metal chelates in IIC has been elucidated as a function of eluent composition [82] in respect to the significant differences found in methanol–water and acetonitrile–water systems as a function of the volume fraction of water [83].

Octadecyl-bonded silica permanently coated with sodium dodecylsulphate in the presence of complexing agents was proposed for the separation of transition metals [84]. In that work it is proved that an ion-exchange mechanism similar to that of fixed-site exchangers occurs.

The separation performance of an ion separation according to an anion- or cation-exchange mechanism, both based on an ion-interaction system, has been compared by Cassidy and Sun [85]. Cetylpyridinium chloride or *n*-octanesulphonate were used to modify a reversed-phase stationary phase. The anion-exchange system provided column efficiencies comparable to that for the cation system in the separation of transition metals (Mn, Co, Ni, Cu and Zn).

The separation and the detection of metal species have been optimized by reversed-phase ion-pair procedures involving EDTA with tetraethylammonium (TEA) [86], tetrapropylammonium (TPA) [32] and tetrabutylammonium (TBA) [86–88] bromide ion-pairing agents. Techniques like pre-column derivatization without complexing agent in the eluent or on-column derivatization may be less efficient and give rise to peak broadening. The data obtained throughout experiments [86] clarify some aspects of the separation mechanism of ion-interaction chromatography for different oxidation state metal ions and confirm that the retention of divalent and trivalent metal ions complexed with EDTA takes place through an ion-exchange mechanism in which the ion exchanger is dynamically generated by the retention of the counterion in the stationary phase [88].

Studies on the separation and determination of metal ions (Cu, Co, Fe, Ni, V(V), Pd) as 5-Br-PADAP chelates (5-Br-2-pyridylazo-5-diethylaminophenol) by RP-HPLC showed that only the retention of the Co(III)-5-Br-PADAP complex is affected by variation of the concentration of the surfactant added to the eluent [89]. Stronger interactions are shown to result for tetrabutylammonium (TBA) in respect to cetyltrimethylammonium (CTA) and cetylpyridinium. An alkyl group, such as that of CTA or TBA, may interact with the C_{18} chain on the stationary phase by molecular interaction, so that the charged part of the surfactant is exposed on the surface, increasing its polarity so that the Co chelate is eluted earlier.

Dynamic ion-exchange allows the use of bonded microparticulate alkyl silicas as non-polar stationary phases with aqueous buffers containing low concentrations of hydrophobic ions for the separation of rare earth elements.

Lanthanide separation was pioneered by Cassidy [90] using gradient elution with sodium octanesulphonate as an ion-interaction reagent, which provides virtual ion-exchange sites by adsorption on a non-polar C_{18} stationary phase with α-hydroxyisobutyric acid as complexing eluting component. Quantitation is achieved by post-column reaction with arsenazo III and optical absorbance detection at 658 nm, with typical detection limits below 1 ng for each element. Alternatively, 2-(4-pyridylazo)resorcinol (PAR) can be used as post-column reagent.

In most cases lanthanides need to be precomplexed with proper ligands and then separated. The most widely used ligands are lactic [91], glycolic [92,93], HIBA [94–97], nitrilotriacetic [98] acids. In certain cases, the achievement of complete resolution among all the rare earths is difficult. Kuroda and co-workers [92,93] showed that glycolato complexes are eluted within 20–30 min, but their method suffers from incomplete resolution between Ho and Sm, Eu, Gd, Tb and Dy which coelute. The same authors showed that better resolution and separation is achieved using lactate and laurylsulphate as hydrophobic ion in the eluent [91].

Dodecylsulphate [94,99] and 1-octanesulphonate [100] have been employed in the determination of

some lanthanide elements in apatite, bastnesite and monazite samples. A coupled column chromatographic procedure based on a semi-preparative reversed-phase column and a dynamic cation-exchange column has been proposed by Lucy et al. [101]. This configuration has been applied to the determination of lanthanides in uranium matrix, due to the problem of degradation of neutron economy of nuclear reactors by impurities of rare earths. A C_{18} column and HIBA eluent has been used to remove uranium, while a C_{18} column loaded with $C_{20}SO_4$ and a HIBA gradient enabled lanthanide determination after post-column reaction with arsenazo III.

Several examples of coupling both isocratic and gradient reversed-phase separations with ICP [102–104] and ICP-MS [105] detections are available.

Two laser spectroscopic methods (thermal lens effect and laser-induced fluorescence) have been used for rare earth trace determination after HPLC separation [106] with a Whatman Partisil 5 ODS-3 column and a CH_3OH–water (60:40) mixture.

6. Metal speciation

The determination of the form in which an element exists, 'speciation', has become a basic requirement in many chemical investigations today. Since variations in the chemical form define the toxicity or essentiality of the analyte, this information is particularly crucial in environmental and toxicological investigations. Metal speciation refers to the identification and quantitation of organometallic, chelated or free metal ion forms or its oxidation states in a particular sample. The occurrence of organometallic compounds in the environment has received increased attention due to their toxicity and to the lipid-phase association in biological systems. With the recent developments in speciation techniques, significant advances in the knowledge of natural occurrence, formation and environmental pathways of elements have been gained.

Trace metal speciation is achieved through the combination of two different techniques: one providing an efficient and reliable separation procedure and the other detection and quantification. Direct speciation of metals has been performed by separation of the molecular species by chromatography (gas and liquid), followed by detection and determination by an element-specific detector.

Since liquid chromatography is suitable for the separation of ionic, polar and non-polar compounds, it is recognized to be especially efficient in the speciation of organometallics. Conventional LC detectors can be used for detection during speciation. Pre-column complexation with 1,5-diphenylcarbazide and elution on a C_{18} analytical column by sulphuric acid and acetonitrile [107] has been used for Cr speciation. Rutin (quercetin-3-β-D-rutinoside) [108] has been used as chelating reagent in an ion interaction technique using a Nucleosil C_{18} column and proper concentrations of cetyltrimethylammonium bromide in the presence of tetrahydrofuran. Under these conditions, direct detection of the Cr(VI) chelate is achieved by Visible spectrophotometry at 408 nm.

On-column complexation with common ligands (pyridine-2,6-dicarboxylic acid) and post-column reaction with 4-(2-pyridylazo)resorcinol or 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol [109] are used in the UV–Vis detection for speciation of metal ions such as Fe(II)/Fe(III) after ion-exchange on mixed-bead columns. Electrochemical detection through conductivity [110] or amperometry [111] and voltammetry can also be exploited.

Nevertheless, detection and quantification of the chromatographed *species* at trace levels is generally performed by atomic spectroscopic techniques. When these are coupled with a separation system, information on speciation instead of the total concentration or amount of element(s) of interest is provided.

Among atomic emission techniques, the majority of applications have been based on ICP emission spectroscopy. This source is more suitable for LC since the chromatographic flow-rate is compatible with the conventional (i.e. pneumatic chamber) ICP interface. The use of an ultrasonic nebulizer or direct injection nebulization (DIN) with microbore columns increased transport efficiency to the ICP interface. Finally, ICP has been used as a source for MS and ICP-MS has become one of the most powerful techniques for speciation analysis when coupled with separation procedures. When used as a detector for HPLC, ICP offers good sensitivity, a dynamic range of over 5 orders of magnitude and

multi-element detection capabilities. However, conventional direct connection HPLC–ICP couplings, can suffer from poor transport efficiency, particularly when pneumatic nebulizers are used. Such couplings also demonstrated low tolerance for many of the organic solvents commonly employed in HPLC eluents. Investigations have, therefore, been performed to characterize the effect of mobile-phase composition and flow-rate on HPLC–ICP methods.

As mentioned, ICP atomic emission spectrometry (AES) and ICP-MS are two different and sensitive methods used in trace metal speciation. The lower limits of detection (sub-ng to sub-pg levels), the wide linear ranges and isotope analysis capability and the high precisions (0.1–5.0% RSD) associated with ICP-MS make it more advantageous than ICP-AES. In addition, the capability of ICP-MS for isotope ratio determinations allows isotope dilution analysis to be used. Atomic absorption spectrometry can also be used as a sensitive detector after chromatographic separation.

As an example and due to their environmental impact, speciation methods and advances will be detailed hereafter mainly for Cr and As species.

Chromium is a ubiquitous element, not only through its occurrence in nature, but also due to many anthropogenic influences resulting from its widespread industrial applications. In environmental studies, its analytical determination is connected with the differences in biological and toxicological behavior of its two main oxidation states, Cr(III) and Cr(VI). Cr(III) is essential for the maintenance of the glucose tolerance factor in the human body. Cr(VI), due to its high oxidation potential and to its relatively small size which enables it to penetrate cell membranes, is toxic and carcinogenic. On the other hand, the occurrence of Cr(III) in the biotic environment as the aquo-hydroxocomplexes $[Cr(H_2O)_n(OH)_{6n}]_{n-3}$, due to its size, makes it almost entirely excluded from penetrating cell membranes.

Speciation of chromium using HPLC with conductometric detection provides low sensitivity (~100 μg/l) and poor selectivity when higher amounts of common inorganic anions are present, due to their interference with chromate [110]. Better selectivity was achieved by Arar and Pfaff [112] using a reversed-phase guard column NG1 coupled with an IonPac AS7 anion-exchange column. The eluent used was 250 m*M* ammonium sulfate and 100 m*M* ammonium hydroxide, as developed by Dionex. Under these conditions, good sensitivity has been obtained by spectrophotometric detection (0.3 μg/l for Cr(VI) after post-column reaction with diphenylcarbazide). The method developed has been proven suitable to be applied in wastewater analysis. Post-column catalytic oxidation of luminol allowed the chemiluminescence detection of Cr(III) and Cr(VI) at detection limits of 0.1 and 0.3 μg/l, respectively [113], after separation by anion-exchange on IonPac AS4A column and an acidic eluent of 0.28 *M* KCl. The technique developed was not applied to a real environmental matrix, but to a Certified Reference Material IAEA/W4 Simulated Fresh Water.

Current literature provides a wide number of applications for Cr speciation by hyphenated HPLC methods. On-line coupling of a LC system to direct current plasma has been shown by Krull et al. [114]. In this approach, an ion-pair mechanism using tetrabutylammonium phosphate, a *d*-1-camphor sulfonate or pentane sulfonate on a C_{18} support has been used for the sequential determination of Cr(III) and Cr(VI). Throughout the optimization of the method, stability problems of the DCP plume by pentane sulfonate were shown to occur. Flame atomic absorption spectrometry (AAS) has been used in a flow injection system after chelating ion-exchange with a poly(aminophosphonic acid) resin selective for Cr(III) ions [115]. Through the reduction of Cr(VI) to Cr(III) by ascorbic acid, speciation of Cr has been optimized and applied to tap, mineral and river waters. An ion-interaction mechanism performed on a C_{18} column (5-cm bed length, 5-μm packing material) by tetrabutylammonium acetate, coupled with a flame AAS system by a high-pressure capillary with a hydraulic high-pressure nebulization [116], allowed Cr preconcentration and speciation.

Very low detection limits (0.3–0.5 μg/l) can be achieved using HPLC in combination with ICP-MS [115–117]. An anion-exchange column (Waters IC-Pak A, 50×4.6 mm, 10 μm) containing trimethylammonium functionalized groups on polymethacrylate and a cation-exchange column (Waters Guard-Pak, 5×3.9 mm, 5 μm) containing sulphonic groups on polybutadiene maleic anhydride silica

were coupled for the simultaneous chromatographic separation of chromium species [117]. A step gradient with increasing nitric acid concentration and decreasing pH was used as the eluent. The analytical method has been optimized through the use of different Cr isotopes for data acquisition, comparing the interferences of some species (e.g. chloride, chlorate, perchlorate, sulphate, sulphite, sulphide, thiosulphate, carbonate, cyanide and organic species) at different m/z values. A m/z 52 has been chosen as the ideal isotope for Cr speciation in wastewaters.

Ion-pair chromatography with tetrabutylammonium acetate and 25% methanol on a Eurospher 100 C_{18} 5-μm column was applied for the separation of Cr(III) and Cr(VI) [118]. Speciation analysis was studied using hydraulic high-pressure nebulization in combination with ICP-MS. Addition of oxygen to the aerosol gas and effective desolvation were necessary prerequisites in order to apply ICP-MS as a selective and sensitive detection technique, thus reducing interferences from carbon.

Exploiting the residual cationic exchange capacity of a conventional anion-exchange column (IonPac AG5, 50×4 mm, 15 μm), Cr(VI) anions and Cr(III) cations were retained and eluted with discontinuous elution in two steps by 0.3 *M* nitric acid for Cr(VI) and 1.0 *M* nitric acid for Cr(III) [119]. Detection was achieved by coupling ICP-MS with a sample introduction technique using hydraulic high-pressure nebulization.

Despite the good detection limits, ICP-MS is prone to some interferences when real samples have to be analyzed, the most serious problem being the formation of polyatomic ions, expecially below atomic mass number 80. For example the determination of iron is normally difficult using a quadrupole ICP-MS instrument because of the strong interference by $^{40}Ar^{16}O^{+}$ at m/z 56, which is identical with the most abundant iron isotope [120]. In a similar way with organic liquids, owing to the presence of carbon, the abundant molecule $^{40}Ar^{12}C^{+}$ obscures $^{52}Cr^{+}$, the main isotope of chromium, as mentioned above [7,118].

An anion chromatographic separation (polymer-based anion-exchanger; eluent, EDTA–oxalic acid) has been optimized by working at 40°C column temperature [121]. Detection was obtained by direct introduction of eluate into an ICP-MS system. This method enabled 80–88 ng/l detection limits for Cr(III) and Cr(VI), respectively, within a linear range from 0.5 to 5000 μg Cr/l and simultaneous determination of chromium species and Mn, Fe, Ni, Cu, Mg and Ca in water samples. Total chromium, Cr(III) and Cr(VI) speciation was achieved by Powell et al. [122] by coupling high-pressure liquid anion chromatography (eluent nitric acid) with direct injection nebulization and ICP-MS. The detection limits obtained were 30 and 60 ng/l for Cr(III) and Cr(VI), respectively, without particular chromatographic approach. Caruso and co-workers [123] used mixed-mode columns, namely IonPac AS7, for Cr(III) and Cr(VI) separation with $(NH_4)_2SO_4$ eluent (pH 9.2). The Cr(III) species was stabilized with EDTA before sample analysis and detection was performed both by ICP-AES and ICP-MS equipped with a high-performance interface and a concentric nebulizer. Relative detection limits were 0.40 μg/l for Cr(III) and 1.0 μg/l for Cr(VI) within a 4% RSD and a linear dynamic range from 3 to 600 μg/l and from 5 to 1000 μg/l for Cr(III) and Cr(VI), respectively, in aqueous media.

Arsenic-containing compounds subject to speciation analysis are anions, i.e. arsenite As(III), arsenate As(V), monomethylarsonate (MMA) dimethylarsinate (DMA) or cations, e.g. arsenobetaine (AsB), arsenocholine (AsC) and tetramethylarsonium ion (TMAs) or uncharged compounds at neutral pH, e.g. arsenous acid. There is no general rule or trend that relates toxicity with oxidation state or chemical form and number of substituents linked to the central atom. A decreasing order of toxicity is as follows: arsenite, arsenate, MMA, DMA. The lethal doses, LD_{50} (rats), for these compounds are 1.5, 5, 50, and 500 mg/kg, respectively. AsB and AsC have been found to be non-toxic for rats [124].

An IC–HPLC procedure for As(III), As(V), MMA and DMA separation and ICP-AES determination has been developed by Rauret et al. [125] by coupling the systems with the hydride generation sample introduction technique. The procedure was improved [126] by checking two different kinds of column (Nucleosil-5SB and Hamilton PRP X-100) and by comparing isocratic and gradient elution. The peak profile was improved by filtering the data corresponding to low concentration with Fourier transform. With such a procedure detection limits

between 2.7 As(III) and 11.4 As(V) μg/l were obtained. Ion chromatographic methods for elemental speciation (As, Se and Cr) using microbore columns with direct-injection nebulization by IC-PAES have been described by Gjerde et al. [127]. Arsenite, arsenate, MMA, DMA, AsB and AsC have been separated by an anion HPLC procedure with a phosphate eluent and the analytes were determined by hydride generation atomic absorption spectrometry (HG-AAS) [128]. An HPLC–UV–HG–AFS (AFS, atomic fluorescence spectrometry) method has also been applied to investigate the stability of arsenic species in relation to food treatment procedures (seafoods and mushrooms) [129]. Reversed-phase microbore columns and eluents containing ion-pairing agents could be coupled with mass spectrometric detection for arsenic speciation by the use of a direct injection nebulizer interface [130,131]. Good efficiency was also obtained using interfaces based on hydride generation manifolds. Hydride generation was used in order to avoid the poor efficiency of conventional pneumatic nebulizers in LC–ICP-MS studies of arsenic speciation [132,133], but even by using a membrane gas separator [134] the determination was subject to the interference by $ArCl^+$ molecular ion. The removal of the $^{40}Ar^{35}Cl^+$ interference was optimized, by Caruso and co-workers [135,136], by coupling ion chromatography and ICP-MS detectors and lowered detection limits for As(III), As(V), DMA and MMA with the use of an He–Ar gas mixture as ionization source: the limits ranged between 0.032 and 0.080 ng for DMA and MMA, respectively. More recently [137] a speciation of these compounds was obtained by micellar liquid chromatography coupled with ICP-MS detection. The method, based on micellar mobile phase (CTAB, propanol and borate buffer) and on a PRP-1 separation column coupled with the ICP-MS system, allowed linear dynamic ranges of 3 orders of magnitude and detection limits in the picogram range (90–300) and overcame the problems of chloride since it is not co-eluted with any of the four arsenic species. A detailed study must also be mentioned on the suitability of the ion spray (IS) technique for arsenic speciation analysis in biological samples. A cation-exchange HPLC has been coupled with IS-MS–MS detection for analysis of organoarsenic species. Dual mode, elemental and molecular, analysis is presented using standard mixtures. Although detection limits are not as low as those obtained by HPLC–ICP-MS the results indicate IS-MS–MS as a complementary technique to ICP-MS for speciation analysis [138].

A simple methanol–chloroform extraction on a dogfish muscle reference material followed by LC–ICP-MS allowed the identification of AsB as the final metabolic product of arsenic in that sample [139]. Chromatographic separation was based on a reversed-phase mechanism on C_{18} column with 10 m*M* dodecylsulphate in the presence of 5% methanol and 2.5% glacial acetic acid. Although these separation conditions did not provide separation between As(III), As(V) and MMA, they provided complete resolution between DMA and AsB. Method development in arsenic speciation by ion-exchange was also attempted by Beauchemin et al. [140]. Two analytical columns (Radial-Pak (SAX) and PRP-X 100) have been compared with mobile phases containing phosphate buffers at pH values included between 7 and 8. The mobile phases studied did not provide simultaneous baseline resolution between the arsenic-containing compounds considered (As(III), As(V), MMA and DMA) but, due to the buffering capacity of the eluents, the system allowed the injection of a strong acid matrix with minor modifications in retention times.

Finally, a comprehensive review on ICP-MS detection for chromatography and capillary electrophoresis, must be mentioned [141].

7. Abbreviations

AAS	atomic absorption spectroscopy
AES	atomic emission spectroscopy
AFS	atomic fluorescence spectrometry
CIC	chelation ion chromatography
CTA	cetyltrimethylammonium
CTAB	cetyltrimethylammonium bromide
DCP	direct current plasma
DEDTC	diethyldithiocarbamate
DIN	direct injection nebulizer
ED	electrochemical detection
EDTA	ethylenediamine tetracetic acid
GFAA	graphite furnace atomic absorption
HG	hydride generation

HIBA	α-hydroxyisobutyric acid
HPCIC	high-performance chelation ion chromatography
HPLC	high-performance liquid chromatography
HQ	8-quinolinol
IC	ion chromatography
ICP	inductively coupled plasma
IIC	ion interaction chromatography
IIR	ion interaction reagent
IS	ion spray
LC	liquid chromatography
MS	mass spectrometry
ODS	octadecyl silica
PAR	4-(2-pyridylazo)-resorcinol
PBMA	poly(butadiene–maleic acid)
PS–DVB	polystyrene–divinylbenzene
RP	reversed-phase
TBA	tetrabutylammonium
TEA	tetraethylammonium
TPA	tetrapropylammonium

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