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Review

Complexation ion chromatography —an overview of developments and trends in trace metal analysis☆

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ABSTRACT

Complexation ion chromatography (IC), including all ion chromatographic modes in which complexation is exploited for the separation and detection of metal ions in different ways, is now a widely accepted method of trace metal analysis. Some of the significant advances in the theoretical aspects and practical applications of complexation IC modifications (non-suppressed cation chromatography with complex-forming mobile phases, coordination chromatography with chelate-forming bonded phases, ion-exchange and ion-pair chromatography of anionic metal chelates) recently developed in the authors' laboratories are reviewed. The retention behaviour and separation mechanism of non-complexed and complexed metal analytes are discussed from the point of view of basic coordination chemistry (stability of metal complexes, effective charge of metal atom, ligand complexing ability, etc.). Comparisons and contrasts between various metal complexation IC techniques and their common features and advantages relative to other methods used in analyses for transition and heavy metal ions are evaluated.

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☆ Dedicated to Professor Oleg M. Petrukhin on the occasion of his 60th birthday.

1. INTRODUCTION

The development and substantial practical achievements of ion chromatography (IC) in the analysis of metal ions in the last decade have been achieved mainly by the exploitation of metal complexation (*e.g.*, refs. 1–7 among many others recently published). The complexation reactions can take place in either the mobile or stationary phase and sometimes simultaneously in both. Chromatographic separations of metal complexes formed before chromatography and detection by postcolumn complex-forming reactions are also within the scope of complexation IC. Hence it appears that a major advantage of the chromatographic determination of metals in the form of various complexes is that a variety of separation modes and detection methods can be utilized.

In general, complexation IC is considered to include all metal ion chromatographic separation techniques based on or dependent on complexation processes [5]. Among the diverse possible chromatographic variants, cation chromatography with complex-forming mobile phases and coordination chromatography of metal ions on chelate-forming bonded stationary phases, ion-exchange and ion-pair chromatography of anionic metal chelates with direct conductivity or spectrophotometric detection, including postcolumn complexation derivatization, has been the focus of our recent interest.

This review is devoted to a critical evaluation of separations recently obtained in the authors' laboratories and some recently published literature data on this subject. Fundamentals of coordination chemistry related to the consideration for the retention behaviour of metal chelates and complexed metal ions are discussed. Comparisons and contrasts are discussed between the various metal ion chromatographic techniques. The resolution and detection parameters are also compared and illustrated by some applications of practical relevance, mainly in trace metal determinations.

2. RESULTS AND DISCUSSION

2.1. *Chromatographic methods*

In our research on the determination of transition and heavy metals, we have relied mainly on the four IC methods summarized in Table 1.

Cation-exchange IC is the most obvious and therefore the most common chromatographic method for the determination of metal ion. When using silica- [8,9] or polymer-based [10] sulphonated ion-exchange stationary phases, selective and efficient separations of bivalent metal ions require the addition of a complexing ligand (*e.g.*, di- or tribasic carboxylic acids) to the eluent, which is able to decrease differentially via complexation the effective charge of the metal cation and, hence, the retention. When chelating chemically bonded phases (such as an iminodiacetic acid functional silica-bonded material [11,12]) are used instead of cation exchangers, complexation reactions in the stationary phase are responsible for the separation (sometimes together with complexation in the eluent and the ion-exchange mechanism due to free or protonated chelating groups, which act as ion-exchange sites) and offer a number of advantages in terms of selectivity.

Another promising approach to the ion chromatographic determination of metal ions is their precolumn conversion into the negatively charged chelates, followed by anion-exchange chromatographic separation [13]. The separation of the same type of metal chelates {mostly of heterocyclic azo dyes, such as 4-(2-pyridylazo) resorcinol (PAR) [14,15]}, on dynamicaly coated anion-exchange columns, *i.e.*, in the ion-pairing mode, introduces addi-

TABLE 1

COMPLEXATION ION CHROMATOGRAPHY OF METAL IONS

Chromatographic technique	Metal forms separated[a]	Chelating ligand (L)	Metal ions determined
Cation-exchange IC	$M^{n+} \ldots ML_m^{(n-m)\pm}$	Carboxylic acids (citric, tartaric, oxalic)	Cd(II), Co(II), Cu(II), Fe(II), Fe(III), Mn(II), Ni(II), Pb(II), Zn(II), alkaline earths
Coordination IC	$M^{n+} \ldots ML_m^{(n-m)\pm}$	Carboxylic acids, pyridine-2,6-dicarboxylic acid, nitrilotriacetic acid	Cd(II), Co(II), Fe(II), Mn(II), Pb(II), Zn(II), alkaline earths
Anion-exchange IC	ML_n^{n-}	4-(2-Pyridylazo)-resorcinol (PAR), EDTA	Cd(II), Co(II), Cu(II), Fe(III), Ni(II), Pb(II), Zn(II)
Ion-pair chromatography	$ML_n^{n-} \ldots ML_nB_n$	PAR	Co(II), Cu(II), Fe(III), Ni(II)

[a] B = Quaternary ammonium ion.

tional possibilities for improving the resolution [16,17].

2.2. *Retention behaviour and separation mechanism*

2.2.1. *Cation-exchange IC*

To overcome the strong electrostatic interactions of transition metal ions with the column, ethylenediamine (EDA) has been used in the eluent as a competing ion. EDA is highly protonated at the pH level used (usually between 3 and 5) and loses its ability to act as a ligand. A schematic representation of the cation-exchange interactions between EDA and the bivalent sample ion, M^{2+}, on a stationary phase, R, with complexing eluents (H_2L) is shown by the following equilibria:

$$\begin{aligned} &\text{R–M} + \text{EDAH}_2^{2+} + \text{L}^{2-} \\ &\quad \rightleftharpoons \text{R–EDAH}_2 + \text{M}^{2+} + \text{L}^{2-} \\ &\quad \rightleftharpoons \text{R–EDAH}_2 + \text{ML} \end{aligned}$$

In the absence of a complexing ligand, L^{2-}, transition metal ions are bound firmly to the sulphonic acid groups, and elution by the ethylenediammonium cation is ruled only by the EDA concentration. If a complexing agent is added to the eluent, its anionic carboxylate groups compete for the metal ion, thus favouring release from the stationary phase by EDA, resulting in a decrease in retention time.

Under conditions where the same solute cation, M^{2+}, is eluted from the same column with varying concentrations of competing cation, En^{2+}, in the eluent, a plot of log k' *versus* log [En^{2+}] will be a straight line with a slope equal to -1 [18]. The slopes of the experimentally observed dependences shown in Fig. 1 are in a good agreement with the

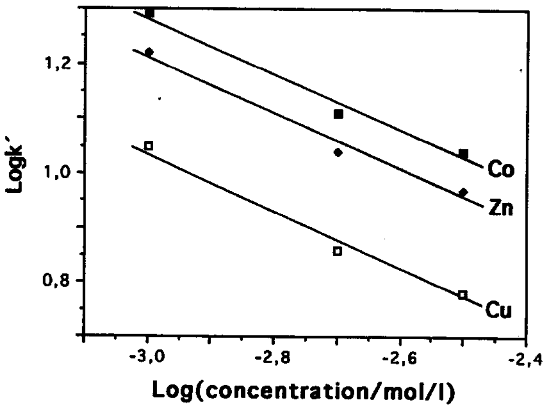

Fig. 1. Dependence of log k' values of metal ions on the concentration of ethylenediamine in the mobile phase. Stationary phase, G-253 macroporous methacrylate ion exchanger; column, 100 × 6 mm I.D.; mobile phase, EDA–2 mmol/l tartaric acid; flow-rate, 2.0 ml/min.

theoretical value. This suggests that in the presence of complex-forming ligands cation exchange remains the operating retention mechanism.

The effect of the eluent complexing agent on retention behaviour is equivalent to reducing the effective charge of the metal ion. This results in a decrease in the retention times of all bivalent cation studied with increase in the concentration of a carboxylic acid [10]. The selectivity of the separation is also changed owing to complexing effects. This is attributable to the fact that the distribution coefficients of transition metals (K_D) with a charge and size of the same magnitude differ only slightly in the absence of complexing acids. On the other hand, when complexation becomes a part of the separation mechanism the relative retention of two metal ions is determined by

$$K_D^{\mathrm{compl}}(1)/K_D^{\mathrm{compl}}(2) = [K_D(1) / K_D(2)]\,[\beta_n(1) / \beta_n(2)]$$

where β_n is the stability constant of the corresponding complex [10,19]. The differences between the values of the stability constants of transition metal complexes with carboxylic acids are usually larger than the variations in the distribution coefficients. The dominant impact of complexation effects on the selectivity of separation can be confirmed by the linear correlation between the log k' and log β_n values observed in different chromatographic systems (Fig. 2). The more stable the metal ion–carboxylate complex, the more rapidly it will be eluted.

The dependence of the resolution on the pH of the mobile phase must also be carefully controlled to optimize the separation. Increasing the pH increases the complex-forming ability of hydroxy acids and the effective constant of complex formation, resulting in decreased retention times and better separations. On the other hand, the pH of the eluent influences the degree of protonation of the amino groups of EDA and thereby interactions between the stationary phase and the metal ions. The result of optimizing the pH of the EDA–citrate mobile phase is illustrated by the separation of a number of transition metal ions of environmental importance (and also some mono- and bivalent cations) with a baseline resolution within 20 min (Fig. 3).

Obviously, the separation of transition metal ions in cation-exchange IC is based on the combined effects of ion-exchange elution with the eluent cation and complexation with the eluent ligand, followed by the formation of complexes of different stability in the mobile phase.

2.2.2. Coordination IC

Among many combinations of immobilized chelating ligand and base material examined [5], we

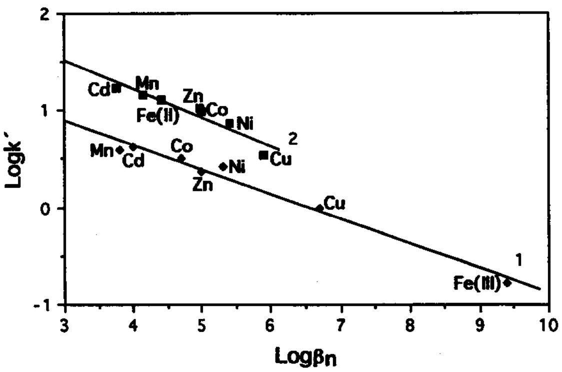

Fig. 2. Log k' *vs.* log β_n plots. 1 = G-253 column with 2 mmol/l EDA–2 mmol/l oxalic acid (pH 4.0) mobile phase; 2 = TSK IC cation SW column with 3.5 mmol/l EDA–10 mmol/l citric acid (pH 2.8) mobile phase.

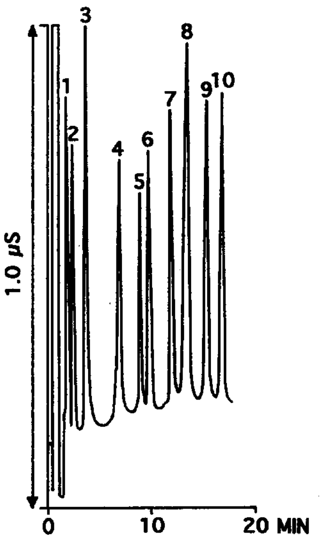

Fig. 3. Simultaneous separation of alkali, alkaline earth and transition metal ions. Column, TSK IC cation SW; mobile phase, 3.5 mmol/l EDA–10 mmol/l citric acid (pH 2.8); flow-rate, 1.0 ml/min; detection, conductivity. Peaks: 1 = Na; 2 = K; 3 = Cu; 4 = Ni; 5 = Co; 6 = Zn; 7 = Fe^{2+}; 8 = Mn; 9 = Cd; 10 = Ca. From ref. 9.

considered a stationary phase with silica-bound iminodiacetic acid (IDA) functions to be one of the most favourable from the point of view of the kinetics and specificity of chelate formation [11,12]. IDA exhibits different complexing abilities for different transition metals, most of the complexes being kinetically labile and moderately stable. These properties ensure the reversible sorption of metal ions.

The most important parameters controlling the retention of cations in coordination chromatography are the concentration of a complexing agent and the pH of the mobile phase. All eluents can be classified into two main types, depending on their complexing ability.

2.2.2.1. Weak complexing eluents. The basic function of these eluents (*e.g.*, citric or tartaric acid) is to regulate the dissociation of free carboxylate groups acting as ion-exchange sites, owing to the pH variations with the changes in eluent concentration (Fig. 4). Nevertheless, although in the pH range investigated (2.5–4.0) there is evidence for ion-exchange processes taking place additionally (also with partially protonated IDA moieties), the retention behaviour reflects the affinity of metal ions to IDA as a complexing ligand. The elution order of metal ions is consistent with the stability constants of metal–IDA complexes and confirms the dominating contribution of complexation reactions within the IDA column to the elution mechanism. Therefore, both citric and tartaric acid can elute only certain transition metal ions from the column, whereas other ions, which form more stable IDA complexes (*e.g.*, Cu^{2+}, Ni^{2+} and Pb^{2+}), remain irreversibly retained.

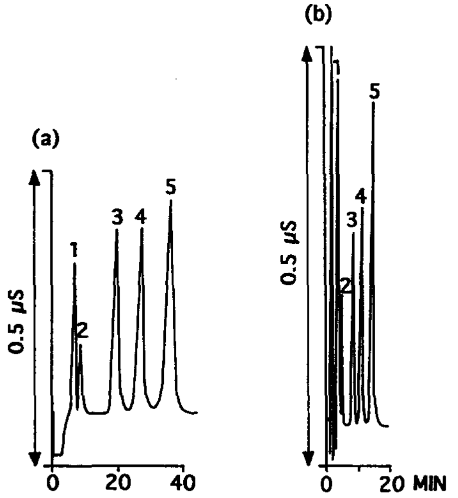

Fig. 4. Chromatograms of metal ions with different concentrations of citric acid: (a) 10 and (b) 25 m*M*. Stationary phase, 7-µm porous silica coupled with IDA; column, 100 × 4.6 I.D.; flow-rate, 1.0 ml/min; detection, conductivity. Peaks: 1 = Mg; 2 = Fe^{2+}; 3 = Co; 4 = Cd; 5 = Zn. From ref. 12.

2.2.2.2. Strong complexing eluents. A high complexing ability of the IDA phase requires the use of sufficiently strong complexing agents in the mobile phase, such as pyridine-2,6-dicarboxylic acid (DPA) or nitrilotriacetic acid (NTA). Both DPA and NTA have the same three coordination centres in complexation as IDA (*i.e.*, the two carboxylate groups and the nitrogen atom), which are apparently very similarly arranged within the molecules. Consequently, under these conditions, complex formation in the IDA phase becomes comparable to that in the eluent.

DPA proved to be a particularly useful eluent for the IDA column [11,20]. Its anion shows a unique degree of complexation owing to the following steric effect. The nitrogen atom of the DPA anion is part of an aromatic system, which rigidly holds the carboxylate groups in a position coplanar to the pyridine ring, thus favouring complex formation (whereas IDA and NTA molecules have a flexible structure).

However, at low DPA concentrations, even transition metals of high complex stability exhibit a retention behaviour similar to that of alkaline earth metal ions, being eluted mainly through an ion-exchange mechanism. Only if higher concentrations of DPA are incorporated in the eluent, complexation processes are favoured. The log k' values plotted against the logarithm of the DPA concentration give straight lines, and the slopes of these dependences are proportional to the formation constants of the metal–DPA complexes (Fig. 5). The $\Delta \log k' / \Delta \log [DPA]$ values increase with increaisng pH, reflecting the increasing dissociation and complexing ability of the ligand. Calculations of conditional stability constants in the pH range studied (2.5–5.0) show that the stability of metal–DPA complexes is fairly high, confirming the ability of this complexing agent to accelerate the elution of transition metal ions under acidic conditions.

Thus, two complexation reactions can take place

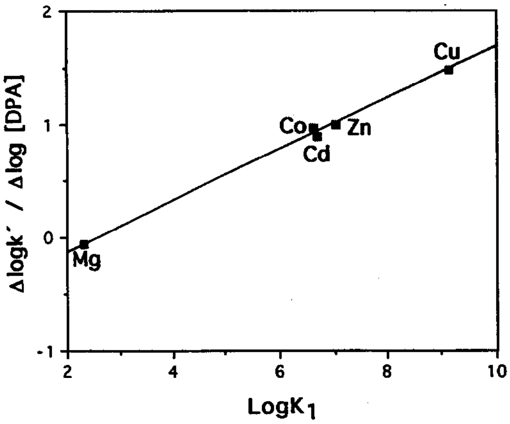

Fig. 5. Correlation between the slope of log k' *vs.* log [DPA] dependences for metal ions and the first formation constant of metal–DPA complexes. Stationary phase, 7-μm porous silica coupled with IDA; column, 100 × 4.6 mm I.D.; flow-rate, 1.0 ml/min; detection, conductivity.

in coordination IC, and the retention and separation of metal ions are governed predominately by the ratio of the stability constants of their complexes with the chelating groups immobilized on the stationary phase and with the complexing agent incorporated in the eluent.

2.2.3. *Anion-exchange IC*

Anion-exchange chromatography of transition metals as previously formed, stable, anionic chelates is a comparatively new chromatographic method. This method has decided advantages, such as a stable baseline and simple instrumentation (see also below). This chromatographic mode can also be achieved by using the on-column complexation technique where the complexing agent is added to the mobile phase [21] or injected into the column before the separation [22,23].

When light-absorbing chelating reagents, such as PAR, are used, the main problem of optimizing the elution conditions is that the metal complexes are strongly retained because of non-ion-exchange sorption, especially on polymer-based ion exchangers [13,17]. We have overcome this problem by adding organic solvents (up to 40 vol. % of 2-propanol or acetone) to the mobile phase. The complete dissociation of the complexes under these conditions requires the use of strongly alkaline eluents,

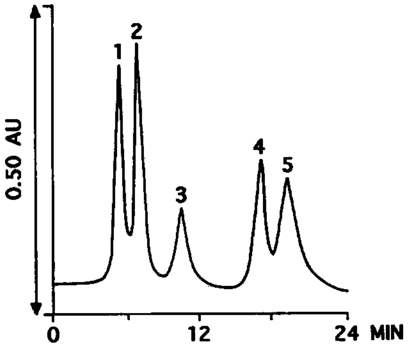

Fig. 6. Chromatogram of metal pyridylazoresorcinolates. Stationary phase, HIKS-1 resin-based anion-exchanger; column, 200 × 6 mm I.D.; mobile phase, 50 mmol/l sodium carbonate–acetone (60:40, v/v) (pH 11.8); flow-rate, 1.0 ml/min; detection, 500 nm. Peaks: 1 = Co; 2 = Cu; 3 = Zn; 4 = Fe^{3+}; 5 = Ni. From ref. 17.

e.g., sodium carbonate. A typical chromatogram is presented in Fig. 6. It should be emphasized that a sufficiently high chromatographic stability of transition metal–PAR chelates in these eluents is provided by their extremely high thermodynamic stability.

Fig. 7 shows the effect of sodium carbonate concentration on the retention. As the eluent concentration increases, the retention time decreases. The linear character of these dependences is indicative of the ion-exchange mechanism of separation, although the slope (−0.4 to −0.7) is less than the theoretical value of −1 [24] (most of the complexes

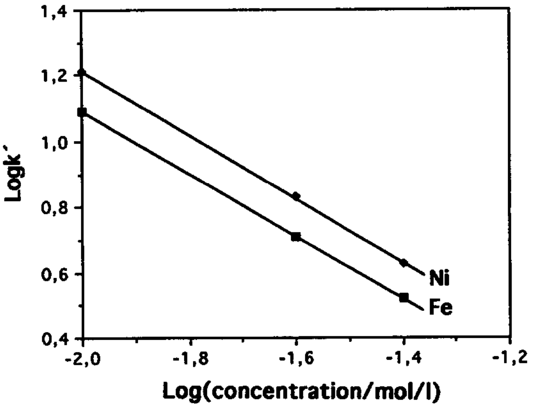

Fig. 7. Dependences of the capacity factors of Fe–PAR and Ni–PAR on the concentration of sodium carbonate in the mobile phase. Mobile phase, sodium carbonate–2-propanol (60:40, v/v). Other conditions as in Fig. 6.

studied have a charge of −2, and the eluent competing ion is also doubly charged in this pH range). The pH increase also considerably decreases the retention time of complexes; the Na_2CO_3 concentration is less and the influence of pH is stronger.

Hence the retention of metal pyridylazoresorcinolates in anion-exchange IC is obviously due to both ion-exchange and adsorption mechanisms. This peculiarity of their chromatographic behaviour allows one to regulate the selectivity of separation by varying independently the eluent competing ion and organic modifier concentration. The metal complexes of aminopolycarbonic acids (*e.g.*, EDTA), on the other hand, experience a comparatively low hydrophobic sorption effect and can be separated by elution with standard carbonate–hydrogencarbonate eluents [13,17].

2.2.4. *Ion-pair chromatography (IPC)*

This HPLC mode, in which mobile phases containing an ion-pairing reagent are used along with a reversed-phase stationary phase, can be considered as a more promising alternative to anion-exchange IC on fixed-site ion-exchange columns. According to the abundant literature on the use of IPC for the separation of metal ions recently reviewed [15–17], two main retention models, the ion-interaction and the ion-partition model, may serve to explain the retention behaviour of metal chelates. Normally, the real retention mechanism is a mixed one, but under certain conditions, ion exchange or adsorption may occur preferentially. The relative contributions of these mechanisms depend on the mobile phase composition and, as we have shown on the example of metal–PAR chelates [16,17], the eluting strength and concentration of the organic modifier are the most important parameters in controlling elution and selectivity in IPC.

When water–organic eluents with comparatively low eluting ability are used, the adsorption of the ion-pairing counter ion on a non-polar stationary phase and the ion-exchange sorption of anionic solutes dominate. This is why the retention of metal complexes is greatly affected by the basicity of an appropriate ionized group of the ligand, participating in ion-exchange interactions, and, thereby, by the electron-acceptor ability of the metal atom. Hence, the higher the effective charge of the metal atom, the stronger is the retention (Fig. 8a). An increase in organic modifier concentration or hydrophobicity transfers the ion-pair formation from the stationary phase to the mobile phase. Consequently, the absorption of ion pairs becomes the predominant separation mechanism and, as in reversed-phase HPLC systems [25,26], the complexes of more electronegative metals have the longer retention times (Fig. 8b). Fig. 9 shows that the stability constant as a universal structure-dependent characteristic of the metal complex can also be suitable for the *a priori* evaluation of the retention values in IPC.

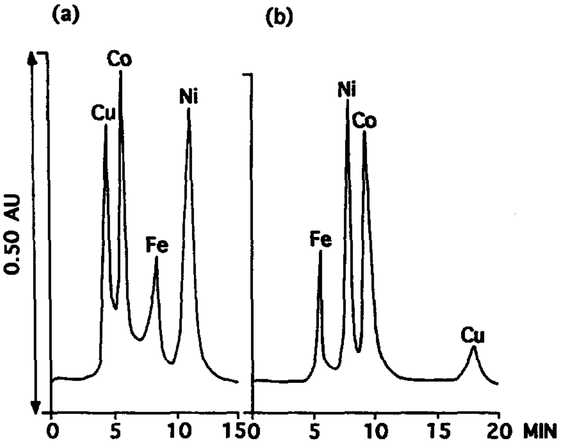

Fig. 8. Effect of the nature of the organic modifier on the retention mechanism of metal–PAR complexes. (a) Ethanol; (b) acetone. Stationary phase, Silasorb C_8 (10 μm); column, 200 × 6 mm I.D.; mobile phase, organic solvent–water (20:80, v/v) containing 8 mmol/l of tetrabutylammonium hydroxide (pH 11.8); flow-rate, 1 ml/min; detection, 500 nm.

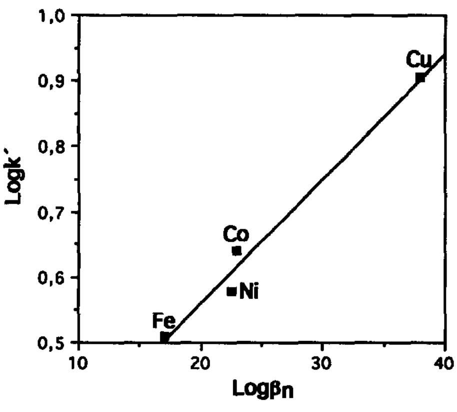

Fig. 9. Plot of log k' *versus* log β_n values for metal–PAR complexes. Chromatographic conditions as in Fig. 8b.

TABLE 2

RESULTS FOR METAL DETERMINATION IN DIFFERENT SAMPLES

Sample	Metals determined	Found (ppm ± S.D.)[a]	Certified value (ppm)
Waste water from electroplating plant	Cu	12.8 ± 0.4	12.0
	Zn	8.8 ± 0.3	9.0
Drinking water	Fe	2.0 ± 0.1	2.1
Wine	Cu	1.4 ± 0.1	1.3
	Fe	8.4 ± 0.3	8.2
Apple–grape juice	Cu	2.0 ± 0.1	1.9
	Fe	13.4 ± 0.5	13.6

[a] n = 3–4.

Thus, changing of the retention mechanism leads to changes in the elution order of metal complexes and, moreover, can result in a complete reversal of the retention. The other numerous parameters of the mobile phase (nature and concentration of the ion-pairing agent, pH, concentration of the eluent co-ion) govern the IPC separation at the same time and with different effects [16,17]. Therefore, this complexation chromatographic technique is characterized by distinct possibilities for regulating the separation due to both multi-component eluent composition and complex retention mechanism.

2.3. Analytical applications

2.3.1. Cation-exchange IC

Post-column reaction detection is now the most commonly applied detection mode for the determination of trace amounts of transition metals by non-suppressed cation chromatography [27,28]. In practical applications we also prefer spectrophotometric detection to direct conductivity measurements. PAR was used as a postcolumn derivatizing reagent [10] and the method was successfully applied to the analysis of waste waters, drinking water, food and beverage products, fertilizers and other matrices. Analytical data for some of these samples are given in Table 2. Further, the potential of the method in the differential analysis of iron species in real samples is demonstrated in Fig. 10.

2.3.2. Coordination IC

The affinity of the IDA stationary phase towards transition metal ions is of great advantage for the determination of trace metals in samples containing high levels of alkali and alkaline earth metal ions. Fig. 11 gives a chromatogram of a sea-water sample, spiked with transition metals, as an example of alkali and alkaline earth metal-rich matrices (*e.g.*, sodium and magnesium are present in more than a 10^4-fold excess over sub-ppm concentrations of transition metals).

A further advantage of coordination IC is the possibility of simultaneous preconcentration and separation on the same column [29]. In this way, zinc was determined in a pretreated urine sample [30]. Using a fully automatic chromatographic system, including a separate concentrator IDA column [12], matrix interferences can also be eliminated. As a result, the detection limits were improved by about three orders of magnitude, reaching the 0.1–

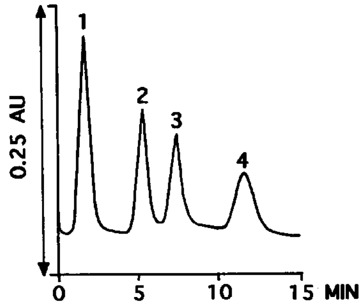

Fig. 10. Cation-exchange IC analysis of waste water. Stationary phase, G-953; column, 100 × 6 mm I.D.; mobile phase, 2 mmol/l EDA–2 mmol/l tartaric acid (pH 4.5); flow-rate, 2.0 ml/min; postcolumn reagent, PAR, 0.001% in 0.5% ammonia solution, flow-rate 1.5 ml/min; detection, 500 nm. Peaks: 1 = Fe^{3+} (18 ppm); 2 = Cu (13 ppm); 3 = Zn (9 ppm); 4 = Fe^{2+} (16 ppm).

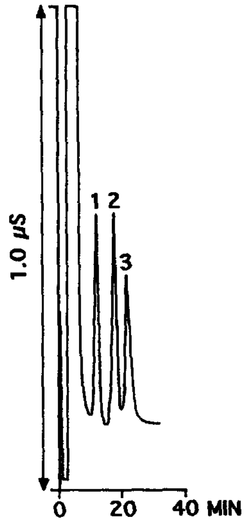

Fig. 11. Separation of transition metals in sea water by coordination IC. Stationary phase, IDA-silica; mobile phase, 10 mmol/l tartaric acid (pH 2.5); flow-rate, 1.0 ml/min; detection, conductivity. Sample: sea water spiked with (1) 5 ppm of Co, (2) 5 ppm of Zn and (3) 10 ppm of Cd. From ref. 11.

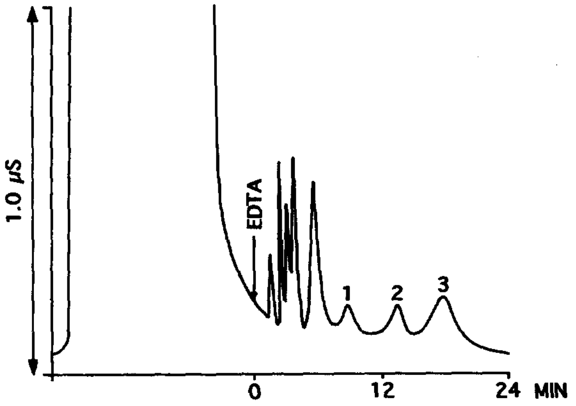

Fig. 12. Chromatogram of the determination of heavy metals in sea water. Stationary phase, KanK-Ast (15 µm); column, 100 × 3 mm I.D.; mobile phase, 2 mmol/l sodium carbonate–0.5 mmol/l ammonium hydrogencarbonate; flow-rate, 0.9 ml/min; detection, conductivity. Peaks: 1 = Cd + Pb ($2 \cdot 10^{-6}$ *M*); 2 = Zn ($\leqslant 5 \cdot 10^{-6}$ *M*); 3 = Cu ($4 \cdot 10^{-6}$ *M*). The first eluted overscaled peak corresponds to the anions. The arrow shows the time of EDTA injection.

0.2 ppb level for zinc, cadmiun, and cobalt with conductivity detection.

2.3.3. *Anion-exchange IC*

Owing to the high sensitivity with respect to intensely coloured metal–PAR chelates, this method can be used in combination with spectrophotometric detection to analyse some real samples without preconcentration, *e.g.*, waste waters from electroplating wash tanks [13,17]. When the iron content was high [*e.g.*, in the purification of effluents by coprecipitation of metals with hydrous iron(III) oxide], a modification by on-column complexation proved to be effective [26]. In contrast to other metals, iron(III) does not form a complex under these conditions, probably for kinetic reasons, and does not interfere.

The simultaneous ion chromatographic determinations of transition metal cations and inorganic anions on a central ion exchanger (KanK-Ast) possessing both anion- and cation-exchange capacity, proposed by Dolgonosov [31], using conductivity detection were recently described [22,23]. The successive separation of anions and metal cations retained on the column during the elution of anions is achieved by the subsequent injection of EDTA and anion-exchange separation of the metal–EDTA complexes formed. The method was applied to the analysis of natural samples. Fig. 12 shows the chromatogram of a sea-water sample. The bifunctional ion-exchange properties of the proposed ion-exchange material permit a multi-step on-column preconcentration of metal ions and, as a result, the detection limits were lowered to the mid-ppb level.

2.3.4. *Ion-pair chromatography*

In comparison with anion-exchange IC, the limits of detection provided by IPC are lower by about one order of magnitude and, therefore, sufficiently low to analyse directly samples containing low levels of transition metals, such as purified waste waters, drinking water and beverages. An example of the application of IPC to water and beverage quality control is presented in Fig. 13. As for anion-exchange IC, the use of the simplest and fastest precolumn complex formation by direct mixing of the sample and reagent solution does not adversely affect the analysis time (the sample pretreatment is usually less than 15 min).

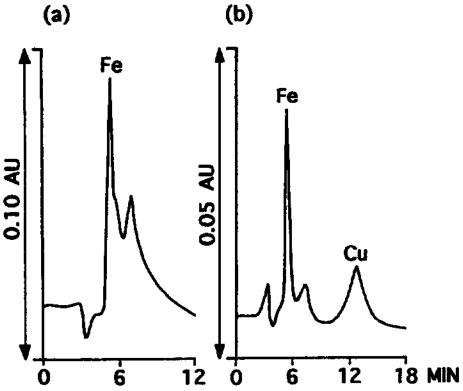

Fig. 13. Analysis of real samples by IPC. (a) Tap water; (b) apple–grape juice. Mobile phase, acetone–water (20:80, v/v) containing 6 mmol/l of tetrabutylammonium hydroxide (pH 12.0). Other conditions as in Fig. 8. Peaks: Fe^{3+}, (a) 8.6 ppm, (b) 13.5 ppm; Cu, 2.1 ppm. From ref. 17.

2.4. Comparison of different complexation IC methods

The complexation IC methods described above are distinguished by a complicated retention behaviour of metal ions and their complexed forms in a chromatographic system. This common feature is related to a number of complexation reactions, accompanying the chromatographic process, and/or a complex retention mechanism. As a consequence, the enormous possibilities of varying the separation selectivity within wide limits by changing the mobile phase composition are apparent. One more distinguishing feature, confirming a relationship between all IC methods based on complexation reactions, is that the main elution regularities and separation mechanisms are determined by the stability of metal complexes. This basic characteristic is of great importance in regulating interactions between the metal ion or complex and the mobile and stationary phases. The stability constants play an important role in the prediction of retention and in the theoretical analysis of chromatographic behaviour in HPLC of metal chelates based on one-dimensional retention models [25,32] and, as shown above, they can also be widely applied for these purposes in complexation IC. Naturally, each of the complexation IC variants considered also has its own peculiarities, accounting for their advantages and disadvantages.

Cation-exchange IC allows the separation and subsequent detection of a large number of transition metals in one analysis step, simultaneously with some alkali and alkaline earth cations. Tedious sample clean-up procedures may be omitted in many instances to shorten analysis times. Additionally, in comparison with precolumn complexation IC techniques, cation-exchange IC has the advantage of avoiding the preliminary complex formation, and this also saves analysis time. However, only metals that undergo complexation rapidly enough can be determined. Such benefits as the ability to separate interferents from peaks of interest, multi-element capability, minimum sample pretreatment and low detection levels are valuable assets that make this chromatographic method very attractive for the speciation of trace elements.

Coordination IC has practically the same merits as cation-exchange IC. Further, the use of stationary phases with higher selectivities for transition metal ions facilitates the solution of an important problem often encountered in the IC of environmental or biological samples, *i.e.*, matrix interferences due to other constituents. However, the gain in selectivity is achieved at the cost of increased analysis times, and this is an adverse aspect of the high affinity of chelating stationary phases towards transition metal ions. The lack of commercially available chelating columns also hinders the application of coordination IC to routine analyse for transition metals.

Both ion-exchange an ion-pair IC are distinguished by the application of metal complexes formed before introduction into the column, so that there is no need for a postcolumn derivatization system. As a result, the solvent-delivery system and handling are simpler, and the analytical precision is better. Further, selective precolumn complexation reactions can largely eliminate interferences from environmental sample matrices, while by varying the complex-forming conditions the selectivity of metal determination can be increased.

The ability to use light-absorbing reagents to form highly coloured and stable chelates with numerous transition metals often makes the preconcentration stage unnecessary, and this speeds up and simplifies the analysis. In addition, the detection limits are low, because an extra dispersion of the chromatographic zones is not introduced by the mixing procedure. The lower sensitivity of ion-exchange IC (because of the lower efficiency) can be compensated for by the higher sample volumes that can be used (*i.e.*, due to the preconcentration directly on the analytical column). IPC has the advantage of possessing a larger number of parameters that can control the retention and regulate the selectivity of the separation. Finally, the separation of transition metals as anionic species by these chromatographic techniques opens up new possibilities for the determination of metal ions and various anions in one run.

Summarizing, the four possibilities for separating and detecting metal ions presented in this review represent a good potential of complexation IC as a versatile tool for the monitoring and routine determination of transition and heavy metals. The versatility of complexation IC is due to the vast range of different metal-complexing forms and complex cation equilibria that can be utilized to control the resolution. Depending on the particular analytes and samples, different chromatographic techniques can be chosen and applied in order to obtain the greatest selectivity. Therefore, it may be concluded that at present complexation IC modifications do not compete but complement each other well.

Limits of detection of a few nanograms are typical in complexation IC, but after preconcentration limits as low as the parts per billion level can be obtained. For many metals these detection limits are comparable to those of instrumental methods such as atomic absorption, X-ray fluorescence or X-ray emission spectrometry. Further, in many instances the chromatographic separations achieved are difficult to obtain by other analytical methods. In comparison with multi-element techniques, such as inductively coupled plasma, atomic emission, and atomic fluorescence spectrometry, HPLC instrumentation is much less expensive and more accessible for many laboratories. The possibility of determining several elements without large-scale decomposition operations is one of the advantages of chromatographic methods over atomic absorption spectrometry. Electrochemical methods demand careful control over all experimental parameters and are more susceptible to matrix interferences than is complexation IC.

With regard to the relative merits of complexation IC and the tranditional techniques used in the determination of metal ions, it should be noted that complexation IC (i) is easily adapted to a variety of analytical problems and (ii) to the consecutive determination of metals in different oxidation states and non-metals in different chemicals forms; (iii) it can be combined with metal preconcentration methods; (iv) the analytical results hardly depend on sample treatment; (v) handling and maintenance are simple; and (vi) good technical equipment is available at comparatively low cost.

In fairness, some disadvantages of complexation IC should also be enumerated: the analysis times are comparatively long, ultrapure chemicals (reagents, buffers, solvents) and even metal-free HPLC systems must be used for ultra-trace analyses and considerable chemical expertise and knowledge are required for method development [5]. These are the major objectives of future improvements.

3. CONCLUSIONS

The HPLC determination of metal ions was first developed in the mid-1970s, the earliest achievements being largely attributable to normal- and reversed-phase HPLC of metal chelates. More recently, a shift has occurred towards the use of ion-pair reversed-phase HPLC of charged chelates, and subsequently IC methods, in which complex formation is also utilized to a greater or lesser extent. These are principally cation-exchange IC with postcolumn reaction detection, IC of metal complexes of chelate or non-chelate nature and coordination IC on chelating bonded phases. These chromatographic variants have rapidly gained importance owing to the growing demand for rapid methods for the determination of transition and heavy metals in complex matrices. The intense development of complexation IC seen recently is related to its main field of practical application, namely the analysis of environmental water samples [26,33,34], where this method is becoming the technique of choice.

An analysis of basic trends in the chromatographic determination of trace metals by complexation techniques suggests that adsorption HPLC of neutral metal chelates is gradually declining in popularity, although its modern modifications (supercritical fluid chromatography, chromatography with micellar mobile phases) look very promising. Among the complexation chromatographic methods considered here, non-suppressed cation chromatography already holds (or will soon take) the key positions. Ion-interaction IC on dynamically coated columns undoubtedly occupies a worthy place in the rank of complexation IC techniques. Nevertheless, at this stage it is difficult to evaluate the prospects of these methods, as they are highly developed technically and methodologically. The present situation with several powerful chromatographic methods competing in the practice of metal ion determinations may last for a long time, and only the future can tell which method will become the favourite, and when.

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