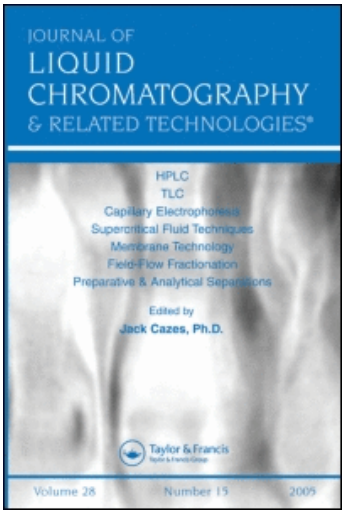

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REVIEW

ANALYSIS OF METAL IONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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1. INTRODUCTION

Metal cations are of great importance in biological and environmental systems. In living organisms many trace metals are micro-nutrients and are required for the functioning of certain enzymes, proteins, and electron transport systems. Other inorganic cations can be toxic and cause damage to cells or the functioning of carefully balanced biological systems. Not only the total concentration but also the chemical form or species of the metal can affect the functions. The term *species* refers to the actual form in which a molecule or ion is present in solution. Biological availability of metals and the physiological and toxicological effects of the metals depend on the species of the individual metal present. In some cases, one form of the metal ion can be an activator of an enzyme whereas another form is not an activator. In other cases a metal ion in a complex with a certain ligand can be toxic whereas the same metal ion in a different form is not toxic. For example, organic mercury is much more toxic to biological organisms than elemental mercury. The oxidation state of a metal can also determine the effectiveness or toxicity of a metal. For example, iron which is found in small quantities in cells is a necessary element for cultures of

unicellular algae. Recently it was hypothesized that the iron in seawater may limit the primary productivity in some oceanic areas where there is a relatively high concentration of major nutrients such as nitrate. However, the phytoplankton in seawater can only take up the truly dissolved Fe as its micro-nutrients. Fe(II) is much more soluble than Fe(III) in seawater, and Fe(II) can easily be taken up by the phytoplankton. Thus the truly dissolved iron, especially Fe(II) concentration, instead of the total iron concentration in atmosphere and in seawater, may directly affect not only the geochemical cycling of iron but also the biological productivity in the ocean. Therefore the determination of the concentration of specific species of trace metals, such as Fe(II) instead of total iron, is essential for the understanding of the biochemical behavior or toxicity to organisms and the geochemical cycling of trace metals in the environment.

Since metal ions of interest are often present in trace or ultra-trace levels, very sensitive and reproducible techniques are required to achieve accurate and reliable measurements. Many sensitive analytical methods, which can be classified as non-separation and separation methods, have been used for the determination of trace metals. . Non-separation methods include atomic absorption spectroscopy (AAS), inductively coupled plasma emission spectrometry (ICPES), ultraviolet and visible spectrophotometry (UV/VIS), flow injection chemiluminescence analysis (FIA), gas-phase atomic fluorescence spectrometry (AFS), neutron activation analysis (NAA), and analytical voltametry. Separation methods include chromatographic methods and capillary electrophoresis (CE). In this paper the determination of metal ions by high performance liquid chromatography will be reviewed and other methods used in the determination of metal ions will be discussed briefly.

1.1. Non-separation Techniques

1.1.1. Atomic absorption spectroscopy*(AAS)*

Atomic absorption spectroscopy has been widely used for the determination of metals because of its sensitivity and specificity. In addition it is relatively inexpensive (1-35). In principle, AAS employs a narrow-line source emitting the energy required for the transition of an element from the ground electronic state to an upper excited electronic state. Unexcited atoms of the element absorb radiation from the source and transfer it to the excited state. Absorption is measured by the difference in the transmitted signal in the presence and absence of the element of interest. Since the energy source is specific for the element of interest, only a single element can be analyzed at a time. The experimental conditions for the AAS of most metal elements have been established. However, AAS does not give different signal responses for different states of the same element; thus only

the total amount of the metal ion is measured and the different chemical forms and/or oxidation states of the same element cannot be distinguished, e.g., Cr^{3+} vs. Cr^{6+} and Fe^{2+} vs. Fe^{3+}. In addition, AAS cannot be used for simultaneous multi-element determination and a separate lamp source is needed for each metal. These shortcomings limit the use of AAS.

1.1.2. Inductively coupled plasma emission spectrometry*(ICPES)*

Inductively coupled plasma emission spectrometry is now being used for the rapid multi-element analyses of metal ions (1, 36-38). The plasma, e.g., an argon plasma, is a gas which is partially ionized by high temperature or is formed electromagnetically by radio frequency induction-coupling of argon. The analyte is introduced directly into the center of the plasma and is heated for about 2 msec. The high temperature ensures the complete breakdown of chemical compounds and prevents the formation of other interfering compounds. The plasma excites the ions or the elements of the analyte to the point of light emission and the ion lines or neutral atom lines are detected. Since no electrode is in contact with the plasma, there is low background and a high signal-to-noise ratio; thus detection limits are low, typically in the parts-per-billion range. However, the instrument requires substantial initial capital investment and skilled operators, which limits the availability of ICPES in many laboratories, both for routine work and research.

1.1.3. Ultraviolet and visible spectrophotometry *(UV/VIS)*

The direct photometric determination of metals is not possible because low wavelengths (<200 nm) must be used and other inorganic species, which may be present in the analyte, absorb at these wavelengths. Ultraviolet and visible spectrophotometry can be used for the determination of metal ions if the metal ions form stable colored complexes which absorb UV/VIS light. The absorption is measured in the presence and absence of the colored complex and the amount of the metal ion is determined by the difference in absorption. Quantitative analysis must be carried out in the linear range where the concentration of the metal ion of interest is proportional to the absorption of the complex. Because there is no separation in the method, the complexing reagent must be specific for the metal ion of interest and caution must be taken to prevent interference from other metal ions. Thus, only a single metal ion can be analyzed at a time. For example, manganese was analyzed by complexation with 1-(2-pyridylazo)-2-naphthol (PAN) and iron(II) was analyzed by complexation with ferrozine (27, 28, 39-43). The methods are simple and inexpensive. However, a complexing reagent, such as ferrozine, absorbs radiation at the same wavelength as the complex, thus considerable error can be introduced since an excess of the complexing reagent must be used and the amount is unknown. In addition, UV/VIS

spectrophotometry is a less sensitive method than AAS or ICP and simultaneous multi-element analyses cannot be achieved.

1.1.4. Flow injection chemiluminescence analysis *(FIA)*

Flow injection analysis with chemiluminescence detection has been used for the determination of metal ions, such as cobalt, manganese, and iron(II) (44-46). The FIA method employs a chemiluminescence reaction between a metal ion and a reagent to produce photons at a rate proportional to the concentration of the metal ion in the solution. As an example, the reagent used for the analysis of Fe^{2+} was a solution of H_2O_2 and alkaline brilliant sulfoflavin (BSF, sodium 4-amino-N-(*p*-tolyl)-naphthalimide-3-sulfonate) (46). The FIA method is sensitive and the metal ions can be detected in the subnanomolar to picomolar range. In addition, the FIA system is simple and can be readily adapted to automated analyses. However, interference by other ions, such as alkaline-earth cations or Fe^{3+} in the analysis of Fe^{2+} complicates the sample handling and data analysis, and the FIA method is not suitable for simultaneous multi-element analyses.

1.1.5. Gas-phase atomic fluorescence spectrometer *(AFS)*

Gas-phase atomic fluorescence detection has been applied to the determination of the concentration and chemical speciation of mercury in surface waters (47). Because this detection system does not require a monochromator, focusing lenses, or housing to eliminate ambient light, the light attenuation associated with dispersive systems is reduced and the sensitivity of this system is increased. The sensitivity of the atomic fluorescence detection system is about 0.5 pg of Hg and the linear working range extends between 0.01 and 20 ng of Hg.. However, at present this method can only be used for the determination of mercury because the metal ions must be in the gas phase.

1.1.6. Neutron activation analysis *(NAA)*

Neutron activation analysis is a mature technique used to determine the concentration of metals in various samples, including biological, environmental, geological, and industrial product samples (48-68). The sample is placed in a flux of energetic neutrons which had been produced by a reactor, and a radio-nuclide product is generated. The radio-nuclide product is then put in a radioactivity counter for the measurement of each metal with the desired statistical precision. With this technique the concentrations of many elements can be measured in one sample and the detection limits for many elements are in the subnanomolar to picomolar level. In addition the sample is not destroyed in the analysis. However neutron activation analysis requires the use of a reactor, which is expensive and not available to many researchers, and the concentration of different species of metals cannot be determined.

1.1.7. Analytical voltammetry

Analytical voltammetry has been successfully used in the determination of the concentrations of metal ions. In principle, voltammetry is a two step technique. In the first step a portion of the metal ions in the solution is deposited electrolytically on an electrode, e.g., a hanging mercury drop electrode. In the second step the electrode is stripped by the application of a linear potential sweep and the metals are changed back to their ionic forms. Current or potential is measured during the second step. The most widely used voltammetric techniques in the determination of trace metals are adsorptive stripping and anodic or cathodic stripping methods (69-86). The adsorptive stripping method includes coprecipitation of a metal with a ligand or the use of modified electrodes that preferentially adsorb a given ion. These techniques are very sensitive and highly selective, and the concentration of some species, including the free ions of some metal ions can be measured. However they usually need specially modified electrodes.

1.2. Separation Techniques

The speciation of trace metals is of prime importance in the field of biogeochemical cycling and in studies of the toxic effects of metals in the environment. In many cases the toxicity of metals is related to the concentration of a specific species of the metal instead of the total metal concentration. In addition the biogeochemical cycling of some metals is often dependent on the redox states of the metals . For the determination of speciation as well as total concentration of trace metals, separation techniques such as capillary electrophoresis (CE), gas chromatography (GC), thin-layer chromatography (TLC), gel permeation chromatography (GPC), ion chromatography (IC), normal phase and reversed-phase high performance liquid chromatography (RPHPLC) have been used

1.2.1. Capillary electrophoresis *(CE)*

Capillary electrophoresis is a separation technique that is characterized by high speed, high efficiency, great mass sensitivity, low sample consumption, and high resolution. Since the publication of the early articles on high performance capillary electrophoresis (HPCE) by Hjerten (87) in 1967, Virtanen (88) in 1974 and Mikkers et al. (89)in 1979, capillary electrophoresis has been applied successfully to the separation and analysis of a variety of simple and complex molecules, including metal ions (87-102). Electrophoresis is an old technique in which the separation of charged molecules is based on differential migration in an applied potential field. While the neutral molecules remain at the site of sample introduction, the cations migrate to the negative electrode and the anions migrate to the positive electrode. For capillary electrophoresis the technique was modified so that the separation takes place in a capillary tube. The instrumentation in capillary

electrophoresis is simple and consists of a high voltage power supply (5-30 kV with low current of 20-200 μA), two buffer reservoirs, a capillary, a detector, and a data acquisition system. Although CE is only a little over 20 years old, the recent development of CE techniques has made possible the separation and the detection of various species of metals. Capillary electrophoretic separations of metal ions are based on the different electrophoretic mobilities of metal ions in the capillary under a positive power supply. With CE many metal ions (e.g., alkali, alkaline earth, transition, and lanthanide metals) can be separated in a few minutes with good resolution (90). Although detection limits with CE are still a problem in the determination of ultra-trace amount of metals, the recent work by Weston et al. (91) has shown that there is a real potential for the use of CE in environmental and biological studies.

1.2.2. Gas chromatography *(GC)*

Gas chromatography is an established technique for the separation and determination of volatile, heat stable, inorganic and organometallic substances (103-125). Forty years ago Lederer suggested that the formation of neutral metal chelate complexes might be used for solving the problem of the lack of volatility of metal ions (126); for example, the determination of aluminum at the nanomolar level by the formation of a neutral compound with 1,1,1-trifluoro-2,4-pentanedione (127). However for most metal ions, the problem of volatility still remains. Because useful ligands are readily not available for forming neutral, highly stable species with metal ions, application of GC for the determination of metal ions is limited. Similarly, GC is severely limited for analyzing samples of metal-organic compounds because the method usually requires high temperatures at which many of these species either lack volatility or undergo decomposition.

1.2.3. Thin-layer chromatography *(TLC)*

Thin-layer chromatography (TLC) has been used for the separation of metallic substances (128, 129). For quantitative analysis of metals, a second technique such as AAS and UV/VIS must be used after the TLC separation. Therefore it is not convenient to determine metal ions using TLC separations, although the technique was used for the separation and detection of mercuric chloride, copper sulfate, cadmium sulfate, and silver nitrate in fresh water (128). TLC was also found useful in the identification of nickel(II) mixed-ligand complexes from single-ligand complexes (129). However TLC is not generally used for the analysis of metal ions because it is time consuming and not as quantitative as other techniques.

1.2.4. High performance liquid chromatography *(HPLC)*

High performance liquid chromatography is a routine method for the determination of organic and biological compounds, but it has not been widely used for the separation and determination of inorganic metal ions because of a lack of suitable detection techniques. However, with the development of element selective and sensitive detectors and indirect detection (130) HPLC is becoming more important in trace metal analysis. In addition, the formation of metal complexes can increase the sensitivity of UV/VIS, fluorescence, and electrochemical detectors commonly used with HPLC. Therefore, HPLC can be an effective means for the separation and determination of many metals as metal coordination complexes. Metal ions can be resolved on the basis of size (gel permeation chromatography, GPC), charge (ion chromatography, IC), absorption (normal phase, NP), and hydrophobicity (reversed phase, RP). In this review we will discuss in detail the analysis of metal ions by high performance liquid chromatography.

2. ANALYSIS OF METAL IONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

2.1. Modes of Separation

The HPLC separation of metal species can be achieved using gel permeation, ion exchange, normal phase, or reversed phase chromatography. Since reversed phase HPLC is now the most predominant HPLC mode, metal species are usually separated by the reversed phase mode.

2.1.1. Gel permeation chromatography *(GPC)*

Gel permeation chromatography has been used for the separation of metallic substances (131-136). It is often applied to the separation of proteins containing metal ions and reveals the molecular weight ranges for the proteins at the same time. For example, GPC was used for the separation of iron proteins in plasma (131), cadmium proteins in rainbow trout hepatocytes (132), copper proteins in the marine bacterium Vibrio alginolyticus (133), heavy metals (Cd, Cu, Zn) proteins in the squid Onychoteuthis borealijaponica (134), zinc and cadmium proteins in the sea star Asterias rubens L (135), and cytosolic binding of Cd, Cu, Zn and Ni in four polychaete species (136). GPC also is a technique which requires another technique to identify and determine quantitatively the metal ions. Dual techniques complicate the analysis of metal ions and the determinations are usually not sensitive enough for ultra-trace analyses in environmental and biological investigations. Thus the use of GPC is limited.

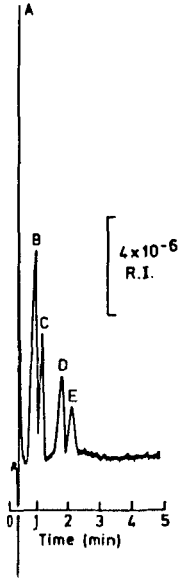

Figure 1. Separation of some inorganic cations, with indirect refractive index detection. Conditions: column, Wescan cation column; flow rate, 2.0 mL/min; mobile phase, 2.74 mM anilinium ion at pH 4.65; chart speed, 1.0 cm/min; injection volume, 20 μL; detector sensitivity, R.I. x 1. Peaks: A = solvent peak, B = Li^+ (0.4 μg), C = Na^+ (0.4 μg), D = NH_4^+ (0.4 μg), E = K^+ (0.4 μg). (Ref. 86, reprinted with permission of the publisher.)

2.1.2. Ion (exchange) chromatography *(IC)*

Cation species are analyzed by ion chromatography less frequently than are anionic species. Cations are generally analyzed by spectroscopic methods such as AAS or ICPES and by reversed phase HPLC. However, IC methods have been reported for the separation of alkali, alkaline earth, transition, and post-transition metals, including the highly radioactive and unstable man-made elements (137, 138). IC has been applied to the analysis of metal ions in various natural waters (139-143), in industrial or treated water (144, 145), and in the atmosphere (146).

An example of the separation of three alkali metal ions and ammonium ion by IC is shown in Figure 1 (147). Li^+, Na^+, NH_4^+, and K^+ were separated in less than 3 minutes on a 250 x 2.1 mm I.D. Wescan 269-004 cation column. The mobile phase was a 2.74 mM anilinium ion solution at pH 4.65 with a flow rate of 2.0 mL/min. Indirect refractive

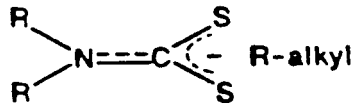

Figure 2. The structure of the dithiocarbamate ligand.

index detection was used with a sensitivity of R.I. x 1. The separation was rapid and the peaks were mostly baseline resolved.

Metal ions have been derivatized for IC (137). For example, dithiocarbamate was used as a derivatizing agent for metal ions prior to electrochemical detection (148). The structure of the dethiocarbamate ligand (dtc) is shown in Figure 2. The method employed a Dionex CS2 ion chromatography column. Under the applied potential, dtc can be oxidized. Post-column reaction of the metal ions with excess dtc ligand decreases the oxidation current. Thus indirect amperometric detection can be applied. Separation and determination of Ni^{2+}, Pb^{2+}, Cd^{2+}, and Fe^{2+} with indirect amperometric detection is shown in Figure 3. The metal ions were eluted with a solution containing 0.040 M tartaric acid and 0.012 M citric acid at the pH of 4.3 with NaOH. The flow rate was 1 mL/min. The detection limits of these metal ions was in the sub-ppm and low ppm levels. The separation is rapid and was completed in 8 minutes.

Another complexing agent, 4-(2-pyridylazo)resorcinol (PAR) (Figure 4), is often used in IC analysis (137, 149). A separation of Co^{3+} and Cu^{2+}+ using PAR is shown in Figure 5. The metal-PAR complexes will exist as anions or neutral species at a slightly acidic pH and can be detected by visible detection at 546 nm. Simultaneous determination of Co^{3+}, Fe^{3+}, and Cu^{2+} was achieved with a 100 x 4 mm weak anion exchange amino silica column coupled with a 10 μ, 100 x 4 mm C18 column. The mobile phase was a 50% aqueous solution containing 0.005 M Na_2HPO_4 at pH 6.5 and 50% methanol. The flow rate was 1.0 mL/min. The coupled columns offered good resolution of Co^{3+} and Fe^{3+}, a separation which cannot be achieved by the amino column alone. The detection limits were 0.017 ppm, 0.21 ppm, and 0.018 ppm for Co^{3+}, Fe^{3+}, and Cu^{2+}, respectively.

Because IC methods are simple and sensitive, it is a useful mode of separation for the determination of a number of metal species.

2.1.3. Normal phase HPLC

Normal phase HPLC has been used for the separation of organometallic compounds and metal complexes with organic ligands (137, 150-153). The method uses a polar stationary phase, e.g., silica, and a nonpolar eluent, e.g., hexane or benzene. A

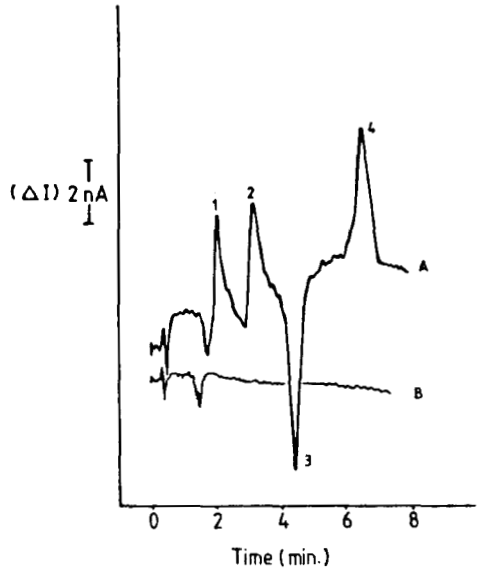

Figure 3. Chromatographic separation of metal ions with indirect amperometric detection (applied E = +0.65 V vs. Ag/AgCl). (A) 1.0 ppm Ni^{2+} (peak 1), 1.0 ppm Pb^{2+} (peak 2), 8.0 ppm Fe^{2+} (peak 3), and 1.0 ppm Cd^{2+} (peak 4) and (B) blank deionized water. (Ref. 87, reprinted with permission of the publisher.)

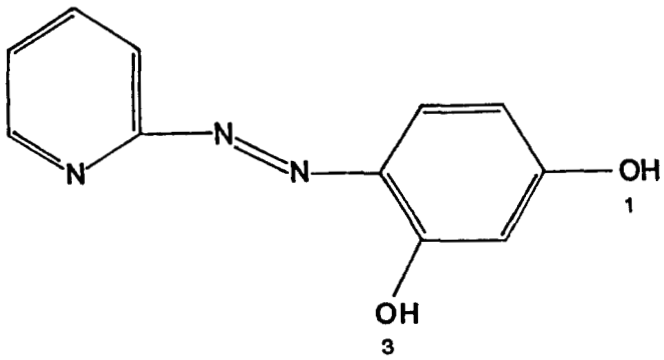

Figure 4. Structure of 4-(2-pyridylazo)resorcinol (PAR).

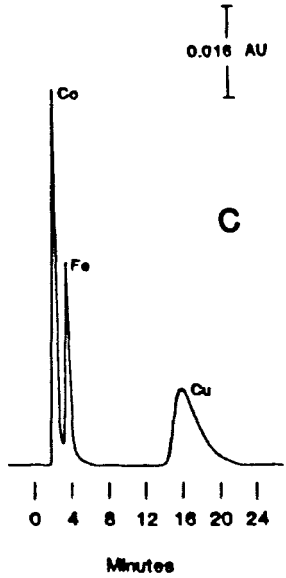

Figure 5. Separation of 1.1 ppm Co^{3+}, 3.0 ppm Fe^{3+}, and 5.1 ppm Cu^{2+} complexes of PAR on an amino silica column coupled with a C18 column. (Ref. 88, reprinted with permission of the publisher.)

separation of transition metals complexed with N,N-diethyl-dithiocarbamate (N,N-diethyl-dtc) is given in Figure 6. The chromatogram shows the simultaneous determination of Zn^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+} Co^{2+}, Cd^{2+} and Fe^{2+} complexed with N,N-diethyl-dtc on a 10 μm 200 x 4.5 mm LiChrosorb SI 60 column (152). The mobile phase was 10% chloroform in cyclohexane. The metal-complexes were detected with a UV detector at 254 nm. The separation was completed in 20 minutes.

Edward-Inatimi (153) also reported a HPLC method with a UV detector for the multi-element determination of trace metals. Dithizone or diethylammonium diethyldithiocarbamate (DDDTC) was used as the coordination ligand to complex five metals (Ni^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+}) and chloroform was used as a pre-column liquid extraction solvent. The metal-dithizone or metal-DDDTC complexes were baseline separated on a stainless steel column (150 x 4.6 mm) packed with Hypersil 5 μm diameter silica gel. The method was applied to water samples collected from the River Thames at the estuary of the river in the North Sea and to other samples from kale and fish. Figure 7 shows the separation of dtc complexes of Cu^{2+}, Ni^{2+}, and Mn^{2+} from a fish sample. The

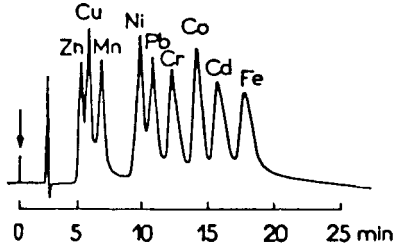

Figure. 6. Separation of a synthetic mixture of N,N-diethyl-dtc complexes of Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(III), Co(II), Cd(II) and Fe(II). (Ref. 91, reprinted with permission of the publisher.)

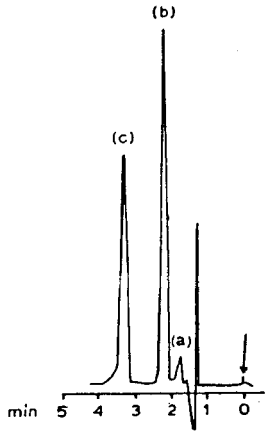

Figure 7. Fish sample extracted at pH 8.5 with diethylammonium diethyldithiocarbamate solution in chloroform. (a) complex of Cu, (b) complex of Ni, and (c) complex of Mn. (Ref 92, reprinted with permission of the publisher.)

mobile phase was Spectragrade benzene. The separation was rapid and was completed in 4 minutes. This method was simple but the detection limit of 50 ppb was not satisfactory.

2.1.4. Reversed phase HPLC

In recent years, the HPLC analysis of metal ions has been dominated by reversed phase HPLC (RPHPLC). By forming metal coordination complexes, sensitive analyses of metal ions can be obtained using various types of detectors.

2.2. Detection Methods

2.2.1. UV/VIS spectrophotometric detection

UV/VIS detection is one of the most popular detection methods for high performance liquid chromatography. Direct UV/VIS detection is attractive whenever it is applicable. Unfortunately, only mercury has sufficient optical absorption to permit sensitive detection directly. The method of choice has been to form a highly absorbing stable metal complex and then to monitor the optical absorption of the complex. UV/VIS detection of metal complexes has been used for many applications and showed excellent agreement with results obtained by AAS analyses (154-171). Ligands used for complexation in the analysis of metal ions will be reviewed later in this paper.

Some natural occurring metal organic species, e.g., copper-organic complexes, can produce sufficient optical absorption for sensitive UV/VIS detection (172, 173). Thus direct UV/VIS detection can be applied.

2.2.2. Electrochemical detection

Electrochemical detection is attractive because of its high sensitivity. It is another major detection method for the determination of metal complexes. Electrochemical detection of metal complexes is based on the oxidation or reduction of the ligands. Many applications of electrochemical detection have been reported (164-170). A three electrode system, consisting of a platinum auxiliary, a Ag/AgCl (3 M KCl) reference and a glassy carbon working electrode, is often the choice for the electrochemical detection of metal complexes (164-170, 173, 174). However, a mercury electrode was used by Bond and Mclachlan (175) as the working electrode for the direct determination of tetraethyllead (TEL) and tetramethyllead (TML) in gasoline by HPLC with electrochemical detection. The separation of the tetraalkyllead compounds was achieved on a 150 x 3.9 mm C18 column (Figure 8).

2.2.3. Indirect detection

Indirect detection has been applied to liquid chromatography since the early 1980s (130). It is especially useful for the detection of non-absorbing species. With indirect detection, a highly absorbing species is added to the mobile phase and offers a constant large background absorbance. Non-absorbing analytes displace equivalent amount of mobile phase from the absorbing species and lower the absorbance in the flow cell. The difference of absorbance generates a negative signal and can be recorded on the chromatogram. Similarly, indirect fluorescence detection can be achieved by introducing a fluorophore into the mobile phase.

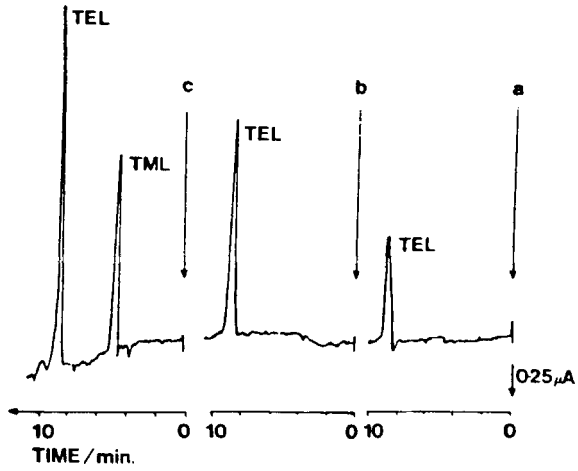

Figure 8. Chromatograms with electrochemical detection (+0.63 V) at a hanging mercury drop electrode for 5 μL injection of different gasoline samples using acetonitrile (0.05 M Et4NClO4) as eluent. (a) premium motor gasoline containing 0.30 g/L Pb (1.5 μg) as TEL, (b) aviation gasoline containing 0.55 g/L Pb (2.7 μg) as TEL, and (c) premium motor gasoline containing 0.84 g/L Pb (4.2 μg) as TEL and 0.32 g/L Pb (1.6 μg) as TEL. (Ref. 114, reprinted with permission of the publisher.)

Indirect detection is well-suited for measurements in systems with small volumes and/or short light paths. It has been well adapted in the analysis of metal ions by capillary electrophoresis (90, 91). A highly UV absorbing aromatic amine was used to obtain indirect photometric detection and to ensure symmetrical peak shape for the determination of many inorganic divalent metal ions. Detection limits were in the low to mid-parts per billion ranges. With micro-HPLC, indirect detection is a promising method of detection.

2.2.4. Other Detection Methods

Other sensitive detection methods, e.g., AA, ICP, atomic fluorescence, and molecular fluorescence, have been used for the detection of metal species. The determination of organoarsenicals and organotin compounds by LC-AA have been reported in a number of papers (176-179). However, since AA can only detect a single element, it is not applicable for multi-element detection. Although ICP limits of detection are very low (180, 181), the high cost of an ICP makes detection by that technique not available for many applications. Fluorescence detection is very sensitive, an order of 3 magnitudes more sensitive than that obtained with UV and atomic fluorescence detection has been used

for the detection of marine metal-organic complexes (182-184). High-intensity lamps were used to improve the sensitivity by 1-2 orders of magnitude, e.g., 0.05 μg/L for Zn^{2+} and 7 μg/L for Fe^{3+} (182). Molecular fluorescence detection was also used for the determination of metal ions, such as Zn^{2+}, Cd^{2+}, and Pb^{2+} with the complexation of fluorescent 4-aminophenyl-EDTA (185). Because metal complexes that fluoresce often precipitate (e.g., 8-hydroxyquinoline complexes), the use of fluorescence detection is limited for metal ion analysis. With the appearance of new fluorescent ligands, more applications of sensitive fluorescence detection are expected.

2.3. Metal Coordination Complexes

2.3.1. General

Early in 1969, HPLC was first applied to metal-organic systems (186). There are two methods of introducing ligands for the formation of metal complexes. (1) *In situ* formation in which excess ligands are added to the mobile phase and complexes are formed upon the injection of metal ion samples (164-168, 173, 174). *In situ* formation of metal complexes requires complete, rapid reactions between the metal ions and the ligands. (2) External formation in which the metal complexes are formed before the injection onto the HPLC column (154-162, 166, 169, 174, 187). Sample preconcentration can be applied by using the external formation of metal complexes. The preconcentration methods include liquid-liquid extraction and solid phase extraction. Since most metal complexes are neutral, chloroform (154-156, 158-160) or acetonitrile (157) are used as the solvent for liquid-liquid extraction. Solid phase extraction can be achieved by using Sep-Pak C18 cartridges (174, 187).

Bond and Wallace (166, 174) investigated the methods for the formation of metal-dithiocarbamate complexes in detail and concluded the following: (1) external formation was simple but it was not applicable to the multi-metal determination of eight metals (Cu^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Se^{4+}, and Cr^{3+}); (2) liquid-liquid extraction was recommended for the determination of the eight metals with chromium in two oxidation states. The extraction procedures have the advantage of sample clean-up and preconcentration; (3) solid phase extraction was a better procedure than the liquid-liquid extraction for clean-up and to concentrate the samples.

Organic compounds often used as coordination ligands are dithiocarbamate (154-161, 164-167, 173, 174, 188, 189), 4-(2-pyridylazo)resorcinol (162, 163), 8-quinolinol (168, 169, 190, 191), cupferron (170), ferrozine (187), *b*-diketonates (192, 193), and dithizonate (194, 195).

2.3.2. Specific coordination reagents and separations

2.3.2.1. Dithiocarbamate *(dtc)*

Dithiocarbamate is the most widely used coordination organic compounds. Its structure is shown in Figure 2. Dithiocarbamate can complex rapidly with many metals and form neutral complexes which are extractable into organic solvents. The advantage of the dithiocarbamate complexes is their insolubility in inorganic solvents; thus, the coordination complexes can be extracted with organic solvents and the neutral chelate can be separated from the excess ligand. Because of this advantage, this method has widespread use in the HPLC separation of trace amounts of metals. In addition, dithiocarbamate is often used for the *in situ* formation of metal complexes since the complexing reaction is rapid and complete. Metal complexes of dithiocarbamate are thermodynamically stable and kinetically inert even when the concentrations of the complexes are at the 10 nM level (159).

Liska *et al.* (134, 150-152) conducted a series of HPLC analyses of metal-complexes of N-dissubstituted dithiocarbamic acids and reported a normal-phase separation of Zn^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+}, Co^{2+}, Cd^{2+} and Fe^{2+} on a stainless-steel column with a UV detector at 254 nm. Bond and Wallace (164, 166, 167, 174, 188, 189) developed a series of RPHPLC methods to determine metal concentrations, in which dithiocarbamate was used as a coordination ligand to complex the trace metals. These methods included the simultaneous determination of Cu^{2+}, Ni^{2+}, Co^{2+}, Cr^{6+} and Cr^{3+} with an electrochemical detector, the simultaneous determination of Cd^{2+}, Co^{2+}, $Cu,^{2+}$ Pb^{2+}, Hg^{2+}, and Ni^{2+} with UV and EC detectors, and the automated determination of Pb^{2+}, Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+} by HPLC with both UV and EC detectors.

Hutchins *et al.* (160) reported a reversed-phase HPLC separation of diethyldithiocarbamate complexes of Co^{3+}, Cr^{3+}, Cu^{2+}, Hg^{2+}, Ni^{2+} Pb^{2+}, Se^{4+}, and Te^{4+} using a radial compression column with a UV detector at 254 nm. However, they also found that the diethyldithiocarbamate complexes of Pb^{2+}, Cd^{2+}, and Fe^{3+} gave poor peak shapes because of the substitution reaction with nickel from the stainless steel components of the chromatographic system.

Shih and Carr (159) synthesized a novel dithiocarbamate ligand, n-butyl-2-naphthylmethyldithiocarbamate, and used it in the determination of trace metals by HPLC. The dithiocarbamate complexes of Ni^{2+}, Fe^{3+}, Cu^{2+}, Hg^{2+}, and Co^{2+} have been "baseline" separated on a C18 column with a variable-wavelength absorbance detector at 221 nm. They indicated that the metal-dithiocarbamate complexes were thermodynamically stable and kinetically inert at 10^{-8} M level and the detection limits were about 1-2 ng.

King and Fritz (161) used bis(2-hydroxyethyl)dithiocarbamate as a pre-column derivatizing reagent and determined its complexes with Ni^{2+}, Cu^{2+}, Hg^{2+}, and Co^{2+} by

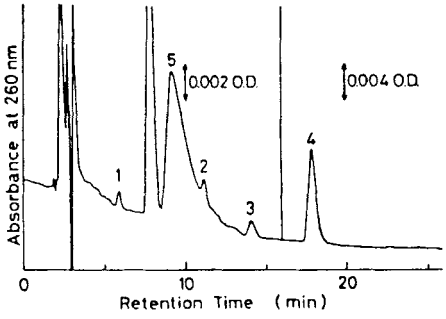

Figure 9. Typical chromatogram obtained from river water samples. (1) Cd (0.7 ppb), (2) Ni (1.4 ppb), (3) Co (1.4 ppb), (4) Cu (40 ppb), (5) Zn. (Ref. 94, reprinted with permission of the publisher.)

reverse phase HPLC on a C18 column with a UV detector. Because the metal complexes were water soluble, they were not extracted with an organic solvent prior to injection, but little interference was found in the determination.

Mueller and Lovett (157) introduced a salt-induced phase separation of acetonitrile for direct injection in the determination of Pt^{2+}, Pd^{2+}, Rh^{2+}, Co^{2+} Ru^{3+} and Ir^{3+} as their diethyldithiocarbamate complexes by reverse phase HPLC with UV detector. The detection limit for the metals was < 3 ng of metal/mL of original aqueous sample.

Ichinoki *et al.* (155) developed a simple, rapid method for the simultaneous determination of Ni^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+} Bi^{3+}, and Co^{2+} in water samples. Hexamethylenedithiocarbamate (HMDC) was used as the chelate to complex the metals in the river water samples. The HMDC chelates were extracted into chloroform by shaking for 15 min and then separated on a 150 x 4.6 mm C18 column by HPLC with a UV detector set at 260 nm. A typical chromatogram of the metal ions in river water samples is shown in Figure 9. Ichinoki *et al.* reported a recovery of 99.2-101.5% and a precision of 0.5-1.2%. The detection limits were 45-600 pg of metals and the linear standard curve for the six metals were in the concentration of 0.3-2000 ppb with the correlation coefficients of over 0.998. With this method the concentrations of the trace metals were determined in water samples collected from the Tamatani, Gotani, and Kakehashi rivers in Japan. The results from this method were in a good agreement with those measured by flameless AAS method (155).

Bond and Wallace (167) developed a microprocessor-based instrumental method for automated monitoring of Ni^{2+} and Cu^{2+} by RPHPLC with electrochemical and

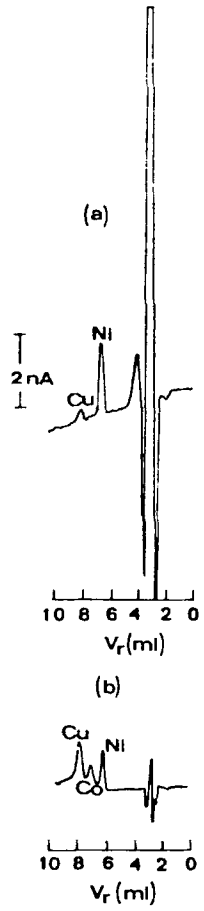

Figure 10. Determination of copper and nickel in an industrial effluent sample. (a) electrochmical detection at +0.75 V vs. Ag/AgCl. (b) UV/VIS detection at 400 nm. Chromatographic eluent: 70%:30% acetonitrile: acetate buffer (0.02 M) pH 6, 0.005 M $NaNO_3$, 0.1 mM $[dedtc]^-$; flow rate, 1 mL/min. (Ref. 106, reprinted with permission of the publisher.)

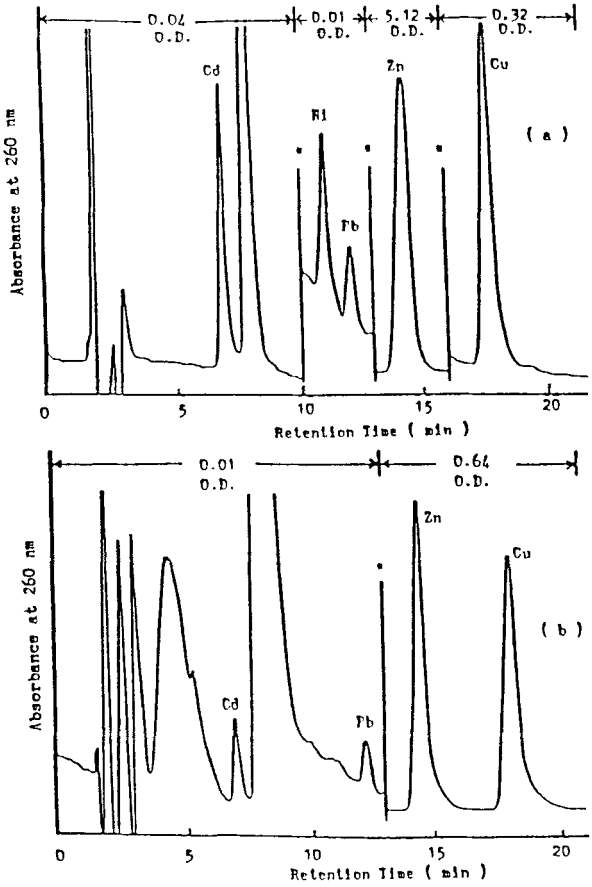

Figure 11. Chromatograms of samples of (a) oyster tissue and (b) bovine liver. (Ref. 95, reprinted with permission of the publisher.)

spectrophotometric detection in a wide variety of samples collected from industrial plants. They used dithiocarbamate as the coordination ligand and the *in situ* formation method to complex Ni^{2+} and Cu^{2+} in the samples. The results measured by this method for various industrial plant solutions were compared with data obtained by AAS (167) and there was an excellent agreement. Chromatograms with UV/VIS and electrochemical detection are shown in Figure 10.

Ichinoki *et al.* (156) measured simultaneously the concentrations of Cd^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Bi^{2+} in bovine liver and oyster tissue by RPHPLC with

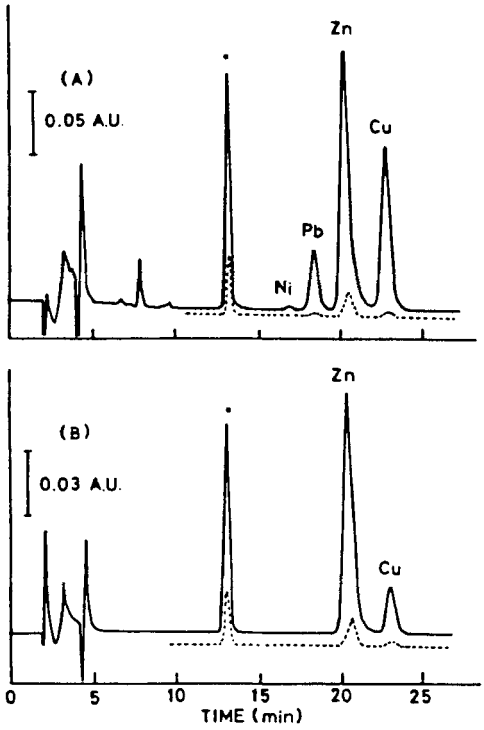

Figure 12. Chromatograms of samples of (A) citrus leaves and (B) rice flour. (Ref. 97, reprinted with permission of the publisher., reprinted with permission of the publisher.)

UV detector at 260 nm (Figure 11). They used hexamethyleneammonium hexamethylenedithiocarbamate (HMA-HMDC) as the coordination ligand. The metal-HMA-HMDC complexes were extracted into chloroform and separated on a 150 x 4.6 mm C18 column. The bovine liver or oyster tissue samples was ashed in a muffle furnace over night at 500-550°C. The ash was treated with 2 N HCl. The metal concentrations were determined accurately over a range of 0.5-850 ppm with a standard deviation of 7%.

Ichinoki and Yamazaki (158) also reported a simultaneous determination of Ni^{2+}, Pb^{2+}, Zn^{2+}, and Cu^{2+} in citrus and rice flour by HPLC with hexamethylenedithiocarbamate extraction (Figure 12). A 250 x 4.6 mm C18 column was used and the UV detection was at 260 nm. The results showed that the detection limit of this method was in the parts-per-million levels of the trace metals in biological samples.

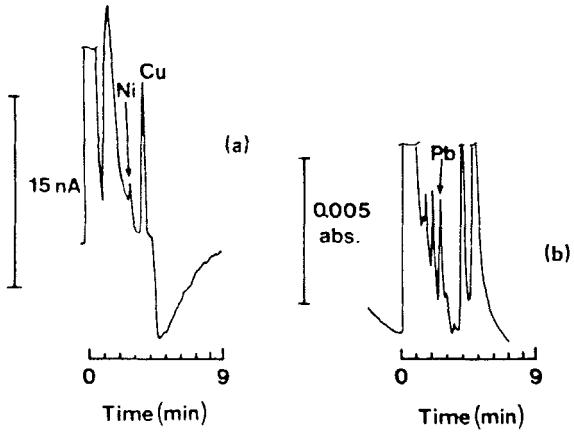

Figure 13. Chromatograms for the simultaneously determination of (a) copper and nickel (electrochemical detection, +0.8 V vs. Ag/AgCl) and (b) Lead (spctrophotometric detection, 260 nm) in a freshly acidified urine sample. (Ref. 104, reprinted with permission of the publisher.)

2.3.2.2. 4-(2-Pyridylazo)resorcinol *(PAR)*

The structure of 4-(2-Pyridylazo)resorcinol is shown in Figure 4. Because of its sensitivity and solubility in water, 4-(2-Pyridylazo)resorcinol is one of the best chelating reagents for external complexation of many metal ions. Zhang *et al.* (163) reported the simultaneous determination of Mo^{3+}, Cr^{3+}, and V^{3+} with 4-(2-Pyridylazo)resorcinol by ion-pair HPLC. A 6 μm 250 x 4.6 mm Zorbax CN column was used. Hydroxylamine and tetrabutylammonium iodide were used as ion-pairing reagent. The eluted metal-PAR complexes were monitored by a spectrophotometric detector at 540 nm. The peaks of the complexes were well resolved and the analysis was completed in less than 10 minutes.

Hoshino and Yotsuyanagi (162) studied the extraction behavior of Fe^{2+}-PAR and Fe^{3+}-PAR. They also reported the separation of PAR complexes of Co^{2+}, Fe^{2+}, V^{3+}, and Ni on a 250 x 4 mm Yanapak ODS-T column with a spectrophotometric detector at 500 nm.

Bond *et al.* (165) developed a rapid, simultaneous determination method for analyses of the metal concentrations in urine by HPLC with EC or UV detectors (Figure 13). They injected directly the acidified and filtered urine samples onto a μBondapak C18 column with a guard column packed with Bondapak C18/Corasil for sample clean-up. The metal chelate ligand, dithiocarbamate, was included in the mobile phase. They were able to

determine simultaneously the concentration of Cu^{2+}, Ni^{2+}, and Pb^{2+} in fresh acidified urine with EC detection (0.8 V vs. Ag/AgCl) for Cu^{2+} and Ni^{2+} and UV detection (260 nm) for Pb^{2+} at the concentration levels where health problems may arise.

2.3.2.3. 8-Quinolinol (8-Q)

8-Quinolinol is a good ligand for use in the separation of multi-element mixtures by HPLC. 8-Quinolinol can form complexes with many metal ions to produce neutral chelates. Since several of these chelates posses native fluorescence, the sensitivity of the determination can be improved considerably by using fluorescence detection.

Berthod *et al.* (190) first reported the separation of multi-metal ions, including Cu^{2+}, Co^{2+} Ni^{2+}, Hg^{2+}, and Fe^{2+}, by complexation with 8-hydroxyquinolates. RPHPLC with three modes of detection were used: UV absorption, atomic absorption, and electrochemical detection. Al^{3+} and Co^{3+} were also separated with a silica column by using 8-hydroxyquinolates as a chelate (190). Methanol-chloroform (5%) was used as the mobile phase with UV detection at 254 nm. The two metal ions can be separated in less than 5 minutes and determined at nanogram levels.

Bond and his colleagues (168) successfully used 8-quinolinol as the coordination ligand to separate and determine simultaneously Al^{3+}, Cu^{2+}, Fe^{3+}, and Mn^{2+} in oyster tissue and bovine liver samples, as well as drinking water, river water, and coastal seawater using RPHPLC with EC or UV detector. Recently, Nagaosa *et al.* (169) also developed a separation and simultaneous determination of Al^{3+}, Fe^{3+}, and Mn^{2+} in river and coastal waters by using HPLC with either spectrophotometric or electrochemical detection (Figure 14). They used 8-quinolinol as the coordination ligand and the metal-8-quinolinol complexes were separated on a Bondasphere ODS column. The mobile phase was acetonitrile/20 mM acetate buffer solution (2:3) containing 5 mM 8-quinolinol reagent. The concentrations of the three metals were monitored by UV/Visible detector at 390 nm. The detection limits of these metals were at the part per billion levels. Using this method they measured the concentrations of these metals in rivers and seawater and the results were in agreement with the data obtained by AAS. The method was simple and there was no interference in the matrices from the alkali and alkaline-earth metals. However, the detection limits of this method were not satisfactory to measure the concentrations of these metals in the open ocean. With a preconcentration techniques such as liquid-liquid extraction or a C18 cartridge column, the detection limits and the sensitivity of this method could be improved.

Bond and Nagaosa (168) determined the concentrations of Al^{3+}, Cu^{2+}, Fe^{3+}, and Mn^{2+} in oyster tissue and bovine liver using 8-quinolinol as the chelate ligand to complex the metal ions by RPHPLC with EC and UV detectors. The mobile phase was 1:1

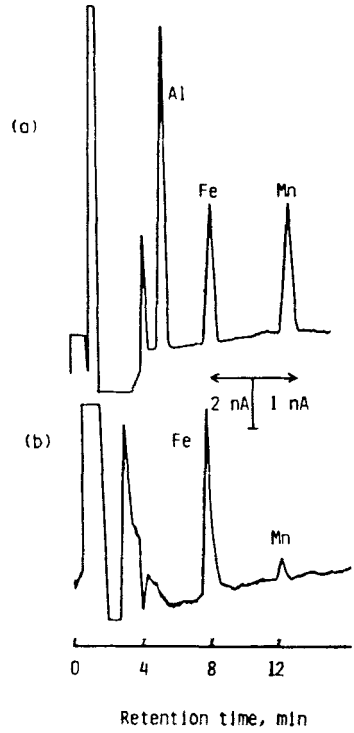

Figure 14. Chromatograms for the simultaneous determination of Al(III), Fe(III), and Mn(II) in Asuwa River water with (a) spectrophotometric and (b) electrochemical detection. Sensitivity: 0.02 AUFS for Al(III); 0.01 AUFS for Fe(III); 0.0002 AUFS for Mn(II). (Ref. 108, reprinted with permission of the publisher.)

acetonitrile/water containing 5 x 10^{-3} M 8-quinolinol, 0.4 M potassium nitrate and 0.02 M acetate buffer. The EC detection was at -0.5 V vs. Ag/AgCl and UV detection was at 400 nm. They were able to determine <2 ng Cu and < 1 ng Fe with injection volumes of 20 µL. They also used Sep-Pak cartridges for preconcentration after dichloromethane extraction. The results in the bovine and oyster tissue obtained by this method were in a good agreement with the certified values provided by NBS or determined by AAS and differential pulse polarography methods.

2.3.2.4. Cupferron

Cupferron ($C_6H_5N(NO)(ONH_4)$) was recently used to form complexes with Cu^{2+} and Fe^{3+} for the determination of the two ions in biological and river water samples.

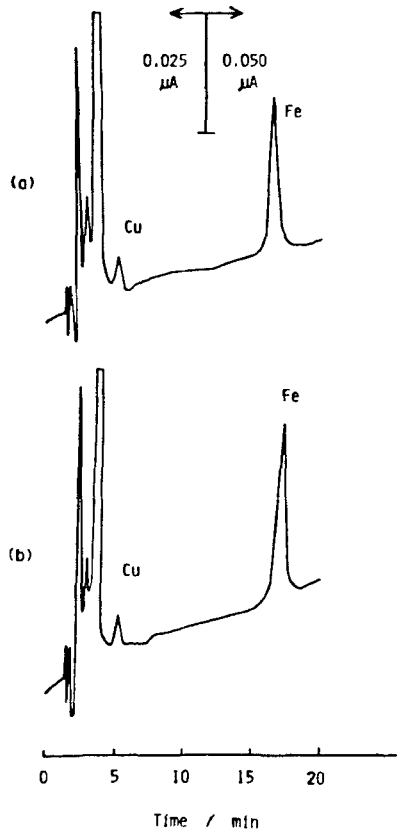

Figure 15. Chromatograms of the simultaneous determination of Cu and Fe in drinking water after ethyl acetate extraction with electrochemical detection (-0.40 V vs. Ag?AgCl). (a) Sample solution; (b) standard solution containing 12.5 μg/L Cu(II) and 200 μg/L Fe(III). (Ref. 109, reprinted with permission of the publisher.)

Nagaosa *et al.* (170) simultaneously determined Cu^{2+} and Fe^{3+} concentrations in river waters by reversed-phase HPLC with EC and UV detectors, They used cupferron as a pre-column coordination ligand. The metal cupferronates formed in acetonitrile-water (1:1) were injected on a 5 μm, 150 x 4.6 mm ODS column. The mobile phase was acetonitrile-water (7:3) that contained 10^{-3} M cupferron, 0.02 M sodium acetate buffer and 0.2 M potassium nitrate. Amperometric detection with a glassy carbon electrode at -0.40 V vs. Ag/AgCl was used (Figure 15). This work indicated that the trace metals Cu^{2+} and Fe^{3+}

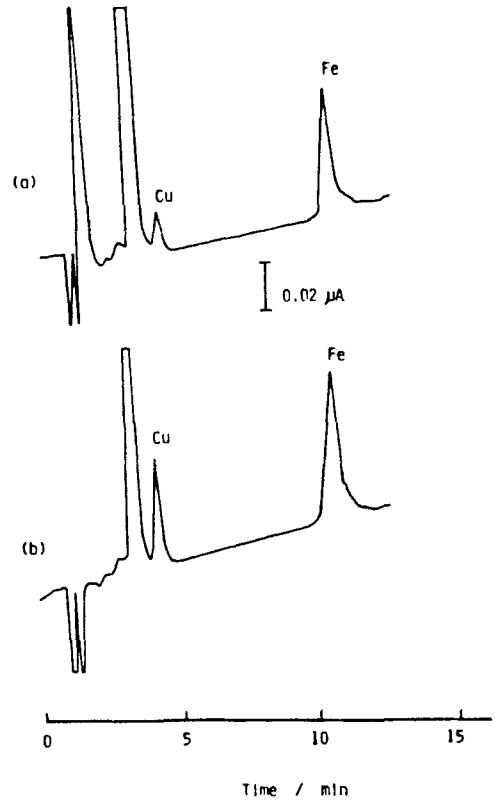

Figure 16. Chromatograms for the simultaneous determination of Cu and Fe in (a) oyster tissue and (b) bovine liver with electrochemical detection (-0.40 V). (Ref. 109, reprinted with permission of the publisher.)

could be measured by HPLC with reductive amperometric detection, following ethyl acetate extraction of their cupferron complexes.

Nagaosa *et al.* (170) also determined the Cu^{2+} and Fe^{3+} concentrations in two NBS biological samples, bovine and oyster tissue by using RPHPLC with EC and UV detectors. Cupferron was used as a pre-column derivatizing agent (Figure 16).

2.3.2.5. Ferrozine *(FZ)*

The structure of ferrozine is shown in Figure 17. Yi *et al.* (187) recently developed a novel reversed-phase HPLC method with UV detection to measure ultra-trace amounts of

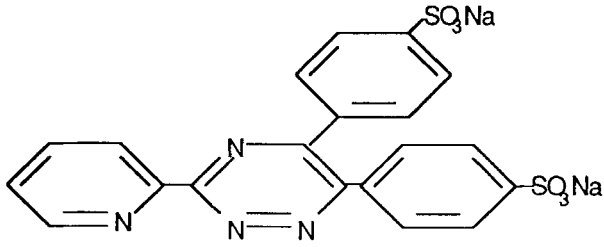

Figure 17. Structure of ferrozine (FZ).

Fe^{2+} in aerosols, rainwater, and seawater. This method was isocratic, rapid, sensitive, selective and reproducible. Fe^{2+} forms a stable complex ion, $[Fe(FZ)_3]^{2+}$, with the reagent ferrozine (FZ) in a pH range of 4 to 10. By measuring the absorbance of $[Fe(FZ)_3]^{2+}$ at 254 nm, Fe^{2+} concentrations were determined. The iron complex, which was separated from ferrozine, was detected in 6 minutes (Figure 18). Reversed phase C_{18} solid phase extraction cartridges, which were used for the sample preparation, concentrated the $[Fe(FZ)_3]^{2+}$ and increased the sensitivity of the analysis. A detection limit of 0.1 nM Fe^{2+} was obtained. Retention time, addition of a known amount of standard, and peak area ratios were used for peak identification of the $[Fe(FZ)_3]^{2+}$. There was no interference from Fe^{3+}, Ni^{2+}, Co^{2+} and Cu^{+}. The recovery of 10^{-8}-10^{-10} M $[Fe(FZ)_3]^{2+}$ using the preconcentration step was in the range of 92%-99%. The Fe(II) concentration in remote marine aerosols were determined for the first time by using this method (17, 196).

2.3.2.6. *b*-Diketonates

IN 1972, Huber *et al.* (192) first reported the HPLC separation of coordination complexes of metal ions with *b*-diketonates. Six metal ions, Be^{2+}, Cu^{2+}, Al^{3+}, Cr^{3+} Ru^{3+}, and Co^{3+} were separated in 25 min with A UV detector set at 310 nm. Tollinche and Rosby (193) conducted an extensive study of the elution behavior of a series of metal-*b*-diketonates on alumina, silica gel, bonded-phase, and open-pore polyurethane HPLC columns. They found that the best separations were obtained on normal-phase silica gel columns with non-polar eluents and detection at 280 nm.

2.3.2.7. Dithizonate

Dithizonate (DZ, $C_6H_5N{=}NCSNHNHC_6H_5$), which is an excellent solvent-extraction reagent, can form neutral chelates with many divalent metal ions. The complexes formed have high chelate molar absorptivities (30,000-100,000 l/mol.cm) in the visible

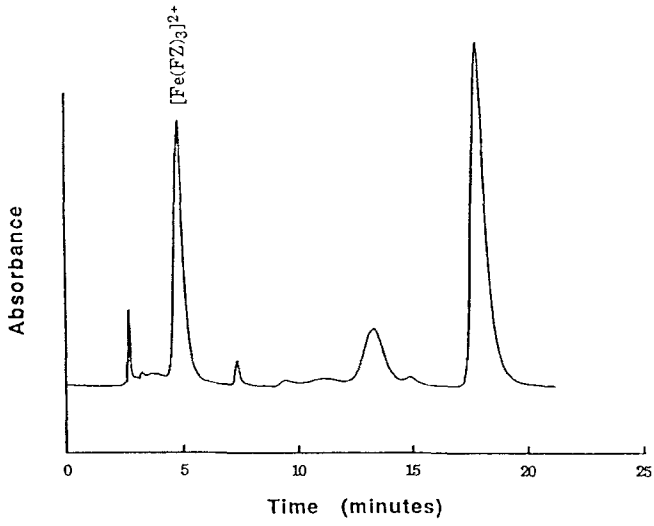

Figure 18. A chromatogram of rainwater collected from Narragansett, Rhode Island, USA (sampling date 12/8/1990). Sample preparation and chromatographic conditions are described in the Experimental Section. Fe(II) in rainwater was preconcentrated 50 times with C18 Sep-Pak. UV/VIS detector: 254 nm; Sensitivity: 0.02 AUFS; Response time: 500 msec. (Ref. 126, reprinted with permission of the publisher.)

region of the spectrum (500-530 nm). This property makes it easy to detect trace amounts of metal ions using a UV/Visible detector.

A separation of DZ chelates with Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, and Co^{2+} was accomplished using normal-phase HPLC on 30-μm silica particles with UV detection at 525 nm (194). A number of divalent dithizonates of Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+} were separated using non-aromatic solvents and a glass-lined column with UV/Visible detection at 475-525 nm (195).

3. CONCLUSIONS

A number of separation and non-separation methods have been used for the determination of trace and ultra trace amounts of metal ions. Each method has its own advantages and limitations. Atomic absorption spectroscopy, ultraviolet/visible spectrophotometry, flow injection chemiluminescence detection, and gas-phase atomic fluorescence detection can be used only for single metal analysis. Atomic absorption spectroscopy and gas-phase atomic fluorescence detection have high sensitivity and

selectivity, and other metal ions in the analyte do not interfere with the determination of the metal ion of interest. Although the ultraviolet/visible spectrophotometric method is simple and inexpensive, it is less sensitive and other UV/VIS absorbing species in the analyte may introduce error. Flow injection chemiluminescence detection is sensitive and can be easily adapted to automated analysis. However, other species in the analyte, which produce fluorescence under the same conditions of the test metal ion, may interfere the analysis.

Inductively coupled plasma emission spectrometry, neutron activation analysis, and analytical voltammetry are the non-separation methods of choice for multi-metal analysis. With inductively coupled plasma emission spectrometry and neutron activation analysis many metal ions can be determined, while with analytical voltammetry only a few metals can be determined concomitantly. However, the lack of the instrumental availability of ICPES and NAA as well as the high price of ICPES and the paucity of NAA facilities limits their popularity.

All the separation methods mentioned in this paper can be applied to the determination of single and multi-metal ion analysis. Among them gas chromatography is unpopular because volatile and heat stable metal species are rarely available. Thin-layer chromatography is time consuming and is complicated by the need to use another method for quantitative detection. Therefore it is not a method of choice. Capillary electrophoresis is a promising method for the separation and determination of metal ions. With the rapid development of CE techniques and the improvement of injection and detection methods as well as detection limits, CE can become a dominant method for the routine analysis of trace and ultra trace amounts of metal ions because of its high resolution, high efficiency, high speed, low sample consumption, quantitative results, simplicity, and relatively low cost.

In liquid chromatography, gel permeation chromatography is a good method for the separation of metallic proteins. However, to detect low levels of metal ions in proteins, another method must be used. Thus the application of GPC for the sensitive analysis of metal ions in environmental and biological samples is limited. The future challenge lies in coupling of a highly sensitive detection method with GPC separation so that GPC can be used in metal ion analyses.

Ion exchange, normal phase, and reversed phase high performance liquid chromatographic analyses with the use of proper coordination ligands for complexing the trace metals has been an effective methods in the determination of trace metals. The major advantage of such method is the multi-metal determination in a single run and the great potential to measure the speciation of a specific metal. Many analytical columns with high resolution power for metal species are available. It is anticipated that the determination of inorganic species by HPLC will be developed in the near future with new developments in HPLC techniques.

Improvements in the sensitivity of detectors are needed to solve challenging problems, such as the measurement of trace metals in the open ocean. One approach is to couple a very sensitive spectroscopic method, e.g., ICP detection, to achieve the lowest detection limits possible. However, the HPLC-ICP system will not be available for many researchers in the near future because of its cost. Another option is to synthesize new fluorescence ligands for the use in direct or indirect fluorescence detection. Although preconcentration of metal complexes before HPLC analysis, e.g., with Sep-Pak cartridges, is a useful method for the determination of ultra-trace amounts of metal ions, caution must be taken not to introduce during the preconcentration procedures unknown contaminants which will interfere with the determination of the metals of interest.

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