AN ABSTRACT OF THE THESIS OF

Yan Liu for the degree of Doctor of Philosophy in Chemistry presented on October 26, 1987.

TITLE: Development of Automated On-line Ion Exchange Systems for the Determination of Chemical Speciation of Trace Metals in Natural Waters

Redacted for privacy

Abstract approved: _______________________

James D. Ingle, Jr.

An automated on-line ion exchange trace enrichment system has been developed to improve the detection limits of flame atomic absorption (AA) spectrophotometry in the determination of trace metals in natural waters. The sample solution is passed through a column of Chelex-100 chelating resin by a carrier buffer stream delivered from a constant flow pump. Trace metal ions retained by the Chelex-100 resin in the column are subsequently eluted off the column using complexing agents such as cysteine and EDTA. The variables that affect the performance of the system are studied. The detection limits of the system with flame AA detection are 0.09 µg/L for Cu and Cd and 0.08 µg/L for Mn with a 10-mL sample loop.

The trace enrichment system has also been expanded to an automated two-column ion exchange system to study the speciation of trace metals in natural waters. In the two-column system, the sample solution is passed sequentially through a column of Chelex-100 resin
and a column of AG MP-1 macroporous anion resin. The Chelex-100 column retains free metal ions and metal ions dissociated from labile metal complexes. The AG MP-1 column retains anionic non-labile metal complexes and metals strongly associated with negatively charged organic matter such as humic acid. The dissolved metal species are classified into three fractions by the proposed measurement scheme. The variables that affect the results obtained by the two-column system are studied using model complexing agents. The measurement scheme is shown to be rapid and simple (about 10 min per sample with a 10-mL sample loop) and is used to determine the speciation of Cu(II), Cd(II) and Zn(II) in natural water samples.

The two-column ion exchange system is a versatile tool to study the trace metal complexation in natural waters. The system is used to determine the trace metal complexing capacity and conditional stability constants of synthetic ligands and ligands in natural waters and to study the dissociation kinetics of trace metal complexes in natural waters. The average Cu(II) complexing capacity of a Willamette River water sample is found to be about 4 \( \mu \text{M} \) and the conditional stability constant of Cu(II) complexes is about \( 10^7 \) at pH 7. The apparent 1st-order dissociation rate constant of Cu-humate complexes is determined to be about 0.3 \( \text{s}^{-1} \) at pH 6.5.
DEVELOPMENT OF AUTOMATED ON-LINE ION EXCHANGE SYSTEMS
FOR THE DETERMINATION OF CHEMICAL SPECIATION OF TRACE METALS
IN NATURAL WATERS

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Yan Liu

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III.1 Calculated Concentrations of CuL at the First
Three Titration Points in the Determination of
Cu(II) Complexing Capacity and Conditional
Stability Constants of Ligands in Water Samples.
Trace metals such as copper, lead and cadmium exist in many different physico-chemical forms in natural waters. They may include metal associated with particulate matter and dissolved forms such as hydrated ions, inorganic and organic complexes, and metal associated with a variety of inorganic and organic colloidal matter. The chemical speciation of an element can be defined as the distribution of concentrations of the different physico-chemical forms of that element in a sample (1).

It is now well recognized that speciation information is essential to understand the biological and geochemical impact of trace metals in natural water systems. Studies have shown that hydrated metal ion and simple inorganic metal complexes are usually the form most toxic to aquatic organisms, whereas stable organic metal complexes and metals associated with colloidal particles are much less toxic (2-4). Lipid-soluble metal complexes such as
alkylmercury compounds are particularly toxic forms of trace metals because they can diffuse through a biomembrane (5,6). The chemical form of trace metals also affects their adsorption on suspended matter and their sedimentation and transport in natural water systems (7).

The determination of chemical speciation of trace metals in natural waters is a very challenging problem. The distribution of a trace metal among different physico-chemical forms is highly complex due to the large number of possible interactions with ill-defined dissolved and particulate organic and inorganic substances and non-equilibrium conditions in natural waters. In addition, the total concentrations of dissolved forms of trace metals such as Cu, Pb, Zn, and Cd in natural waters are often below 1 µg/L, and sometime even below 0.1 µg/L. The determination of chemical speciation of trace metals at these very low concentrations is obviously an extremely difficult task.

Two basic approaches have been used to study the speciation of trace metals in natural waters: (1) calculation of the distribution of metal species using chemical equilibrium models and (2) experimental measurement of species or groups of species in samples. Calculation methods are based on computing the equilibrium concentrations of all species using published stability constant data and the known total concentrations of various metal ions, ligands and suspended solids in the solution. Experimental methods are based on various combinations of analytical measurement and separation techniques. Different physico-chemical forms of metal species in the
water sample are divided into several experimentally defined fractions based on their behavior during chemical separation and measurement.

Considerable research effort has been devoted to the determination of speciation of trace metals in natural waters and to the understanding of their profound impact on aquatic biota. Recently, several comprehensive reviews and conference proceedings on the subject have been published (1, 8-13). The correlation of speciation results and toxicities of different forms of trace metals is of current research interest (9, 11). Most experimental schemes reported are very time-consuming and difficult to adopt as routine methods. The classification provided by some schemes may be more detailed than is needed to characterize some effects of interest such as the toxicity of trace metals to aquatic organisms (11). The development of relatively simple but rapid speciation methods will be valuable in providing routine methods for the determination of trace metal speciation in natural waters and establishing the correlation of metal speciation and its biological effects, particularly the toxicity.

The primary goal of this research project is to develop rapid, versatile and sensitive methods to study the interactions between trace metals and organic ligands in natural waters and to determine the speciation of trace metals in natural waters. An automated on-line ion exchange trace enrichment system was developed to improve the detection limits of flame atomic absorption spectrophotometry in the determination of trace metals in natural waters. This system was
then converted to an automated two-column ion exchange system to study the speciation of trace metals in natural waters. In the two-column ion exchange system, the sample solution is passed sequentially through two ion exchange microcolumns of Chelex-100 chelating resin and AG MP-1 macroporous anion resin. Different dissolved forms of metal species are separated and preconcentrated by the two ion exchange columns for the determination with an on-line flame atomic absorption spectrophotometer. A experimental scheme based on the two-column ion exchange system is proposed and applied to study the speciation of Cu(II), Cd(II), and Zn(II) in natural waters.

In this thesis, the chemical speciation of trace metals in natural waters is first reviewed (chapter 2). Then the design, construction and application of automated on-line ion exchange trace enrichment system (chapter 3) and automated on-line two-column ion exchange speciation system (chapter 4) are discussed. The last part of the thesis is focused on the use of two-column ion exchange system in the determination of trace metal complexing capacity and conditional stability constants of ligands in natural waters (chapter 5) and in the study of dissociation kinetics of trace metal complexes in natural waters (chapter 6). In the appendices, the hardware and software developed are discussed in more detail and some supplementary data are presented.
REFERENCES


Trace metals of primary interest for speciation studies in natural waters are As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, and Zn because they are either potentially harmful or nutritionally important elements (1). Other elements such as Se and Ni are also sometimes of interest. In natural waters trace metals are distributed between the dissolved and particulate forms. By convention, dissolved metal species are those which pass through a 0.45 \(\mu\)m filter. This is an operational definition as metals may be sorbed onto particulates smaller than 0.45 \(\mu\)m. Although the nature of the dissolved metal species is very complex, they may be operationally divided into six classes: simple hydrated metal ions, inorganic and organic complexes, metals adsorbed on inorganic and organic colloids, metals adsorbed on mixed organic/inorganic colloids as shown in Table 2.1 (2).

Dissolved inorganic and organic species, colloidal and particulate matter, pH, pE, etc. have significant effects on the speciation of trace metals in natural waters. Because of the high concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) ions in seawater, the formation of simple, molecular organic complexes of most trace metals in seawater is believed to be unfavorable. Chloro complexes are often important species for some metals such as Hg and Cd. Florence and Batley (3)
Table 2.1. Possible Physico-chemical Forms of Trace Metals in Natural Waters\textsuperscript{a}.

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<th>Physico-chemical form</th>
<th>Possible example</th>
<th>Diameter/ nm</th>
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<td>Particulate</td>
<td>Retained by 0.45-(\mu)m filter</td>
<td>&gt;450</td>
</tr>
<tr>
<td>Simple hydrated metal ion</td>
<td>(\text{Cd(H}_2\text{O)}_6^{2+})</td>
<td>0.8</td>
</tr>
<tr>
<td>Inorganic complex</td>
<td>(\text{CdCl}^+, \text{PbCO}_3)</td>
<td>1</td>
</tr>
<tr>
<td>Organic complex</td>
<td>Cu - fulvic acid</td>
<td>2-4</td>
</tr>
<tr>
<td>Adsorbed on inorganic colloids</td>
<td>(\text{Pb}^{2+}/\text{Fe}_2\text{O}_3)</td>
<td>10-500</td>
</tr>
<tr>
<td>Adsorbed on organic colloids</td>
<td>(\text{Cu}^{2+})</td>
<td>10-500</td>
</tr>
<tr>
<td>Adsorbed on mixed organic/inorganic</td>
<td>(\text{Cu}^{2+}) - humic acid/\text{Fe}_2\text{O}_3</td>
<td>10-500</td>
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\textsuperscript{a} Source: Florence, T. M. \textit{Analyst}, 1986, 111, 489-505.
found that a mixture of organic complexing agents such as EDTA at 2 x 10^{-7} M had no effect on the Cu, Pb, Cd or Zn anodic stripping waves in either synthetic or natural seawater. Freshwater usually has a lower ionic strength and higher content of organic matter. Heavy metals may form complexes with naturally occurring organic ligands such as low molecular weight fulvic acids (4-7). Additionally, carbonate may be a more important ligand than chloride for some metals such as Cu and Pb.

Dissolved organic matter plays an important role in the speciation of trace metals in natural waters. About 50-80% of the dissolved organic matter in natural waters consists of fulvic and humic acids (7). Fulvic acids are of humic substances that are soluble in water under all pH conditions. Humic acids are humic substances that are not soluble below pH 2 but soluble at higher pH. Fulvic and humic acids are highly complex polymeric compounds containing aromatic rings with phenolic OH and benzenecarboxylic groups with molecular weight (MW) ranging from 2000-300,000 (8). Fulvic acids are thought to be lower MW compounds containing a high percentage of COOH group and humic acids are thought to be higher MW compounds containing a high percentage of OH group.

For many heavy metals, a significant fraction of the dissolved metal species in natural waters has been found to be associated with colloidal humic acids (9-11). Studies also have shown that colloidal particles of iron oxide coated with humic acid strongly adsorb trace metal ions (12-14). It has been proposed that the predominate form of dissolved trace metals may be metals adsorbed on mixed organic/
inorganic colloids (15).

The interactions between particulates and trace metals play an important role in the regulation of dissolved metal concentrations. Changing environmental parameters such as pH (e.g., acid rain) and discharge of complexing agents (e.g., NTA) affect adsorption and desorption process of trace metals onto suspended particulates and deposited sediments (16-18).

CHEMICAL EQUILIBRIUM MODELS

The concentrations of various metal species in aqueous systems can be calculated with chemical equilibrium models. The total metal and ligand concentrations and relevant stability constants must be known. This approach to study the speciation of trace metals in natural waters has been pursued by many researchers. More than 50 computer programs have been reported for the calculation of equilibrium distribution in aqueous systems (19). A symposium was devoted to chemical equilibrium models in 1978 (20). The subject of chemical equilibrium models has been reviewed by Nordstorm, etc. (19, 21).

The calculation of concentrations of various species in an aqueous system by computer programs is usually based on solving a set of nonlinear equations involving equilibrium constants and mass balance constraints. All programs can calculate concentrations of various species in a single aqueous phase (19). In addition, many
programs can be used to solve the problem of the distribution of metal between two or more phases such as the precipitation or dissolution of a mineral. A limited number of the programs also consider the effect of mass transport (e.g., the movement of dissolved trace metals in a flowing river) in their models (22-24). Attempts have also been made to solve the more complex problem of calculating concentrations of species in states of partial equilibrium during the reaction path towards complete equilibrium (25-26).

The use of chemical equilibrium models to determine the speciation of trace metals in natural waters is an attractive approach for several reasons. First, the concentration of individual chemical species in a water system can be predicted without having to determine the species experimentally. In many cases, the analytical methods are not available to determine many specific species at the low concentrations in environmental samples. Second, effects of variables such as pH and ligand concentrations on the metal speciation can be rapidly studied. Third, time-consuming and labor-demanding laboratory work and all problems of sample collection and contamination are eliminated.

However, it is clear that the results of calculations with chemical equilibrium models are only as valid as the equilibrium constant data and the model used to describe a particular water system. The approach of chemical equilibrium models suffers from some limitations. First, the most fundamental assumption of most chemical equilibrium models is that the system under study is in equilibrium. This may be true for some reactions in the aqueous phase such as ion
pairing of inorganic species, but it is certainly not valid for all the interactions in natural waters, especially those between heterogeneous phases and involving redox reactions. Second, there are no reliable data and models available for describing the interactions among trace metal ions and natural organic ligands with variable metal binding properties or the adsorption of metal ions on the particulate matter in natural waters. Third, the disagreements about the thermodynamic data for many of the simple inorganic complexes in aqueous solutions and problems with activity coefficient corrections can seriously compromise the accuracy of chemical equilibrium models.

At present, chemical equilibrium models are very useful to predict the speciation of trace metals in aqueous systems. It is most useful where the necessary thermodynamic data is known and equilibrium has been achieved. Clearly experimental techniques must be used in conjunction with chemical equilibrium models to determine when chemical equilibrium models are applicable.

EXPERIMENTAL TECHNIQUES

The experimental techniques used in the determination of chemical speciation of trace metals in natural waters can be classified into three categories: direct measurement techniques, separation techniques and conversion techniques. The number of applicable techniques is limited by the low concentrations of trace metals in natural waters. Ion-selective electrode (ISE) potentiometry is the only technique
responding to a single, well defined metal species (i.e., the hydrated metal ion). However, the use of this technique is limited because the concentrations of trace metals are usually too low in natural waters for direct determination. Also ISEs cannot be made for many metals. Some voltammetric techniques, in particular anodic stripping voltammetry, yield information about groups of species. Measurement techniques such as atomic absorption spectrophotometry, plasma emission spectrometry and neutron activation analysis are unable to differentiate between chemical forms of metal species. They respond to the total metal concentration. However, they can be very useful when employed in conjunction with suitable separation techniques because of the excellent detectability and selectivity for a given metal.

The principal separation techniques used for the determination of trace metal speciation are filtration, ultrafiltration, dialysis, ion exchange, and solvent extraction. These techniques are used to separate and sometimes concentrate selected species or groups of species based on their physical and chemical properties before measurement of the total metal in each fraction separated.

Conversion techniques are based on detecting the metal in a sample before and after sample treatment such as acidification, oxidation/reduction, or UV irradiation. They are often used in conjunction with direct measurement or separation techniques. In the latter case, the sample treatment converts all forms of metal species into one, measurable form in the determination of the total metal concentration.
The techniques most commonly applied to study the speciation of trace metals in natural waters are anodic stripping voltammetry, ion exchange techniques, physical separation techniques (e.g. ultrafiltration, dialysis, etc.). The characteristics of these techniques are discussed in detail below.

**Anodic Stripping Voltammetry**

Anodic stripping voltammetry (ASV) has been widely used in speciation studies because it has some inherent differential response to different chemical forms of a metal, and it is also one of few analytical techniques with sufficiently low detection limits to determine directly trace heavy metals in natural waters. The theory and application of ASV in speciation studies have been reviewed many times (2, 15, 27-29). ASV is the combination of an electrochemical plating process and a voltammetric analysis. The plating step serves to preconcentrate reducible metal species onto an electrode. This is followed by reoxidizing or stripping the metal back into the solution with an anodic voltammetric scan. The anodic stripping current is monitored and the height of the current peak produced is proportional to the concentration of the reducible metal species in the solution.

ASV can be used to distinguish between "ASV labile" and "ASV non-labile" metal species (30, 2). The labile species include hydrated metal ions and metal complexes which dissociate rapidly in the diffusion layer of the electrode to the free electroreducible form, yielding an ASV signal. The degree of the dissociation of complexed metal species at the electrode depends on the dissociation
rate constant and the residence time of metal species in the diffusion layer. The residence time in the diffusion layer decreases as the rotation rate of the electrode (or stirring rate of the solution) increases. The non-labile metal species are metals bound in inert complexes or adsorbed on colloidal matter with a dissociation rate that is slow compared to the time scale of the diffusion layer (on the order of milliseconds).

The labile fraction of metal species determined by ASV is also dependent on pH, the deposition potential and other operating conditions of the electrode system. The labile metal species is usually determined in mildly acid solution (e.g., acetate buffer at pH 4.7). At pH values greater than 7, ASV is insensitive to some ionic species like hydroxycarbonates of Pb and Cd. At lower pH values, metal ions may be dissociated from organic complexes or colloidal matter. The total metal concentration can be measured by ASV after acid digestion or UV irradiation of the sample solution to destroy organic matter and to convert non-labile species into electroactive forms.

The use of ASV suffers a few problems in the determination of trace metal speciation in natural waters (2, 15). First, the organic matter present in natural waters may adsorb on the mercury electrode and therefore hinder the diffusion of metal ions. The adsorption of organic matter can change peak currents and potentials, and even tensammetric peaks may occur due to the adsorption/desorption of organic matter on the mercury surface. Because of this problem and the variable condition of the electrode surface from sample to sample,
calibration is usually achieved by standard addition. Second, a buffer must be added to control the pH and increase the ionic strength of the test solution (to about 0.02 M) to obtain consistent results. This sample treatment can alter the speciation. Third, as most other measurement and separation techniques, the measured concentration of ASV-labile species is operationally defined by the instrumental and solution conditions used.

Nevertheless, ASV is a very important technique in speciation studies because of its ability to measure the labile metal species and its excellent detectability (about \(10^{-10}\) M) for Cu, Pb, Cd and Zn, four common trace metals in natural waters. It has been reported that the ASV-labile fraction of metal species may be correlated to the toxicity of trace metals to aquatic organisms (31, 32).

**Ion Exchange Technique**

The ion exchange technique is one of most important separation techniques in the speciation of trace metals in natural waters. In the ion exchange method, the water sample is mixed with an ion exchange resin in a batch mode or passed through an ion exchange column.

Ion exchange resins usually consist of insoluble beads of highly polymerized, crosslinked styrene-divinylbenzene lattice with attached ionic functional groups. In the ion exchange process, ionic trace metal species in the solution are exchanged with counterions of the same charge associated with the functional groups on the resin. The retained metal species are later eluted off the resin with a suitable
eluting solution and the concentration of the metal in the eluate is determined with measurement techniques such as atomic spectrometry or ASV. Different ion exchange resins can be used to retain and separate one group of metal species from another. Compared to other separation techniques, ion exchange separations can be carried out with little manipulation and opportunity for contamination. Ion exchange techniques also provide some degree of concentration after separation and can be applied to more metals than ASV. These are important considerations in speciation studies because of the very low concentrations of trace metals in natural waters.

Conventional cation and anion resins have been used in the speciation studies. Shuman and Dempsey (33) reported a method in which the water sample was passed sequentially first through a 0.2 μm filter, then a column packed with AG 50W-X8 cation exchange resin and a column packed with AG 1-X8 anion exchange resin. The dissolved metal species were separated into cationic and anionic species. The retained metal species were eluted of with 3 M HCl and their concentrations were determined with atomic absorption spectrophotometry. They applied the method to study the speciation of Cd, Cr, Cu, Pb and Zn in river water. Zorkin and co-workers (34) used a AG 50W-X12 cation exchange column to separate cationic copper species from sea water. They observed the correlation between the copper fraction determined by the ion exchange column and the toxic fraction of copper quantified by a diatom bioassay. Cation and anion resins were also used in the speciation of Cr(III) and Cr(VI) in natural waters by Pankow and co-workers (35-36), and Marino and Ingle
Because of its relatively high selectivity for transition metals, Chelex-100, a chelating resin with iminodiacetate functional group, has been used extensively for studying the speciation of trace metals in natural waters. Since it was first used in the speciation scheme of Batley and Florence (9, 38), ion exchange with Chelex-100 resin has been an important step in most of the speciation schemes reported (14, 39-43). Chelex-100 resin binds hydrated trace transition metal ions strongly. It can compete with some dissolved organic ligands for trace metal ions. Some weak or labile complexed metals species are likely to dissociate and contribute to the fraction of metal species retained by the resin.

Figura and McDuffie (41, 42) showed that the fraction of trace metal species retained by the resin was related to the contact time between the water sample and the resin. However, large molecules and colloidal particles are excluded from the resin beads and are not retained by the resin because the resin has a pore size of about 1.5 nm (15). Therefore, ion exchange with Chelex-100 resin provides a simple, rapid and almost contamination-free method for separation of the dissolved metal species into two classes which have been termed as "Chelex labile" and "Chelex non-labile" species. This classification is important because it can provide the speciation information about the main classes of toxic and non-toxic trace metal, respectively, in natural waters.

Nonpolar polystyrene resins (Amberlite XAD-2, etc.) have also been reported to separate metal species associated with organic matter...
(44-45). Florence (14) used Bio-Rad SM2 resin, a nonpolar polystyrene resin with high surface area, to separate lipid-soluble metal species in the speciation studies. Hiraide and co-workers (46) reported the use of diethylaminoethyl Sephadex A-25, a weak base anion exchanger, to separate heavy metals complexed with humic substances in freshwaters.

Physical Separation Techniques

A number of physical separation techniques including filtration, ultrafiltration (UF), dialysis, gel filtration chromatography and centrifugation have been applied to the study of trace metal speciation in natural waters. With these techniques, the particulates or dissolved metal species are separated according to their sizes. Recently the use of physical separation techniques in the speciation studies has been reviewed by De Mora and Harrison (47) and Steinnes (48).

Filtration of the water sample through membrane filters of different pore sizes is a method widely used to fractionate trace metal species according to their sizes. Commonly, 0.45 μm or 0.40 μm polycarbonate or cellulose acetate membrane filters are used to separate so called "dissolved" (soluble, filterable) and "particulate" (non-filterable) metal species. This classification of dissolved and particulate metal species will also be used in this thesis. This filtration step operationally separates the particulate metal species from metal species in true solution and a large part of the metal species associated with colloidal particles. Although it is certainly
operational by nature, filtration with 0.45 μm filter is often the first separation step before further separation and determination.

A further size fractionation of the dissolved metal species is possible using techniques with UF and dialysis. UF is usually referred to as the technique involving filtration through membrane filters having a pore size of less than 15 nm (0.015 μm). UF membrane filters are available with pore sizes in the range of 1 to 15 nm, corresponding to nominal molecular weight (MW) cut-off from 500 to 300,000. To obtain a satisfactory filtration rate, the UF cell is pressurized under a compressed inert gas such as nitrogen or argon. UF cells are often provided with a magnetic stirrer to prevent filter clogging.

Dialysis is a technique similar to UF in some respects. Larger metal species such as those associated with colloidal particles are separated from smaller species based on the fact that they are unable to pass through the dialysis membrane. In dialysis, the sample solution is not pressurized. The permeable species diffuse across the membrane from the sample solution to the dialysate solution on the other side until equilibrium is established (i.e., the concentration of the permeable species is same in the solutions on both side of the membrane). The dialysis membranes used in the speciation studies are in the range of 1 to 5 nm, corresponding to MW cut-off about 1000 to 10,000. Compared to UF, dialysis is a much slower process because of the long time required for equilibrium to be established.

UF and dialysis have been used by a number of researchers for the size fractionation of dissolved trace metal species in natural waters
Hoffmann and co-workers (52) developed a sequential UF method to characterize the dissolved metal species in Mississippi and Minnesota River water samples. They reported that major fraction of dissolved organic carbon has MW less than 10,000 and that the highest concentrations of trace metals such as Cu, Pb, Cd, and Mn are often found to be associated with species in the intermediate MW range (1000-10,000). Studies (10, 51, 53) also show that a significant fraction of trace metals in natural waters is bound to organic matter of size greater than 100,000 MW.

Even though UF and dialysis are very useful techniques to classify the dissolved metal species on the basis of molecular size, they are associated some problems (15, 47-48). First, pore diameter of UF and dialysis membranes may show considerable variation, especially for those having small pore size. Therefore, precise size fractionation is difficult. Second, UF and dialysis membranes as supplied are often contaminated with trace metals and soluble organic matter. Careful cleaning with diluted nitric acid is recommended before the use. Third, the adsorption of trace metals onto UF or dialysis membranes is particularly a serious problem because of very low concentrations of trace metal species in natural waters. This problem could be avoided in in-situ dialysis since the adsorption capacity can be saturated.

Physical separation techniques used less often include centrifugation, gel filtration chromatography and electrophoresis (54, 55).
A number of measurement schemes to determine the speciation of trace metals in natural waters have been proposed based on various combinations of the available measurement, separation and conversion techniques. Some representative schemes are outlined here.

Batley and Florence (9, 38) reported the first comprehensive scheme in 1976. The water sample is first filtered with 0.45 μm filter to separate the particulate metals from the dissolved metals. ASV is then used to determine the concentrations of various fractions of dissolved metal species in the filtered sample, before treatment, after passage through a Chelex-100 column, after UV irradiation, and after passage of the UV-irradiated sample through the Chelex-100 column. The UV irradiation step is used to release organically bound metals by decomposing organic matter and the Chelex-100 column retains free metal ions and metals from dissociation of metal complexes during interaction with the Chelex-100 resin. Metals associated with colloidal species are considered unretained by Chelex-100 resin because of the limited pore size of the resin (about 1.5 nm). The scheme is used to quantify seven classes of metal species: (a) ASV labile species retained by Chelex-100, (b) ASV labile organic complexes and colloidal species not retained by Chelex-100, (c) ASV labile inorganic complexes and colloidal species not retained by Chelex-100, (d) ASV non-labile organic complexes retained by Chelex-100, (e) ASV non-labile inorganic complexes retained by
Chelex-100, (f) ASV non-labile organic complexes and colloidal species not retained by Chelex-100, (g) ASV non-labile inorganic complexes and colloidal species not retained by Chelex-100. Undoubtedly, there are some overlaps of those classes, where metal complexes are partially dissociated, or some metals associated with colloidal species are dissociable and retained by Chelex-100 resin. Therefore, the results of speciation measurements are operationally defined by nature. Nevertheless, measurements with this scheme have revealed an important, previously undocumented aspect of trace metal speciation, namely that significant fractions of trace metals such as Cu, Pb, and Cd are present in colloidal forms in natural waters. Batley and Gardner (55) reported that, in a study of dissolved Cu, Pb, and Cd species in estuary and coastal waters, 40-60% of Cu, 45-70% of Pb and 15-35% of Cd were found to be associated with colloidal matter.

Figura and McDuffie (41,42) developed a scheme to classify dissolved metal species (unretained by 0.4 μm filter) into four groups according to the dissociation kinetics of the metal species. First, the "very labile" fraction of dissolved metal species is determined directly by an ASV procedure with a diffusion layer residence time of 2 ms. This fraction includes hydrated and metal complexes such as Pb(glycine)⁺ which dissociate in the time scale of the ASV measurement. Second, the filtered water sample is passed through a Chelex-100 column with a contact time of 7 s and then eluted with 2 M HNO₃. The metal species retained by the column is considered to be the "very + moderately labile" fraction, the "moderately labile" fraction may consist of metal species such as metal-NTA complexes
which does not dissociate in the time scale of the ASV measurement.

Third, the eluate from the Chelex-100 column is brought in contact with Chelex-100 resin in a batch procedure for three days; the fraction retained by the resin is called the "slowly labile" fraction which may include relatively stable metal complexes such as Cd-EDTA, and probably some colloidal-bound metal. Finally, the fraction remaining in solution is determined by ASV after acid digestion and is called the "inert" fraction. This fraction is considered to represent extremely stable or non-labile metal complexes and metal adsorbed strongly to colloidal matter.

Using this scheme, Figura and McDuffie found that, in several river waters, more than 80% of dissolved Cd and Zn existed as "very labile" and "moderately labile" species, while more than 40% of dissolved Cu and Pb existed as "slowly labile" and "inert" species. One limitation of the scheme is that species in the "very labile" fraction measured by ASV will not necessarily be fully retained by the Chelex-100 column. "ASV labile" metal species may include both strong and weak complexes with rapid dissociation kinetics. The dissociation of a complex at the electrode surface is an irreversible process as metal ions are removed at the mercury electrode during deposition. "Chelex labile" metal complexes are usually weaker metal complexes and may include some metal complexes stronger than the Chelex-metal complex since the resin is present in considerable excess. Batley and Florence (9) showed that the proportion of this fraction not retained by Chelex-100 resin could be as high as 75% for some metals. Thus the "moderately labile" fraction obtained by difference could be
erroneous.

Among other relatively comprehensive measurement schemes are those of Laxen and Harrison (43) and Hart and Davies (39,40). The Laxen and Harrison scheme adopted a size fractionation approach. Metal species in freshwater samples were fractionated by filtration using Nuclepore filters (12, 1.0, 0.4, 0.1, 0.08 and 0.015 μm pore size). Total concentrations of filterable metal species by each filter were determined. In addition, ASV labile metal species before and after UV irradiation were determined in the filtrates of the 1.0 μm and 0.08 μm filters, and Chelex-100 labile metal species were also determined in the 1 μm filtrate. Further size fractionation attempts were also carried out by UF; however UF membranes were found to be prone to adsorption and, therefore, were not used in the final scheme. They found that, in a river water, 29% of Cd, 25% of Cu, 88% of Mn, 7% of Pb, and 8% of Fe are associated with species smaller than 1 μm. In the 1 μm fraction, 34% of Cd, 36% of Cu, 95% of Mn, 14% of Pb, and 13% of Fe are retained by Chelex-100 resin.

Hart and Davies classified dissolved metal species (0.4 μm filter) into three fractions. The "exchangeable" fraction was obtained by equilibrating the filtered sample with a Chelex-100 ion exchange batch for at least 24 h. The "dialysable" fraction was obtained by a combined dialysis-ion exchange method in which the dialysable metal species in the solution were pumped through a dialysis membrane and were removed from the solution on a Chelex-100 column for 5 to 6 h. The concentrations of metals in these two fractions were determined by atomic absorption spectrophotometry after
the metal ions were eluted from the resin by nitric acid. The "bound" fraction was calculated by the difference of total filterable and "exchangeable" metal species.

The measurement schemes developed so far suffer a few problems. First, the results of speciation measurements are operationally defined. At present, measurement techniques with the selectivity to respond to a single, well defined metal species at sub-μg/L levels are not available. Most of the techniques used do not produce a clear-cut separation of different forms of metal species. In addition, the very act of sampling and measurement may perturb the natural speciation. Second, those operationally defined fractions, in most cases, have not yet been correlated to the effects of interest such as toxicity, although this kind of correlation is a primary goal for pursuing speciation measurements. Third, most schemes are very time consuming in an attempt to classify as many of fractions of metal species as possible. As the number of fractions identified increases, so does the complexity of the scheme, and the risk of loss and contamination of trace metals. A complex scheme is difficult to adopt to experiments necessary to establish the correlation between speciation results and biological effects of trace metals in natural waters. The development of relatively simple but rapid measurement schemes will be valuable in this respect.
DETERMINATION OF TRACE METAL COMPLEXING CAPACITY AND
CONDITIONAL STABILITY CONSTANTS OF LIGANDS IN NATURAL WATERS

In natural waters, many dissolved organic substances such as fulvic and humic acids can complex metal ions and colloidal particles of iron and manganese oxides can adsorb metal ions. These materials can reduce the toxicity of the added trace metal ions by complexing the added metal ions and converting them to forms less available to aquatic organisms. Therefore, the determination of trace metal complexing capacity and conditional stability constants of these ligands in natural waters is clearly of considerable interest. These two parameters provides an indication of the amount of a trace metal can be discharged into a water system before causing possible toxic effects.

The complexing capacity of a water sample is usually referred to as the total concentration of ligands capable of binding the metal ion under study. The common approach to determine complexing capacity involves titration of the water sample with hydrated Cu ion. Cupric ion is usually chosen because it is common trace heavy metal. It is very toxic to aquatic organisms and it binds strongly with natural organic ligands. After each addition of known amount of Cu ion, the concentration of the resulting hydrated Cu ion or labile Cu species is measured with a technique that is capable of discriminating between labile and complexed metal species. A titration curve is obtained by plotting the measured concentration of labile Cu species against the
total added Cu concentration. The initial portion of the titration curve has relatively small slope because most of the added Cu is complexed. The slope of the titration curve increases gradually as the titration proceeds. After all of the complexing sites of ligands in the sample are saturated with Cu, the slope is then unity (i.e., each addition of Cu produces equal amount increase in the concentration of the hydrated or labile Cu). From the titration data, it is possible in principle to obtain not only the complexing capacity but also the conditional stability constant(s) of the ligand(s) by treating the titration data with a suitable equilibrium model.

A variety of techniques including ion selective electrode potentiometry, anodic stripping voltammetry, ion exchange, solubilization, dialysis and biological methods have been used to monitor complexing capacity titrations. Several detailed reviews on the applications of these techniques in the determination of complexing capacity have been reported (56-58). The subject of complexation of trace metals in natural waters was also the theme of a recent international symposium (59).

As for trace metal speciation studies, the detection methods usually imparts an operational character to the measured complexing capacity because most of the detection techniques respond to not only hydrated metal ions but also other labile metal species to different extent. Therefore, different techniques may give different results of complexing capacity for a given water sample. Some techniques may underestimate the complexing capacity because of the short delay between the addition of metal ion and the measurement. If
complexation kinetics are slow, it may take a considerable amount of
time for equilibrium to be reached.

Despite the evident problems, the determination of trace metal
complexing capacity of natural waters is very important in
understanding the likely fate and effects of trace metals added to
natural waters. An ideal method for the determination complexing
capacity would be one where the affinity of the analytical probe for
the metal ion titrant would be the same as that of a biomembrane
(e.g., the gill of a fish).
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CHAPTER 3

AUTOMATED ON-LINE ION EXCHANGE TRACE ENRICHMENT SYSTEM
WITH FLAME ATOMIC ABSORPTION DETECTION

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ABSTRACT

Chelex-100 chelating resin is used for enrichment of trace concentrations of transition metals in an automated flow system. The effects of column dimensions, resin particle sizes and sample loading and eluting flow rates are studied. To avoid problems of using strong acid as the stripping reagent, complexing reagents like cysteine and EDTA are successfully used to elute transition metals such as Cu, Cd, Mn, Zn, and Pb from small columns packed with Chelex-100 resin. To improve detection limits, the system can be configured for multiple injections of a given sample solution before the column is eluted. A stop-flow technique is developed to allow complete elution of transition metals from Chelex-100 resin that react more slowly with the stripping complexing ligands. The detection limits of the system with flame atomic absorption spectrophotometry are 0.09 µg/L for Cu and Cd and 0.08 µg/L for Mn with a 10-mL sample loop.
INTRODUCTION

Chelex-100 resin has been widely used for trace enrichment of transition metal ions from various sample matrices. It is a chelating ion exchange resin with an iminodiacetic acid functional group and has a high selectivity for transition metal ions relative to alkali and alkaline metal ions. The applications of Chelex-100 resin have been reviewed by several researchers (1-3). Compared to other trace enrichment techniques such as solvent extraction or precipitation, trace enrichment with Chelex-100 resin is easier to use and capable of providing higher enrichment factors; also samples are less likely to be contaminated by the heavy metal impurities from buffers and organic solvents.

For the conventional use of Chelex-100 for preconcentration, a large volume of sample solution is percolated through a column containing a bed volume of several milliliters of the resin. The retained metals are eluted or stripped with 5 to 10 mL of 2 to 4 M HNO₃. The metal concentration is then determined by a technique such as atomic absorption (AA) spectrophotometry. There are some disadvantages to this procedure. First, a relatively large amount of sample (usually 100 mL to 1000 mL) is required to obtain enrichment factors of 10 to 100 because the bed volume of the column and the amount of the stripping reagent used are relatively large. Second, it is time-consuming and labor-intensive to carry out the enrichment
Recently, some on-line ion exchange trace enrichment systems have been reported in which the column is directly interfaced to the detection system. In 1981, Ambrose and Ingle (4,5) developed a computer-controlled on-line ion exchange trace enrichment system with flame atomic absorption detection. The cation resin AG 50W-X8 was used to preconcentrate trace transition metal ions from fresh river water. With a sample volume of 50 mL, the detection limit was 0.3 µg/L for Cu, Cd, and Mn. In 1983, Olsen (6) et al. developed an on-line flow injection analysis (FIA)-AA system with Chelex-100 ion exchange preconcentration. In 1984 Fang (7) et al. reported a similar but improved FIA- AA system with Chelex-100 preconcentration. The detection limits for Cu, Zn, Pb, and Cd were reported to be 0.07, 0.03, 0.7 and 0.05 µg/L, respectively; the system provided a 50-105 fold preconcentration at a sampling rate of 60 hr⁻¹. More recently Hartenstein (8) et al. and Hirata (9) et al. reported on-line ion exchange preconcentration systems for ICP atomic emission spectrometry. Other on-line preconcentration systems with different chelating resins or sorbents have also been described (10-13).

The on-line ion exchange trace enrichment techniques decrease the drawbacks of conventional off-line column techniques. First, higher enrichment factors can be attained with a smaller sample volume since minicolumns (< 1 mL bed volume) and a smaller volume of stripping reagent are used. Second, the speed of analysis is much faster because higher sample loading and eluting flow rates are used in...
conjunction with smaller sample volumes. Third, the concentration at the maximum of the elution band is measured instead of the average concentration in 5 to 10 mL of the stripping reagent.

Strong acids such as nitric acid have been used as the stripping reagent in most off- and on-line ion exchange enrichment systems with Chelex-100 resin reported so far. One primary disadvantage of this procedure is that the volume of the Chelex-100 resin changes dramatically when counter ions are changed (e.g., the bed volume decreases about a factor of 2 when going from N\textsubscript{4}H\textsuperscript{+} form to H\textsuperscript{+} form). Therefore, if strong acid is used as the stripping reagent, the resin (usually used in N\textsubscript{4}H\textsuperscript{+} form) is converted to H\textsuperscript{+} form in eluting step and the packing of the column becomes progressively tighter in an unidirectional flow system. This causes the pressure drop across the column to increase gradually and creates difficulty in maintaining constant flow rate. Olsen et al. (6) changed the flow direction in eluting step to overcome this problem partially. Other researchers (9, 10) used different resins to avoid this problem. Another limitation of eluting with strong acid is that H\textsuperscript{+} replaces all metal ions retained by the resin and does not provide any selectivity, which might be preferred sometimes. Using complexing reagents as stripping reagents can be an alternative. L-cysteine was used as stripping reagent for Cu\textsuperscript{2+} ion after preconcentration by Chelex-100 resin in an off-line spectrophotometric technique to determine Cu in seawater (14). A 20 mL solution of 1.65 x 10\textsuperscript{-4} M L-cysteine buffered at pH 9 was used at an eluting rate of 0.6 mL/min.

Although the on-line ion exchange trace enrichment techniques are
more efficient than conventional column techniques, most of the on-line systems noted above have not been automated with microcomputers. Analysis events such as loading the sample, eluting the column, etc. are usually accomplished by activating pumps or valves manually or semiautomatically at given time intervals. Operation of the system can be labor-intensive and exact timing of each event may be difficult. Microcomputers can be interfaced with the on-line ion exchange system to control those analysis events and automate operations for more efficiency (4, 5).

In this paper, a computer-controlled Chelex-100 ion exchange trace enrichment system on-line with a flame atomic absorption spectrophotometer is presented. Instead of strong acid, the complexing reagents EDTA and cysteine were used as stripping reagents. The design of the flow system is changed from that previously described (4) in our laboratory so that only one pump is used to deliver an acetate buffer mobile phase. This solution carries the sample solution, the stripping reagent solution, and the resin regeneration solution to the enrichment column sequentially. To improve detection limits, a multi-injection technique was developed to allow the system to be configured for multiple injections of a given sample solution before the column was eluted. A stop-flow technique was also developed so that some kinetically slow complexing reactions could be used in eluting metal ions from the enrichment column. Several system variables were studied including column dimensions, sample loading and eluting flow rates, resin particle sizes, and pH. The performance of the system for trace enrichment of transition
metals is demonstrated with solutions of Cu(II), Mn(II) and Cd(II). The system was also used to preconcentrate and detect Cu(II) from river water samples.
Reagents and solutions

All reagents were of analytical grade and used as received unless otherwise specified. Deionized water from a Millipore Milli-Q system connected to the house deionized water was used for all aqueous solution preparation. Standard solutions of 1000 mg/L Cu, Cd, Mn, Pb, and Zn were prepared from dissolutions of salts or nitric acid digestion of pure metal. Standard solutions of lower concentrations for making calibration curves were prepared when needed and stored in dedicated volumetric flasks.

Chelex-100 resins were purchased from Bio-Rad in Na\(^+\) form and were converted to the NH\(_4\)\(^+\) form as previously described (15) and used throughout experiments. Particle sizes of Chelex-100 resins of 50-100 mesh, 100-200 mesh, 200-400 mesh and minus 400 mesh were studied.

The carrier buffer used was 5 x 10\(^{-3}\) M NH\(_4\)Ac/1.7 x 10\(^{-3}\) M HAc at pH 5.2 prepared from dilution of a 5.0 M NH\(_4\)Ac/1.7 M HAc stock solution which was made from mixing a concentrated NH\(_4\)OH solution and glacial acetic acid (HAc).

Stripping reagents (SR) used were 0.01 M cysteine solution and 0.025 M EDTA solution. A 0.01 M cysteine solution was prepared by dissolving of L(+) cysteine hydrochloride in 0.5 M NH\(_4\)OH, and a 0.025 M EDTA solution was prepared by dissolving EDTA acid in NH\(_4\)OH.
with the pH adjusted to ~8-9 with 0.1 M HNO₃ and 0.1 M NH₄OH solutions. The basic cysteine solution is unstable and is prepared fresh daily.

River water samples were collected from the Willamette River at the north boat ramp in Corvallis, OR with polyethylene plastic bottles which were cleaned with HNO₃ and deionized water and then conditioned with same river water sample before sampling for analysis. Immediately after sampling, river water samples were filtered through a precleaned 0.4 µm Nuclepore membrane filter. The filtered water samples were buffered at pH 5.2 by addition of small amount of the concentrated NH₄Ac/HAC buffer. To study the recovery of the system, two portions of the filtered and buffered water sample were spiked with Cu²⁺ ions to provide a 2.5 µg/L and a 5.0 µg/L increase in Cu concentration, respectively.

**Column Preparation**

ALTEX microbore glass columns (3 mm i.d. x 50 mm, 3 mm i.d. x 100 mm, 3 mm i.d. x 150 mm) supplied by Rainin were modified and used in the experiments. The stainless steel mesh screens (2 µm) in the Teflon disc bed supports were removed and replaced with porous polyethylene discs cut from polyethylene sheets with a 35 µm pore size to reduce the pressure drop across the trace enrichment columns and metal contamination. Two other columns used were 1.5 mm i.d. x 50 mm and 1.5 mm i.d. x 100 mm (homemade with Teflon tubing). Columns were packed by delivering a water-slurry of Chelex-100 resin into columns with a Pasteur pipet until columns were filled with resin.
Apparatus

The arrangement of the flow and detector components of the on-line ion exchange trace enrichment system built is depicted in Figure 3.1. The major components used in the system are listed and described in Table 3.1. The sampling loops for the sample solution, the stripping reagent solution and the resin regeneration solution (2 M NH₄OH) are made with 1.5 mm i.d. Teflon tubing. The connections among pumps, valves, the column and detectors are made with 0.5 mm i.d. or 1.5 mm i.d. Teflon tubing and Cheminert type fittings. The 1.5 mm i.d. Teflon tubing is used only in connections where the spreading of the sample zone is not critical. All valves used in the flow system are 4-way pneumatically-actuated slide valves made of Teflon. Pneumatic activation of the valves is achieved with the use of 3-way solenoid valves connected to the house high pressure air line.

The system is controlled by an AIM-65 microcomputer as shown in Figure 3.2. The solenoid valves (and thus the position of the 4-way valves) and the on/off state of the different pumps in the system are controlled by switching the ac power to these devices with solid state relays activated by signals from I/O lines of the computer. The computer system also digitizes the recorder output signal (proportional to absorbance) from the AA spectrophotometer.

Procedure

As seen in Figure 3.1, Pump A is used to pump the NH₄Ac buffer solution which serves as the carrier stream to deliver either the
Figure 3.1. Block Diagram of Flow and Detector Components of the Automated On-line Ion Exchange Trace Enrichment System.
<table>
<thead>
<tr>
<th>component</th>
<th>comment</th>
<th>source and model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump A, B</td>
<td>dual-piston constant flow rate pumps</td>
<td>Laboratory</td>
</tr>
<tr>
<td></td>
<td>flow rate: 0-10 mL/min</td>
<td>Data Control</td>
</tr>
<tr>
<td></td>
<td>pressure: 5000 psi max.</td>
<td>Constametric II G</td>
</tr>
<tr>
<td></td>
<td>pumps for delivering carrier buffer stream. Pump B is used only at high</td>
<td>Constametric I</td>
</tr>
<tr>
<td></td>
<td>flow rates</td>
<td></td>
</tr>
<tr>
<td>Per. Pump I</td>
<td>single channel peristaltic pumps</td>
<td>Markson Science</td>
</tr>
<tr>
<td>Per. Pump II</td>
<td>Per. Pump I is used for loading sample solution and Per. Pump II is</td>
<td>Cat. No. V-13003</td>
</tr>
<tr>
<td></td>
<td>for loading SR and NH_4OH solutions via a &quot;Tee&quot; connector</td>
<td></td>
</tr>
<tr>
<td>Guard Column</td>
<td>3 mm i.d. x 150 mm column packed with 100-200 mesh Chelex-100 resin</td>
<td>Rainin</td>
</tr>
<tr>
<td></td>
<td>for removing possible metal contamination in carrier buffer</td>
<td>Cat. No. 251-04</td>
</tr>
<tr>
<td>Enrichment Column</td>
<td>3 mm i.d. x 50 mm column packed with 100-200 mesh resin for trace</td>
<td>Rainin</td>
</tr>
<tr>
<td></td>
<td>enrichment</td>
<td>Cat. No. 251-84</td>
</tr>
<tr>
<td>V1, V2, V3</td>
<td>4-way slide type sample loop valves with pneumatic activation for</td>
<td>Dionex</td>
</tr>
<tr>
<td></td>
<td>loading sample solution, stripping reagent and NH_4OH solution</td>
<td>Cat. No. 030520</td>
</tr>
<tr>
<td>V4, V4'</td>
<td>4-way slide type valves with pneumatic activation configured as 3-way</td>
<td>Dionex</td>
</tr>
<tr>
<td></td>
<td>switching valves</td>
<td>Cat. No. 030520</td>
</tr>
<tr>
<td>UV</td>
<td>fixed wavelength (254 nm) UV monitor with flow cell</td>
<td>Laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Data control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>model 1203</td>
</tr>
<tr>
<td>AAS</td>
<td>flame atomic absorption spectrophotometer with variable flow rate</td>
<td>Varian</td>
</tr>
<tr>
<td></td>
<td>nebulizer</td>
<td>AA6</td>
</tr>
<tr>
<td>Microcomputer</td>
<td>AIM-65 with expanded memory (24K) and I/O capability</td>
<td>Rockwell</td>
</tr>
</tbody>
</table>
Figure 3.2. Block Diagram of System Control and Signal Acquisition Components of the Trace Enrichment System.
sample solution or the SR and NH$_4$OH solutions to the trace enrichment (TE) column sequentially. The carrier buffer solution also maintains the pH of the resin at a suitable value (i.e., pH 5) to retain most transition metal ions. The flow rate of Pump A is manually adjusted to 6.0 mL/min for routine trace enrichment work and to other desired values in flow rate studies. Pump B is used when a flow rate higher than 10 mL/min is needed. The guard column packed with Chelex-100 resin is placed in the outlet of Pump A to remove the trace amounts of transition metal ions which might be present in the carrier buffer.

Valve V1 is a slider-type sample loop (injection) valve for standard or sample solution. Typically the sampling loop volume used is 1.0 mL or 10.0 mL. The 10-mL loop is used when the concentration of transition metal ion is lower than about 20 μg/L. Valves V2 and V3 are sample loop valves for the SR and the NH$_4$OH solution; the volumes of both loops are typically 1.0 mL. The NH$_4$OH loop is loaded with 2.0 M NH$_4$OH. The NH$_4$OH solution serves to convert Chelex-100 resin back to NH$_4^+$ form after the stripping step. The operations of V1, V2 and V3 are similar. These valves have two positions, "load" and "inject". When V1 is in the "load" position, the standard or sample solution is drawn through the sampling loop by Per. Pump I until filled. Similarly the SR and NH$_4$OH solutions are loaded into the SR loop and the NH$_4$OH loop by Per. Pump II (see Figure 3.3). Next the positions of the V1, V2, and V3 are switched simultaneously (i.e., they are all controlled by one pneumatic activator) to the "inject" position and the standard or sample
Figure 3.3. Operation of the Automated Trace Enrichment System: Loading Sample, SR and NH₄OH solutions.
solution in the sampling loop is delivered to the enrichment column by the carrier buffer (see Figure 3.4). After all the standard or sample solution has been sent to the column, switching valves V4 and V4' (configured as 3-way valves and controlled by one pneumatic activator) are changed to their other position such that the SR and the NH₄OH solutions are sent to the enrichment column to elute the metal ions retained by the column and regenerate the column (see Figure 3.5). The plug of the SR solution carries the eluted metal ions directly into the nebulizer capillary tube of the flame atomic absorption spectrophotometer. A transient, peak shape absorbance signal is obtained. With this design, loading sample, eluting and regenerating column are carried out sequentially and only one HPLC pump is required to carry all those solutions to the enrichment column.

A variable flow rate nebulizer is used in the flame atomic absorption spectrophotometer. The aspiration of the sample solution by the nebulizer is adjusted to the point that the nebulization gas just starts blowing out the sample capillary tube of the nebulizer. The carrier buffer stream is forced into the nebulizer by Pump A (usually at 6.0 mL/min). The flame atomic absorption spectrophotometer with an air-acetylene flame is used as a specific detector for transition metal ions. The analysis wavelengths used are 324.8 nm for Cu, 279.8 nm for Mn, 214.5 nm for Cd, 213.9 nm for Zn, and 217.0 nm for Pb. The flow cell of the UV monitor is placed immediately after the column to measure the absorbance of column eluate in order to determine whether the stripping reagent, which absorbs UV radiation, has been washed completely from the column and
Figure 3.4. Operation of the Automated Trace Enrichment System: Injecting Sample.
Figure 3.5. Operation of the Automated Trace Enrichment System: Eluting the Chelex-100 Column.
the system is ready for next run.

The microcomputer system is used to control the operational events of the trace enrichment system such as switching the valves, loading the sample, SR and NH₄OH solutions, signal acquisition, and data processing. The software package developed for the microcomputer system is modeled after the program developed by Ambrose (4). The experimental parameter values input by the user include the execution time of each operational event and the rate of signal acquisition. The signal acquisition is usually performed for 25 s at a rate of 10 data points per second. The program also calculates the peak height, the peak area, and the retention time (relative to the time of initiating the stripping step) for the elution peak. The absorbance profile of the elution peak is also displayed on an oscilloscope and recorded with a chart recorder.

Two other options developed for the trace enrichment system are multi-injection and stop-flow techniques. The multi-injection mode allows the sample solution to be repeatedly loaded and injected to the enrichment column for the number of times specified by the user before injecting stripping reagent to elute metal ions from the column. This technique improves the detection limit and the dynamic range of the sampling loop and the system. It is not convenient to use sampling loops with a volume greater than about 10 mL because of the large length of tubing required. The multi-injection mode is achieved by proper valve switching and timing.

In the stop-flow mode, the carrier buffer stream is stopped when the front of the stripping reagent solution just passes the enrichment
column by turning off Pump A. This point can be easily located by the response of the UV monitor. Pump A is restarted after a given time. The duration of the stop time is adjustable so that some kinetically slow complexing reactions can be used for eluting metal ions from the column. The technique can potentially be used to study the kinetics of reactions between the solution and the resin phase.

The operation flow chart of the enrichment system is shown in Figure 3.6. The loading of the sample and reagents is depicted as sequential in the diagram. They are actually implemented simultaneously. The times for loading and injecting sample and reagent solutions are determined by the sample loop size and the flow rates used. The flow rate of Per. Pump I or Per. Pump II for loading the sample solution, SR and NH₄OH solutions is about 20 mL/min, and Pump A is usually operated at 6.0 mL/min for routine trace enrichment work. Usually 2 to 3 loop volumes of the solution are used to fill or rinse the sample loop. The loading time can be reduced by using a valve-vacuum combination to pull solution through the sample loops at about 30 mL/min.
Figure 3.6. Operation Flow Chart of the Trace Enrichment System.
RESULTS AND DISCUSSION

Design of Flow System

There are several points to consider when designing a flow system for on-line ion exchange trace enrichment. First is the type of pump to use. Both peristaltic pumps and piston pumps are commonly used for delivering solutions in flow systems. Multichannel peristaltic pumps have been used in most on-line trace enrichment systems reported. However, peristaltic pumps are usually operated at pressures lower than 20 psi. They cannot be used in situations requiring higher pressures (e.g., systems with longer columns packed with smaller diameter resins or studies requiring high flow rates). We used an HPLC-type piston pump in our flow system. It provides an accurate and reproducible flow rate even if the pressure required changes to maintain a constant flow rate. This allows the effects of column dimensions, resin particle sizes, sample loading and eluting flow rates, etc., to be studied comprehensively. In our system, the pump pressure required varied between 30 and 150 psi.

Two primary methods are used for delivering defined volumes of sample solution and stripping reagent solution for the trace enrichment procedure: sampling with a sample loop valve or by time and flow rate. In the latter case, the time a sample solution or stripping reagent solution passes through a pump or pump-valve combination and onto the column is controlled. If the pump flow rate
is constant, the volume of the solution delivered to the column is the product of the flow rate and time. Sampling by time allows the sampling volume to be changed readily. With the sample loop method, only one constant flow rate piston pump is required to operate the system. Contamination from the pump is reduced because sample solutions do not pass through the pump. Sample solutions and stripping reagents are loaded into their loops using low-cost single- or double-channel peristaltic pumps. Different volumes of sample solution are injected into the column to achieve the desired preconcentration factors by changing the sizes of sample loops or using the multi-injection mode.

The automation provided by the flow system is also important. The on-line trace enrichment system we report here is fully automated with microcomputer. This allows the system to operate with high throughput and high reproducibility. The automatic regeneration of the column after each run is also critical in providing long term precision.

Several other designs of the flow system were tried. Valves V4 and V4' can be eliminated if the NH₄OH loop, SR loop and sample loop valves are connected in series. The sample solution, the SR and NH₄OH solutions are sequentially carried to the column. The SR and NH₄OH solutions pass through the sample loop before reaching the column. This configuration performs well if the volume of sample loop is small (e.g., 1 mL). A large sample loop (e.g., 10 mL) can cause severe dispersion of the SR solution so that the elution peak is broad and the elution may not be complete if the SR solution is too
diluted. It is also possible to load and inject the sample solution with one sample loop valve and then to load and inject the SR solution with the same valve. This procedure is more time consuming. Longer wash times are required to clean out the sample loop after each run to prevent cross contamination.

Effects of column dimensions, resin particle size and flow rate

From the point of trace enrichment, the retention of trace metal ions by the Chelex-100 column should be as complete as possible. This condition can be achieved with lower sample loading flow rates and longer columns. On the other hand lower sample loading flow rates decrease the sample throughput rate and longer columns degrade the detection limit. The retention of the metal ions by Chelex-100 resin can also be affected by the resin particle size. No comprehensive studies have been reported in on-line preconcentration systems to understand the effects of column dimensions, resin particle sizes and sample loading flow rates on the uptake of metal ions by Chelex-100 resin. Because those are important factors that affect the performance of on-line trace enrichment system, studies were conducted with Cu(II) as the metal ion probe at pH 5.2.

Figure 3.7 shows the effect of the sample loading flow rate and the column length on retention of Cu$^{2+}$ ion by the Chelex-100 resin (50-100 mesh) under the conditions indicated. The stripping flow rate was kept constant in all experiments so that the elution peak height could be compared among different columns. The retention of Cu$^{2+}$ ion by the column strongly depends upon the sample loading flow rate
Figure 3.7. Effects of the Sample Loading Flow Rate and the Column Length on the Retention of Cu\textsuperscript{2+} ion by the Chelex-100 Column.

Sample: 500 \(\mu\)g/L Cu\textsuperscript{2+} at pH 5.2. Resin: 50-100 mesh Chelex-100. SR: 0.01 M cysteine in 0.5 M NH\textsubscript{4}OH.
for the two shortest columns. The decrease in the retention of Cu\(^{2+}\) ion by the resin with increasing sample loading flow rate is most dramatic for the 50 mm long column. The retention efficiency is less dependent on flow rate for the 100 mm long column and larger for all flow rates. For the 150 mm column, it is independent of the loading flow rate up to at least 15 mL/min. However, the 100 mm long column yields a higher peak signal than the 150 mm long column. The same is true for 50 mm long column when the sample loading flow rate is less than 9 mL/min. With the larger bed volume of the longer columns, the metal ion zone or band eluted from the column by the stripping reagent suffers more dispersion or spreading.

The importance of the particle size of Chelex-100 resin is seen in Figure 3.8. When the 100-200 mesh resin is packed in the shortest (50 mm) column, the retention of Cu\(^{2+}\) ion by the column is much better and independent of the loading flow rate up to at least 15 mL/min. The retention of Cu\(^{2+}\) ion was essentially complete since no Cu was detected by the on-line flame AA spectrophotometer during the sample injection step when high concentrations of Cu were injected. This can be attributed to the fact that the total surface area of 100-200 mesh resin (100 to 300 \(\mu\)m in particle size) is much larger than that of 50-100 mesh resin (300 to 800 \(\mu\)m in particle size) such that the probability for contact of the metal ions with the complexing sites on the resin particles is larger.

The two smaller diameter (1.5 mm i.d.) columns were packed with 100-200 mesh resin. It was found that the retention of Cu\(^{2+}\) ion was not complete when the sample loading flow rate was above 6 mL/min in
Figure 3.8. Effect of the Resin Particle Size on the Retention of 
Cu$^{2+}$ Ion By the Chelex-100 Column.

Sample: 500 µg/L Cu$^{2+}$ at pH 5.2. Column: 3 mm i.d. x 50 mm. 
SR: 0.01 M cysteine in 0.5 M NH$_4$OH.
case of the 50 mm column and above 12 mL/min in case of the 100 mm column. Minus 400 mesh and 200-400 mesh Chelex-100 resins were also studied. However, it was found that the pressure drop across the 3 mm i.d. x 50 mm column packed with these resins was too high (exceeding 200 psi in the case of minus 400 mesh resin). After some use, the resins collapsed under such high pressure. This caused the pressure to increase to the point that the pressure limit for the valves and connections is exceeded (about 300 psi) and leaks occurred.

From the above results it is concluded that the column dimensions, resin particle size, and sample loading flow rate have significant effects on the retention of metal ions by Chelex-100 resin. For trace enrichment of Cu$^{2+}$ ion, the 3 mm i.d x 50 mm column packed with 100-200 mesh resin yielded the best results and can be used with sample flow rates up to at least 15 mL/min. Some researchers (8) reported low recovery (about 50%) for Cu$^{2+}$ ion by Chelex-100 in an on-line system. This is probably due to the use of the larger resin particle (50-100 mesh) and smaller dimension columns that are often necessary with low pressure pumps. It is desirable to operate the on-line trace enrichment system under conditions where complete retention is achieved for metal ions so that the performance of system is less likely to be affected by small variations in column packing, flow rates, etc. For different transition metal ions and different solution conditions, the effects of column length, resin particle size and flow rate might be different. Therefore studies of the effect of flow rate on the elution peak heights are recommended for a particular metal-resin-stripping reagent trace enrichment
The response of the on-line flame AA spectrophotometer to eluted metal ions depends on the eluting flow rate (i.e., the rate that eluted metal ions are sent into the AA nebulizer). Figure 3.9 shows how the height and area of the Cu elution peak change with the eluting flow rate. The peak height increases with the flow rate to about 10 mL/min and then decreases afterward. In contrast, the peak area is relatively independent of the flow rate for lower flow rates and then decreases rapidly as the flow rate increases. To increase the sample throughput, it is desirable to use a large eluting flow rate. At a higher eluting flow rate, the elution peak of metal ions is also expected to be sharper. However, the aspiration rate of the AA nebulizer is typically in the 1-7 mL/min range; the nebulization efficiency decreases if the flow rate is too large. In addition, the AA signal is attenuated if the elution peak is too sharp (i.e., small half-width) because of the time constant limit of the nebulizer-burner or the electronic circuitry. Therefore the eluting flow rate is chosen to be 6.0 mL/min, which is usually the sample loading flow rate.

**Uses of Complexing Reagents as Stripping Reagents**

Because Chelex-100 resin is a chelating resin, it is expected that other complexing or chelating reagents can be used as stripping reagents to elute the metal ions retained by resin. To be effective stripping reagents, they must form stronger complexes or chelates with the transition metal ions than with Chelex-100 resin and the kinetics
Figure 3.9. Effect of the Eluting Flow Rate on the AA Response of the Elution Peak.

Sample: 500 μg/L Cu$^{2+}$ at pH 5.2.
of formation of such new complexes must be rapid.

Complexing reagents provide two advantages over strong acid when they are used as stripping reagents. First, the swelling and contraction of Chelex-100 resin that occurs when a strong acid is used as a stripping reagent can be avoided to a large extent if the complexing reagent is prepared in the proper ionic medium. For example, if Chelex-100 resin is used in the NH$_4^+$ form, the complexing reagent can be prepared in a NH$_4^+$ medium. In this case, the ionic form of Chelex-100 resin is maintained in basically the same condition before and after the stripping step (i.e., the counter ion remains the same). Second, complexing reagents can be selective. After trace enrichment, metal ions may be selectively eluted from the column with different complexing reagents. However, in practice, it may be difficult to find suitable experimental conditions to yield selectivity.

There are very few reports on the use of complexing reagents as stripping reagents for on-line ion exchange trace enrichment for transition metal ions. In one (4), a solution of 0.2 M citrate was used to elute trace transition metal ions such Cu, Cd and Mn retained by the strong acid cation resin AG 50W-X8.

One goal of this project was to find complexing reagents suitable as stripping reagents for transition metal ions in the on-line Chelex-100 trace enrichment system. The effect of the concentration of the stripping reagent L-cysteine on the completeness of elution for Cu$^{2+}$ ion with a 1-mL stripping reagent loop and an eluting flow rate of 6.0 mL/min is shown in Figure 3.10. The elution of Cu$^{2+}$ is
Figure 3.10. Effect of the Concentration of Cysteine on the Elution of Cu(II) from the Chelex-100 column.

Sample: 25 μg/L Cu²⁺ at pH 5.2.
Sample Loop: 10.0 mL.
complete when the concentration of L-cysteine at pH 10 is above $5 \times 10^{-3}$ M. A second stripping step produced no observable peak. These results indicate that L-cysteine can be used as stripping reagent for Cu$^{2+}$ ion in the on-line system.

The mechanism of the reaction between L-cysteine and Cu$^{2+}$ ion is proposed to be a two-step process (16) in which L-cysteine reduces Cu(II) to Cu(I) and then forms a complex with Cu(I). In this research, L-cysteine was also used as stripping reagent for other transition metal ions such as Cd, Zn, and Pb. The elution was not complete for these metal ions even though L-cysteine forms complexes with them. Apparently the reduction step is critical in eluting Cu(II) by L-cysteine. It may also be possible to find other reducing reagents to elute Cu(II) selectively.

Since EDTA forms very strong complexes with transition metal ions, the potential of using EDTA as stripping reagent was also studied. It was found that 1.0 mL of 0.02 M EDTA at pH 8 would completely elute Cd(II) and Pb(II). However, the elution of Mn, Zn, and Cu was not complete at the eluting flow rate of 6.0 mL/min. This indicates that the reactions between EDTA and the Mn(II), Cu(II), and Zn(II) complexed on Chelex-100 resin are slow. To study the kinetics, the stop-flow technique was used. Figure 3.11 shows how elution peak height of Mn(II) changes with the stop time and reaches a plateau for stop time above about 30 s. With a 30 s stop period, it was found that the elution of Mn(II), Cu(II), and Zn(II) by the EDTA solution was complete. Those results indicate that, with the stop-flow technique, some kinetically slow complexation reactions may be used in
Figure 3.11. Effect of the Stopping Time on the Elution of Mn(II) From the Chelex-100 column by EDTA.

Sample: 200 μg/L Mn²⁺.
elution of transition metal ions from Chelex-100 column, which makes
the idea of using complexing reagents as stripping reagents more
promising.

The possibility of using different complexing reagents to
separate transition metals retained by Chelex-100 resin in the on-line
was also studied. Results showed that the Cu$^{2+}$ and Mn$^{2+}$ ions
retained by the resin could be sequentially stripped off using 0.02 M
cysteine for Cu and then 0.02 M EDTA in the stop-flow mode for Mn.

Several other stripping reagents were tried to elute Cu$^{2+}$ from
the Chelex-100 column including 1,2-dihydroxybenzene-3,5-disulfonyl-
acid (Tiron), iminodiacetic acid (IDA), xylenol orange, and
diethylenetriaminepentaacetic acid (DTPA). None of these provided
complete elution. It was also demonstrated that 2.0 M HNO$_3$ could be
used as the SR if valves were added to elute the column in the
opposite direction from loading. The backflushing technique decreases
the compression of resin in the column.

Applications in Trace Enrichment of Transition Metal Ions

To demonstrate capabilities of the on-line ion exchange system in
trace enrichment of transition metal ions, the determination of three
metals, Cu, Mn, and Cd were tested. The enrichment studies were
conducted with a 1-mL and a 10-mL sampling loop. A typical elution
profile of a test run with the trace enrichment system is shown in
Figure 3.12. The change in UV response indicates the profile of the
SR and NH$_4$OH solution plug moving through the flow system. The
elution peak of the metal ion is depicted by the AA response. The
Figure 3.12. A Typical Elution Profile of A Test Run with the Trace Enrichment System.

Sample: 200 $\mu$g/L Cu$^{2+}$ at pH 5.2.
metal elution peak is basically Gaussian in shape with a small degree of tailing. The half-width of the elution peak in this case is about 2.5 s or 0.25 mL (eluting flow rate is 6.0 mL/min). The peak height (proportional to the maximum absorbance of the elution peak) is used in the calibration of the trace enrichment system. The enrichment factor is defined as the ratio of peak absorbance obtained with the trace enrichment system to the steady-state absorbance obtained with continuous pumping of the same solution into the nebulizer of the AA spectrophotometer at the same flow rate. It is typically a factor of 6-8 with the 1-mL loop and a factor of 60-80 with the 10-mL loop. The typical analysis time is about 2-3 min for a run with 1-mL loop and 5-6 min with a 10-mL loop, including the time for data processing.

The calibration curves for Cu, Mn, and Cd solutions in 5.0 mM NH₄Ac and 1.7 mM HAc at pH 5.2 were linear over the ranges 1-250 µg/L with the 1-mL loop, and 0.1-25 µg/L with the 10-mL loop. The peak absorbance for 5 µg/L Cu is 0.045 A.U. with the 10-mL sample loop. The RSD of triplicate test runs is typically 2-4% for measurements with the 1-mL loop for concentrations above 25 µg/L and 3-5% for measurements with the 10-mL loop for concentrations above 2.5 µg/L.

Results of the trace enrichment studies are summarized in Table 3.2 for Cu, Mn, and Cd. The detection limit with the 10-mL loop is improved about a factor of 60 relative to conventional flame AA. The standard deviation of measuring the blank is typically 0.0003 to 0.0006 A.U. with the 1 or 10-mL loop. The peak heights of blank peaks observed with the 10-mL sample loop corresponds to about 0.1 µg/L Cu,
Table 3.2. Detection Limits of Flame AA and the Trace Enrichment System for Cu, Cd, and Mn\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Element Condition</th>
<th>Cu</th>
<th>Cd</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varian\textsuperscript{b}</td>
<td>3</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>Experimental\textsuperscript{c}</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1-mL sample loop</td>
<td>0.7</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>10-mL sample loop</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Detection limits in μg/L are calculated from DL = 2s_{bk}/m where s_{bk} is the blank standard deviation in A.U. and m is the calibration curve slope.

\textsuperscript{b}From manufacturer's literature.

\textsuperscript{c}Experimentally determined with 3-s integration time. The aspiration rate of the nebulizer was 6.0 mL/min.
0.2 μg/L Cd, and 0.3 μg/L Mn. The blank peak is barely discernible for Cu and Cd as evidenced by a large standard deviation in the retention time of the blank peak.

The multi-injection technique was tested with 1-mL and 10-mL sample loops. The eluting peak height was found to be linearly related to the number of injections over the range tested (1 to 7 injections with the 1-mL loop and 50 μg/L Cu, and 1 to 5 injections with the 10-mL loop and 5.0 μg/L Cu). After five injections with the 10-mL sample loop, an enrichment factor of 300 was obtained for the solution of 5.0 μg/L Cu, corresponding to a detection limit of 0.02 μg/L. The blank signal also increased with the number of injections. The increase of the blank signal was a factor of 2 after five injections with the 10-mL loop relative to one injection. The multi-injection technique can provide extremely low detection limits if the time is not a factor (5 injections with the 10-mL sample loop take about 35 min). Ultimately this technique is limited by the increase in the blank signal when the standard deviation of the blank is proportional to the number of injections.

The application of the system to preconcentrate transition metal ions from real samples was demonstrated with preconcentration of Cu(II) from river water samples. Some results are summarized in Table 3.3. Clearly, the system can be used to measure very low concentrations with good precision. The recovery of Cu²⁺ added to the river water samples is low. Other researchers (3,17) also reported similar problems when Chelex-100 resin was used in off-line methods to preconcentrate trace metals from natural waters. The low
Table 3.3. Determination of Cu in a River Water Sample.

<table>
<thead>
<tr>
<th>samplea</th>
<th>Cu foundb</th>
</tr>
</thead>
<tbody>
<tr>
<td>river water</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>river water + 2.5 µg/L</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>river water + 5.0 µg/L</td>
<td>2.51 ± 0.14</td>
</tr>
</tbody>
</table>

aAll sample solutions were buffered at pH 5.2.
bResults are in µg/L (av. ± std. dev.) for 3 runs.
recovery in the off-line methods and in our system is attributed to the fact that natural waters contain many natural ligands such as fulvic acid, humic acid that complex trace metals like Cu or particulate matter such as iron oxide colloids that adsorb trace metals. Some of these metal complexes are strong enough that they do not dissociate when passing through Chelex-100 resin. Therefore, they are not retained by Chelex-100 resin. Recoveries may be lower with an on-line system compared to an off-line system because of the smaller contact time between the sample solution and the resin due to higher sample loading flow rates and smaller resin bed volumes (i.e., there is less time for complexes with slower dissociation kinetics to dissociate).

To improve the recovery, the pH of the sample solution can be adjusted to lower values. For example, at pH 4, Cu$^{2+}$ ion is still completely retained in our system but a large fraction of complexed or absorbed metals is released from complexing agents or colloids. To measure the total concentration of a given metal, all chemical forms of the metal of interest in samples should be converted into forms totally retained by the Chelex-100 resin (e.g., acid digestion can be used, but was not in this study).
CONCLUSIONS

Studies with the on-line ion exchange trace enrichment system we report here show that factors such as column dimensions, resin particle sizes, and sample loading and eluting flow rates are critical in performance of on-line trace enrichment systems. These variables should be tested when using different ion exchange resins or stripping reagents. Using complexing reagents rather than acids as stripping reagents has proved to be advantageous. Other complexing reagents might lead to uses of other detection methods such as molecular absorption spectrophotometry, fluorescence spectrometry, etc. The current on-line trace enrichment system is being expanded into an on-line two-column ion exchange system for the determination of trace metal speciation in natural waters.
REFERENCES


CHAPTER 4

AUTOMATED TWO-COLUMN ION EXCHANGE SYSTEM FOR DETERMINATION

OF THE SPECIATION OF TRACE METALS IN NATURAL WATERS

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ABSTRACT

A method for determination of metal speciation based on an automated two-column ion exchange system is described. The dissolved trace metal species are classified into three fractions. Two fractions of dissolved trace metal species are directly determined with an on-line flame atomic absorption (AA) spectrophotometer after preconcentration by a column of Chelex-100 chelating resin and a column of AG MP-1 macroporous anion resin. The third fraction is determined by a standard addition procedure. Variables that affect the results obtained by the two-column system are studied using model complexing agents. The method is shown to be simple and rapid (about 10 minutes per sample with a 10-mL sample loop). Detection limits of the method are 0.1 μg/L for Cu(II), 0.08 μg/L for Cd(II) and 0.2 μg/L for Zn(II) with a 10-mL sample loop and on-line flame atomic absorption detection. The method is used to determine the speciation of Cu(II), Cd(II) and Zn(II) in natural water samples.
INTRODUCTION

Knowledge of chemical speciation of trace metals in natural waters is essential for the interpretation of biological or geochemical cycling of trace metals in natural waters. In recent years, several measurement schemes have been reported for the determination of chemical speciation of trace metals in seawater and freshwater (1-7). Experimental approaches to determine metal speciation are based on various combinations of analytical measurement and separation techniques such as anodic stripping voltammetry (ASV), ion exchange separation, solvent extraction, ultrafiltration, dialysis, etc. Different physico-chemical forms of trace metals in water samples are operationally divided into several groups by separation and measurement procedures. Speciation results are generally method-dependent.

Most measurement schemes reported so far involve rather complex and time-consuming analytical procedures in an attempt to classify as many fractions of metal species in natural waters as possible. For example, Batley and Florence (1,2) developed a comprehensive scheme using various combinations of ASV, ion exchange separation with Chelex-100 resin, and UV irradiation. Their scheme is capable of classifying seven groups of dissolved trace metal species. However, those groups of metal species are not mutually exclusive and most groups are obtained by difference. The sample throughput of eight
hours per sample in this scheme may limit its routine application. In addition, the classification of metal species provided by such a scheme may be more detailed than what is required to define the biological effects of interest such as toxicity of trace metals to aquatic organisms (8). Other measurement schemes which classify a given metal in a smaller number of groups of species are also time-consuming to implement. The development of relatively simple and rapid measurement schemes will be valuable in providing routine methods for establishing the correlation of metal speciation and its biological effects.

Ion exchange separation with Chelex-100 resin has been widely used in the determination of chemical speciation of trace metals in natural waters. The technique is simple and almost free of contamination and is a key step in most measurement schemes. Chelex-100 resin is a chelating ion exchange resin with iminodiacetic acid functional groups and has a high selectivity for transition metal ions relative to alkali and alkaline metal ions. In the method of ion exchange separation with Chelex-100 resin, a water sample (filtered through a 0.45 μm filter) is usually stirred with Chelex-100 resin in a beaker (batch mode) or is passed through a column containing Chelex-100 resin (column mode). Dissolved metal species are separated into two fractions, which have been referred to as "Chelex labile" and "Chelex non-labile" species.

"Chelex labile" species are those retained by the resin, including hydrated metal ions and some weakly complexed or bound metal species that dissociate allowing the metal to be bound by Chelex-100
resin. The labile fraction is determined by stripping the retained metal with acid followed by determination with atomic spectrometry or ASV. "Chelex non-labile" species are not retained by the resin. They are likely to be stable metal complexes with natural organic ligands such as fulvic and humic acids and metals strongly associated with (i.e., complexed by or adsorbed on or occluded in) organic and inorganic colloidal particles. This fraction can be determined by difference if the total metal concentration is known. This classification is important because it may provide the speciation information about the main classes of toxic and non-toxic trace metal, respectively, in natural waters (9).

In all the measurement schemes reported (1-7), ion exchange separation with Chelex-100 is carried out in an off-line batch or column mode. To achieve the preconcentration factor necessary to determine trace metals at μg/L concentrations, a relatively large amount of the water sample must be used. Thus the conventional methods are rather time-consuming (e.g., sometimes hours per sample). Recently, several on-line ion exchange preconcentration systems using Chelex-100 resin have been reported in which the labile fraction is eluted directly into the nebulizer of an atomic spectrometer (10-13). With the on-line ion exchange preconcentration systems, the preconcentration factor is greater for a given volume of sample because the smaller ion exchange columns are used (less dispersion) and the eluted metal is detected on-line (i.e., the maximum concentration of the eluted peak is detected). These advantages coupled with higher sample flow rates allow smaller sample volumes to
be used. The overall advantage is a much higher rate of sample throughput (e.g., minutes per sample) for a given preconcentration factor than that achieved with off-line systems.

In this paper, an automated on-line trace enrichment system in our laboratory (13) has been expanded to an automated two-column ion exchange system to allow rapid determination of trace metal speciation in natural waters. The automated two-column ion exchange system is based on two small columns of Chelex-100 resin and AG MP-1 resin. The AG MP-1 resin is a macroporous anion exchange resin with a large pore size (14). It has been used in separation of proteins and enzymes (15, 16). It is used in the proposed measurement scheme to retain negatively charged metal complexes and metals strongly associated with negatively charged organic and inorganic colloids. In the automated two-column ion exchange system, the water sample filtered through a 0.4 μm filter is passed sequentially through a Chelex-100 column and a AG MP-1 column. Different physico-chemical forms of soluble trace metal species are retained by the Chelex-100 column or the AG MP-1 column accordingly. The measurement scheme based on the two-column system is studied and optimized using model complexing agents. The method is used to determine the speciation of Cu(II), Cd(II), and Zn(II) in natural water samples.
Reagents and solutions

All reagents were of analytical grade and used as received unless otherwise specified. Deionized water from a Millipore Milli-Q system connected to the house deionized water was used for all aqueous solution preparation. Solution were prepared under a class-100 laminar flow hood.

Solutions of 1000 mg/L Cu(II), Cd(II), and Zn(II) in 0.1% HNO₃ were prepared from dissolutions of metal salts. Metal solutions of low concentrations were prepared as need and stored in dedicated volumetric flasks.

Solutions of 0.100 M EDTA, 0.100 M NTA, 0.100 M glycine, and 0.500 M iminodiacetic acid (IDA) were prepared by adding enough 2 M NH₄OH to dissolve these amino acids in water. A humic acid solution was prepared by dissolving 100 mg of humic acid (sodium salt) from Aldrich Chemical Co. in 1 L of deionized water, then filtering the solution through an acid-cleaned 0.40 μm Nuclepore filter.

The carrier buffer solution used in the system was a solution of 5 mM NH₄Ac/1 mM HAc at pH 5.4 from dilution of a 5 M NH₄Ac/1 M HAc stock solution which was made from mixing a concentrated NH₄OH solution and glacial acetic acid (HAc). A solution of 0.025 M cysteine/0.5 M NH₄OH/2.0 M NH₄NO₃ was used as the stripping reagent (SR) for Cu(II), a solution of 0.1 M EDTA/0.5 M NH₄OH/2.0 M
NH₄NO₃ was used as the SR for Cd(II), and a solution of 2.0 M HNO₃ was as the SR for Zn(II).

Chelex-100 resin of 100-200 mesh in Na⁺ form and AG MP-1 resin of 100-200 mesh in Cl⁻ form were purchased from Bio-Rad. The resins were treated with 2.0 M HNO₃ and 2.0 M NH₄OH in a manner similar to that described previously (17). After the treatment, Chelex-100 resin was converted into NH₄⁺ form and AG MP-1 resin was converted into OH⁻ form. The converted resins were used throughout the experiments.

**Column Preparation**

Altex microbore glass columns (3 mm i.d. x 50 mm) modified as reported previously (13) were packed with the water-slurries of Chelex-100 resin and the AG MP-1 resin and used in the experiments.

**Apparatus**

The arrangement of components of the automated two-column ion exchange system is depicted in Figures 4.1 and 4.2. The major components and construction of the system are basically the same as previously reported for the on-line one-column trace enrichment system (13). However, in order to accommodate the second ion exchange column, a second pair of column stripping reagent and regeneration reagent (i.e., NH₄OH) sample loop valves V8 and V9 are added for eluting and regenerating the AG MP-1 ion exchange column. Three 3-way switching valves V5, V6 and V7 are also added to direct the flow path of the carrier buffer stream. Two dual channel peristaltic pumps
Figure 4.1. Block Diagram of Flow and Detector Components of the Automated Two-column Ion Exchange System.
Figure 4.2. Block Diagram of System Control and Signal Acquisition Components of the Two-column Ion Exchange System.
(Per. Pump II and Per. Pump III) were used for loading valves V2, V3, V8, and V9. The AA spectrophotometer was operated with an air-acetylene flame and the conditions described previously (13).

The operation of the automated two-column system consists of five steps. In step one (see Figure 4.3), sample solution, SR and 2 M NH₄OH solutions are loaded into their sampling loops by peristaltic pumps. The process of loading sample, SR and NH₄OH solution usually takes 60 s when a 10-mL sample loop is used, and 20 s when a 2-mL sample loop is used. The peristaltic pumps for loading SR and NH₄OH solutions are turned off after 8 s to conserve these reagents. In step two (see Figure 4.4), the positions of V1, V2, V3, V8, and V9 are switched simultaneously (i.e. they are all controlled by one pneumatic activator) to the "inject" position and carrier buffer from pump A pushes the sample solution plug in the sample loop to the two ion exchange columns sequentially; this process takes 300 s for a 10-mL sample loop and 80 s for a 2-mL sample loop. In step three (see Figure 4.5), the positions of valves V4, V6, and V7 are switched and the carrier buffer stream is directed to push the SR I and 2 M NH₄OH solutions in V2 and V3 to the Chelex-100 column to elute the metal species retained on this column. The plug of SR carries metal species directly into the nebulizer of a Varian flame atomic absorption spectrophotometer and a transient AA signal produced is recorded. The elution of the Chelex-100 column requires 60 s including the time to pass the NH₄OH solution to regenerate the column and wash away all the SR and NH₄OH solutions in the flow path. In step four (see Figure 4.6), the positions of V5 and V7 are switched so that the
Figure 4.3. Operation Sequence of the Two-column Ion Exchange System.
Step One: Loading Sample, SR and NH₄OH.
Figure 4.4. Operation Sequence of the Two-column Ion Exchange System. Step Two: Injecting Sample.
Figure 4.5. Operation Sequence of the Two-column Ion Exchange System.
Step Three: Eluting the Chelex-100 column.
Figure 4.6. Operation Sequence of the Two-column Ion Exchange System. Step Four: Eluting the AG MP-1 column.
carrier buffer stream is redirected to push the SR II and the 2 M NH\textsubscript{4}OH solutions in V8 and V9 to elute metal species retained on the AG MP-1 column. The eluted metal species in the SR plug are carried to the nebulizer of the AAS and the second elution peak is recorded. It also takes 60 s to complete the process. Finally, all the valves in the system are switched back to their initial positions (see Figure 4.3). The carrier buffer stream is passed through the ion exchange columns for 90 s to condition the columns before next run. The flow rate of carrier buffer stream is usually set at 5.0 mL/min. Pump B is used when a flow rate above 10 mL/min is needed.

An AIM-65 microcomputer system is interfaced with the two-column system as seen in Figure 4.2. It is programmed to control completely the operation of the system including switching valves to direct the flow path, loading the sample, SR and NH\textsubscript{4}OH solutions, signal acquisition and data processing. The multi-injection and stop-flow techniques developed in the on-line one-column system (13) are also incorporated in the operation of the two-column ion exchange system. The software was modified to report the peak height, peak area, and retention time (relative to the time of initiating the stripping step) for the AA peaks resulting from the elution of each column and statistical data for repetitive runs.

**Studies of the Two-Column Measurement Scheme**

Solutions of 200 μg/L Cu(II) were prepared in different complexing media including EDTA, IDA, NTA, glycine, and humic acid. These solutions were tested to study and optimize the measurement
scheme based on the two-column ion exchange system. Experiments were conducted at different values of pH, complexing agent concentrations and ionic strengths to study the effects of these variables on the speciation measurements. A 2-mL sample loop was used in these experiments.

Speciation of Trace Metals in Water Samples

Water samples were collected from the Willamette River slightly below the surface of water with 1-L polyethylene plastic bottles and a rural drainage ditch near Corvallis, Oregon. The bottles were cleaned with HNO₃ and deionized water and conditioned with the same water samples before the sampling for analysis. Water samples were filtered through an acid-cleaned 0.40 μm Nuclepore filter immediately after sampling. Speciation measurements were conducted for Cu(II), Cd(II) and Zn(II) in both water samples. The filtered water samples were buffered at pH 6.8 in 0.01 M NH₄Ac by addition of a small volume of concentrated buffer. A portion of filtered and buffered water sample was spiked with each metal ion to provide 6.0 μg/L increase in concentration. Sample solutions were allowed to stand for 8 to 12 h before measurement. Calibration curve data were obtained with solutions of 2.5, 5.0, 10, and 20 μg/L of each metal ion at pH 6.8 in 0.01 M NH₄Ac. A 10-mL sample loop was used in these experiments because of the low metal concentrations.
Two-column Measurement Scheme

The measurement scheme based on the two-column ion exchange system is depicted in Figure 4.7. The dissolved trace metal species in the water sample are separated into three fractions by the two ion exchange columns. The Chelex-100 column retains hydrated metal ions and metal ions from metal species that dissociate when passing through the Chelex-100 column. These metal species may include labile metal complexes and possibly metals loosely associated with organic and inorganic colloidal matter. The metal species retained by the Chelex-100 column are referred to as M1 species in the two-column measurement scheme. Non-labile metal complexes and metals strongly associated with organic and inorganic colloidal matter are not retained by the Chelex-100 column. The AG MP-1 resin is a macroporous strongly basic anion exchange resin with large pore size. Therefore, the AG MP-1 column can retain metal complexes that are negatively charged and some metal ions associated with negatively charged organic matter such as humic acid. These metal species are referred to as M2 species. Metals strongly associated with very large organic or inorganic colloidal particles may not be retained because of the molecular exclusion limit of the AG MP-1 resin (about 75,000 MW (14)). Also neutral non-labile metal complexes may not be retained by the column. These metal species make up the third fraction and are
Figure 4.7. Two-column Ion Exchange Measurement Scheme.
referred to as M3 species.

The concept of using two ion exchange columns in series (i.e., a cation exchange column followed by an anion exchange column or visa versa) to retain two fractions of metal species has been demonstrated by several researchers using conventional and off-line ion exchange techniques (18-21). Anion resins such as AG 1-X8, a strongly basic anion resin (19), and Sephadex A-25, a weakly basic anion exchanger (21), have been used to retain anionic metal species or metals associated with negatively charged humic substances. In our research, several anion resins, including the AG 1-X8, Bio-Rex 5 (intermediate basic), Sephadex A-25, and AG MP-1 resins, were tested for their use in the two-column measurement scheme. The Sephadex A-25 anion exchange resin was found to be not suitable for use in the two-column system because it collapsed under the pressure of the flow system (typically 60 psi) due to its weak gel structure. The AG 1-X8 and Bio-Rex 5 resins were found to be less efficient than the AG MP-1 resin in the retention of the non-labile Cu(II)-humic complexes because they have smaller molecular exclusion limits (e.g., about 2000 MW for the AG 1-X8 resin) than the molecular exclusion limit of the AG MP-1 resin. Therefore the AG MP-1 resin was chosen for the two-column measurement scheme.

The arrangement of two ion exchange columns in the measurement scheme is critical. It is necessary to place the Chelex-100 column in front of the AG MP-1 column because fresh AG MP-1 resin retains trace amounts of free Cu(II) ions. Furthermore, it was also observed that the color of the AG MP-1 resin changed from off-white before use to
beige after a few runs with sample solutions containing humic acid. This indicates that some organic matter such as humic material in sample solutions that is retained by the AG MP-1 resin may not be completely eluted from the resin after each sample run. Some of this permanently retained organic matter is capable of binding trace metal ions. In fact, after the AG MP-1 column was used for a few sample runs with sample solutions containing humic acid, it retained most of the hydrated Cu ions in 1.0 mL of a 200 μg/L Cu$^{2+}$ solution. Therefore, sample solutions are passed through the Chelex-100 column before they enter the AG MP-1 column in the two-column system to avoid the retention of hydrated trace metal ions by the AG MP-1 column.

Strong complexing agents have been used successfully to elute trace metal ions retained by Chelex-100 resin in the on-line ion exchange system reported previously (13). In that study, a solution of 0.01 M cysteine in 0.5 M NH$_4$OH was used as the stripping reagent solution to elute Cu ion retained by Chelex-100 resin, and a solution of 0.025 M EDTA at pH 9 was used to elute other heavy metals such as Cd, Pb, Zn and Mn.

A suitable SR solution is also needed to elute trace metal species retained by the AG MP-1 column in the on-line system. It was found that a solution of 2 M NH$_4$NO$_3$ was able to elute effectively anionic metal complexes such as (CuEDTA)$_2^{-}$ from the AG MP-1 column. However, trace metal species complexed by organic matter such as humic acid that is irreversibly adsorbed on the AG MP-1 resin may not be completely eluted off the resin by the nitrate solution. To strip these metal species, cysteine or EDTA are added to the nitrate
solution since they are sufficiently strong complexing agents to strip trace metals off the adsorbed organic matter. The exact compositions of the SR solutions for either column for Cu(II) and Cd(II) are specified in the experimental section.

Nitric acid solution is often used to elute trace metal ions retained by Chelex-100 resin. A solution of 2.0 M HNO₃ was also tested as the SR for the AG MP-1 column and was found to be effective for elution of metal species retained by the column. A 2 M HNO₃ solution was used as the SR to elute Zn(II) species retained by the two columns. To avoid the pressure increase problem of the Chelex-100 column when using nitric acid as the SR, two valves were added to the system to reverse the flow direction of the carrier buffer stream through the Chelex-100 column and the AG MP-1 column during the time period that the sample solution is loading into the sample loop. The back-flushing technique decreases the compression of the resin in the column. The SR solution for Cd(II) can also be used to strip Zn(II) from the columns. However, the stop-flow technique must be employed (13).

The first two fractions of metal species (M₁ and M₂) in the measurement scheme are preconcentrated by the Chelex-100 column and the AG MP-1 column, respectively. They are determined by the two-column ion exchange system. An example of the AA signal profiles produced by the two-column system is shown in Figure 4.8. Peak I is the elution peak from the Chelex-100 column and Peak II is the elution peak from the AG MP-1 column. If each column is loaded with an equivalent amount of metal species (i.e., same number of moles of
Figure 4.8. AA Signal Profiles of Experimental Runs with the Two-column Ion Exchange System.

Sample: 200 µg/L Cu(II) + 8 mg/L Humic Acid at pH 7.
Cu(II)), the elution peak height for the two columns are generally somewhat different. This occurs because the dispersion of the peaks differs due to the differences in the stripping flow paths to the detector, the column packings, the resin particle sizes, and the mechanisms of elution. As illustrated by the data in Table 4.1, the peak areas for equivalent amounts of free Cu(II) ions and (CuEDTA)$^{2-}$ anionic complexes retained by the Chelex-100 column and the AG MP-1 column are statistically equivalent, but the peak heights differ by about 15%. Therefore, the peak area is used to quantitate the amounts of Cu and other metals. The two-column system is calibrated by measuring the peak areas for the Chelex-100 column with standard solutions of free trace metal ions. Alternatively, the AG MP-1 column can be calibrated with a known amount of a negatively charged metal complex (e.g., (CuEDTA)$^{2-}$) that is completely retained by the column.

**Speciation of Trace metals in Model Complexing Media**

The two-column measurement scheme discussed above was further studied and optimized with Cu(II) as a trace metal probe. Speciation results for 200 μg/L (3.15 μM) Cu(II) in different complexing media are summarized in Table 4.2. Equilibrium calculations show that 100% of the copper is complexed by the corresponding ligands in 1.0 mM glycine, 1.0 mM IDA, and 4.0 μM EDTA solutions, and 64% of the copper is complexed in 2.0 μM EDTA and 2.0 μM NTA solutions. The results in Table 4.2 demonstrates that all Cu(II) species in the NH$_4$Ac/HAc and glycine solutions and 92% of the Cu(II) species in the IDA solution
Table 4.1. Peak Height and Area Data for the Elution Peaks from the Chelex-100 Column and the AG MP-1 Column.

<table>
<thead>
<tr>
<th>Column</th>
<th>Peak Height(^a)</th>
<th>Peak Area(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelex-100(^c)</td>
<td>0.183 ± 0.001</td>
<td>5964 ± 67</td>
</tr>
<tr>
<td>AG MP-1(^d)</td>
<td>0.159 ± 0.003</td>
<td>5902 ± 51</td>
</tr>
</tbody>
</table>

\(^a\)The peak height data are in A.U. (av. ± std. dev. for four runs).
\(^b\)The peak area data are in A.U. x s (av. ± std. dev. for four runs).
\(^c\)Sample solution was 200 µg/L Cu(II) in 0.005 M NH\(_4\)Ac/0.001 M HAc at pH 5.2.
\(^d\)Sample solution was 200 µg/L Cu(II) in 0.001 M EDTA at pH 7.
Table 4.2. Speciation Results of Cu(II) in Some Complexing Media$^a$.

<table>
<thead>
<tr>
<th>Solution$^b$</th>
<th>M1%</th>
<th>M2%</th>
<th>log K'</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM NH$_4$Ac/1 mM HAc</td>
<td>100 ± 2</td>
<td>ND$^c$</td>
<td>—</td>
</tr>
<tr>
<td>1.0 mM Glycine</td>
<td>100 ± 1</td>
<td>ND</td>
<td>9.9</td>
</tr>
<tr>
<td>1.0 mM IDA</td>
<td>92 ± 1</td>
<td>8 ± 1</td>
<td>11.9</td>
</tr>
<tr>
<td>4.0 µM EDTA</td>
<td>ND</td>
<td>100 ± 2</td>
<td>15.5</td>
</tr>
<tr>
<td>2.0 µM EDTA</td>
<td>36 ± 2</td>
<td>64 ± 1</td>
<td>15.5</td>
</tr>
<tr>
<td>2.0 µM NTA</td>
<td>42 ± 2</td>
<td>58 ± 2</td>
<td>10.3</td>
</tr>
<tr>
<td>8.0 mg/L Humic Acid</td>
<td>41 ± 2</td>
<td>52 ± 1</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$Results are in percentage (av. ± std. dev. for three runs), where M1\% is the percentage of the copper retained by the Chelex-100 column and M2\% is the percentage of the copper retained by the AG MP-1 column.

$^b$The concentration of Cu(II) was 3.15 µM in all solutions. All solutions were adjusted to pH 7 except the acetate buffer solution was at pH 5.2.

$^c$ND = not detected.

$^d$log K' is the log of the conditional stability constant of the Cu(II) complex at pH 7.
are M1 species. In contrast, the stable Cu(II)-EDTA complex (i.e., (CuEDTA)$^{2-}$ ion at pH 7) does not dissociate during passage through the Chelex-100 column and is a M2 species. All the Cu species are retained by the AG MP-1 column when EDTA is in molar excess relative to Cu(II) (i.e., 4.0 µM EDTA). If the Cu$^{2+}$ ion is in excess (i.e., 2.0 µM EDTA), only 64% of the Cu(II) is a M2 species as predicted by equilibrium calculations. It was also shown that the retention of (CuEDTA)$^{2-}$ by the AG MP-1 column is independent of the sample loading flow rate up to 15 mL/min. The Cu(II)-NTA complex is less stable than the Cu(II)-EDTA complex. A small fraction of the Cu(II)-NTA complex dissociates during passage through the Chelex-100 column and 42% of the Cu(II) species is a M1 species (rather than 36% if there was no dissociation). The results for Cu(II) species in the solution of 8 mg/L humic acid show that a significant fraction of Cu(II) is strongly associated with humic acid and is a M2 species (52%). In addition, the M3 fraction is about 7%.

The dissociation of Cu(II) complexes in the Chelex-100 column depends on the thermodynamic stability and dissociation kinetics of these complexes. The values of the conditional stability constants of the Cu(II) complexes with glycine, IDA, NTA, and EDTA are given in Table 4.2. The speciation results suggest that the Cu(II) complexes with conditional stability constants similar to that of the Cu(II)-EDTA complex (log $K' = 15.5$ at pH 7) are non-labile and they do not dissociate when passing through the Chelex-100 column. These metal complexes are classified as the M2 species in the two-column speciation scheme. The Cu(II) complexes with conditional stability
constants smaller or similar to those of the Cu(II)-glycinate and Cu(II)-IDA (log $K' = 10-12$ at pH 7) are likely to dissociate in the Chelex-100 column provided that the dissociation rate constant is sufficiently large. These metal species are classified as M1 species. The conditional stability constant of Cu(II)-NTA complex is close to the Cu(II)-glycinate complex. However only a small fraction of the Cu(II)-NTA complex dissociates in the Chelex-100 column possibly due to its slow dissociation kinetics. The metal complexes with slow dissociation kinetics are also classified as primarily M2 species by the two-column system.

Since the AG MP-1 resin is a strongly basic anion resin, the retention of anionic metal species by the AG MP-1 column can be affected by high concentrations of competing anionic species in the solution. The effect of the nitrate concentration on the retention of anionic Cu(II)-EDTA complex by the AG MP-1 column is shown in Figure 4.9. The retention efficiency of the column for (CuEDTA)$^{2-}$ decreases rapidly as the concentration of $\text{NO}_3^-$ in the Cu-EDTA solution is increased above 0.01 M. The unretained (CuEDTA)$^{2-}$ ion was observed with the on-line flame AA detector during the process of loading sample solution when the nitrate concentration was above 0.05 M. The results suggest that the effect of major inorganic anions on the retention of anionic metal species by the AG MP-1 column should be investigated when the two-column system is applied to water samples such as seawater that contain high concentrations of anions. The competition between anion metal species and major anions should not be a significant problem in case of freshwater since the ionic strength
Figure 4.9. Effect of Nitrate Concentration on the Retention of Anionic Cu-EDTA Complex by the AG MP-1 Column.

Sample: 200 µg/L Cu(II) in 0.001 M EDTA at pH 7.
is usually lower than 0.01 M. Other anions are likely to have
different effects since the selectivity of the AG MP-1 resin varies
among anions.

Humic substances are a major component of dissolved organic
matter in natural waters. The complexation of trace metals by humic
substances is very important in their speciation in natural waters.
The effects of the solution pH and the concentration of humic acid on
the speciation of Cu(II) in humic acid solutions were studied with the
two-column system. The pH effect is shown in Figure 4.10. As the pH
value of the Cu(II)-humic solution changes from 5 to 9, the fraction
of the M1 species decreases from 60% to 25%, the fraction of the M2
species increases from 36% to 61%, and the fraction of the M3 species
increases from 4% to 14%. As expected a larger fraction of Cu(II) is
complexed by humic acids at higher pH as there is less H⁺ to compete
for binding sites on the humic acids. From pH 5 to 9, the ratio of
the M3 fraction to the M2 fraction is relatively constant (0.12-
0.22).

The change of Cu(II) speciation with the humic acid concentration
was also studied and the results are shown in Figure 4.11. The degree
of the complexation of Cu(II) by humic acid increases rapidly with the
concentration of humic acid in the solution. The M3 fraction of the
Cu(II) species in the humic solution also increases with the humic
concentration. In addition, the ratio of the M3 fraction to the M2
fraction also stays relatively constant from 4 to 20 mg/L (0.47 to
0.26).

In all measurement schemes involving Chelex-100 ion exchange, the
Figure 4.10. Effect of Solution pH on the Speciation of Cu(II) in the Humic Acid Solution.

Sample: 200 µg/L Cu(II) in 8 mg/L Humic Acid.
Figure 4.11. Effect of the Concentration of Humic Acid on the Speciation of Cu(II).

Sample: 200 µg/L Cu(II) at pH 7.
classification of fractions is operational because the magnitudes of fractions retained and not retained by the Chelex-100 resin depend on the contact time of the test solutions with the resin (22,23). In flow systems, the contact time depends on the sample loading flow rate and the void volume of the column. With longer contact times (or lower flow rates for a given column), the dissociation of some moderately labile metal complexes and thus the so called "Chelex labile" fraction can be greater. The two-column system provides advantages relative to conventional off-line Chelex column techniques. First, the use of an HPLC pump provides precise control of the flow rate and contact time from day to day. Second, the use of small columns and high flow rates decreases the dissociation of moderately labile metal complexes. The contact time is estimated to be about 1.4 s with a 5 mL/min flow rate. For a 200 μg/L Cu(II) solution in 8 mg/L humic acid at pH 6.5, the percentage of the "Chelex labile" fraction changed from 49% to 36% when the flow rate was changed from 5 to 15 mL/min. At a 1 mL/min flow rate, the labile fraction was 67%. The dependence of the speciation on the sample loading flow rate can be used to obtain kinetic information about the dissociation of metal complexes as discussed in another paper (24).

Application to Environmental Water Samples

The speciation results for Cu(II), Cd(II) and Zn(II) in water samples presented in Table 4.3 demonstrate that the two-column ion exchange system is applicable to very low metal concentrations. The results show that only a small fraction of the Cu(II) species is the
Table 4.3. Speciation Results of Cu, Cd, and Zn in Water Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>[M1]</th>
<th>[M2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>0.12 ± 0.05</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>River + 6.0 µg/L</td>
<td>1.84 ± 0.09</td>
<td>2.15 ± 0.05</td>
</tr>
<tr>
<td>Ditch</td>
<td>0.29 ± 0.07</td>
<td>2.56 ± 0.08</td>
</tr>
<tr>
<td>Ditch + 6.0 µg/L</td>
<td>0.52 ± 0.06</td>
<td>6.95 ± 0.28</td>
</tr>
<tr>
<td>Cd(II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>0.12 ± 0.02</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>River + 6.0 µg/L</td>
<td>4.35 ± 0.05</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>Ditch</td>
<td>0.13 ± 0.02</td>
<td>ND</td>
</tr>
<tr>
<td>Ditch + 6.0 µg/L</td>
<td>4.09 ± 0.05</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Zn(II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>2.69 ± 0.23</td>
<td>ND</td>
</tr>
<tr>
<td>River + 6.0 µg/L</td>
<td>7.77 ± 0.09</td>
<td>ND</td>
</tr>
<tr>
<td>Ditch</td>
<td>1.13 ± 0.10</td>
<td>ND</td>
</tr>
<tr>
<td>Ditch + 6.0 µg/L</td>
<td>5.90 ± 0.12</td>
<td>0.30 ± 0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>Results are in µg/L (av. ± std. dev. for three runs).

<sup>b</sup>ND = not detected, the detection limits are 0.1 µg/L for Cu, 0.08 µg/L for Cd, and 0.2 µg/L for Zn. The detection limits are calculated from DL = 2s<sub>bk</sub>/m where s<sub>bk</sub> is the blank standard deviation and m is the calibration slope.
M1 species; a large fraction of Cu(II) species is the M2 species. However, the majority of Cd(II) and Zn(II) species are the M1 species. No M2 fractions of Cd(II) and Zn(II) species were detected from the AG MP-1 column.

The speciation results for water samples with added metals are also presented in Table 4.3. The results show that the distribution of the added Zn(II), Cd(II), and Cu(II) between the M1 and M2 metal fraction generally agrees with the speciation results for the unspiked water samples. These results are consistent with those of other researchers (2-4). They also found that Cd(II) and Zn(II) exist primarily as labile metal species and are retained by Chelex-100 resin, while a significant fraction of Cu(II) is strongly associated with organic matter and not retained by Chelex-100 resin.

One limitation of the two-column ion exchange system is that the concentration of M3 species in the two-column measurement scheme cannot be directly determined. The M3 fraction can be estimated by two methods. One method, as used in other measurement schemes, is to determine the total metal concentration and subtract the concentrations determined for the M1 and M2 fractions. The total metal concentration can be determined by a technique with sufficient detectability such as electrothermal AA spectrophotometry or by sample digestion followed by preconcentration on the Chelex-100 column in the two-column system. It might also be possible to determine the total metal concentration in the two-column system with a strong acid cation resin (e.g., AG 50WX-8) and the adjustment of sample pH to 2 (25).

The second method is based on a standard addition of a known
amount of the trace metal under study. Since the concentration of the added metal is known, the concentration of M3 fraction can be calculated from the difference between the total metal concentration and the concentrations of two fractions directly determined. If the metal added reaches equilibrium with other components in the water sample and does not alter the original metal speciation, the distribution of the added metal among the three metal fraction can be used to predict the original metal speciation in the water sample.

For the added portions of trace metals in the water samples, the percentage distribution among the three fractions of metal species are calculated and summarized in Table 4.4. The ratio of the M1 fraction to the M2 fraction are also calculated and presented in Table 4.4. The M1/M2 ratio should be approximately the same for the spiked and original samples if the standard addition method is valid for predicting the original speciation. The M1/M2 ratio cannot be calculated for Cd(II) and Zn(II) in the original samples because no M2 fractions of these metals were detected. However, the distribution of Cd(II) and Zn(II) between the M1 and M2 fractions in the original samples is clearly similar to that of the added metals as evidenced by the data in Table 4.3. For Cu(II) in the ditch water sample, the M1/M2 ratio of the spiked sample is reasonably close to that of the original sample. In contrast, for Cu(II) in the river water sample, the M1/M2 ratio of the spiked sample is about a factor of 5 larger than that of the original sample.

These results suggest that the distribution of the added metals among the three fractions may be used to estimate the original metal
Table 4.4. Distribution of Cu, Cd, and Zn in Water Samples.

<table>
<thead>
<tr>
<th>sample</th>
<th>M1%a</th>
<th>M2%</th>
<th>M3%</th>
<th>M1/M2b</th>
<th>[M3]c, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>river</td>
<td>29</td>
<td>24</td>
<td>47</td>
<td>0.86 (0.17)</td>
<td>3.54 (0.72)</td>
</tr>
<tr>
<td>ditch</td>
<td>4</td>
<td>73</td>
<td>23</td>
<td>0.075 (0.11)</td>
<td>2.23 (0.85)</td>
</tr>
<tr>
<td>Cd(II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>river</td>
<td>71</td>
<td>4</td>
<td>25</td>
<td>18 (--)</td>
<td>1.53 (0.04)</td>
</tr>
<tr>
<td>ditch</td>
<td>66</td>
<td>4</td>
<td>30</td>
<td>19 (--)</td>
<td>1.84 (0.06)</td>
</tr>
<tr>
<td>Zn(II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>river</td>
<td>85</td>
<td>ND</td>
<td>15</td>
<td>-- (--)</td>
<td>1.37 (0.47)</td>
</tr>
<tr>
<td>ditch</td>
<td>80</td>
<td>5</td>
<td>15</td>
<td>20 (--)</td>
<td>1.09 (0.20)</td>
</tr>
</tbody>
</table>

aM1%, M2%, and M3% are the percentages of M1, M2, and M3 species calculated for the added metals, respectively.

bM1/M2 is the ratio of the M1 concentration to the M2 concentration given in Table III for the spiked and original samples (in the parentheses).

c[M3] is the estimated M3 concentration for the spiked and original samples (in the parentheses), using the relationship:

\[
[M3] = \left(\frac{[M1] + [M2]}{M1\% + M2\%}\right) - [M1] - [M2]
\]
speciation in the water samples except for the case of Cu(II) in the river water sample because the M1/M2 ratio is not constant. In this case, the data suggest that there is a low concentration of natural complexing agents that form strong non-labile complexes with Cu(II) (M2 species). The metal spike might have exceeded the complexing capacity of this fraction of the complexing agents in the sample. A smaller Cu spike might have given a better estimate of the speciation in the original sample. The concentrations of the M3 species in the spiked samples and original samples are also calculated and given in Table 4.4 for each metal under study.

The results in Table 4.4 also show that about 15-20% of the added Zn(II) and 25-30% of the added Cd(II) in water samples appear in the M3 fraction. Also 23% of the added Cu(II) in the ditch water and 47% of the added Cu(II) in river water appear in the M3 fraction. The M3 metal species are metals strongly associated with very large organic and inorganic colloidal matter that do not dissociate in the Chelex-100 column and are not retained by the AG MP-1 column (the molecular exclusion limit about 75,000 MW). Several ultrafiltration and dialysis studies by other researchers (26-29) also have shown that there is a significant fraction of large size dissolved organic matter in natural waters capable of binding trace metals. Guy and Chakrabarti (26) reported that about 30% of Cu(II) in humic acid solution and river water samples were bound to species with sizes greater than 5.1 nm (or above 100,000 MW). Giesy and Briese (28) found that about 30% of Cd(II) and Zn(II) are associated with large size organic matter (300,000 MW).
CONCLUSIONS

The automated two-column ion exchange system presented here offers several advantages. First, the system has the incorporated preconcentration ability for two fractions of dissolved metal species. The concentration factor with a 10-mL sample loop is about 50 compared with direct flame AA measurement. Therefore, with a 10-mL sample loop, the two-column system is able to determine many trace metal species at the 0.1 μg/L level with the on-line flame AA spectrophotometer.

Second, the measurement scheme is simple and rapid. The automated on-line operation requires minimal sample manipulation compared with conventional off-line ion exchange separation operations. The sample throughput of the two-column system is about 10 minutes per sample when using a 10-mL loop. Third, two major fractions of dissolved trace metal species in natural waters are determined directly with a single aliquot of the sample solution in one sample run. In other measurement schemes, at least two separate measurements with two sample aliquots are usually required and one fraction is determined by difference.

One limitation of the method is that the third metal fraction cannot be directly determined with the two-column system. The distribution of trace metals among the three metal fractions can be predicted with a standard addition. Alternatively the third fraction can be determined by the difference between the total metal concentration in the water sample and the concentrations of the first
two fractions.

The two-column ion exchange system provides a versatile tool to study the trace metal complexation in natural waters. The two-column ion exchange system can also be used to determine the dissociation rate constants of metal complexes in natural waters and metal complexing capacity and related conditional stability constants for ligands in natural waters (24, 30). In addition, it is possible to use the two-column system to determine the speciation of trace metals existing in two different oxidation states with different charges such as Cr(III) and Cr(VI).
REFERENCES


CHAPTER 5
TWO-COLUMN ION EXCHANGE METHOD FOR THE DETERMINATION OF COPPER COMPLEXING CAPACITY AND CONDITIONAL STABILITY CONSTANTS OF LIGANDS IN NATURAL WATERS

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for submission to Analytical Chemistry
ABSTRACT

Sample solutions titrated with Cu$^{2+}$ ion are passed sequentially through two ion exchange columns in an automated flow system. The first column is packed with Chelex-100 resin and retains Cu(II) ions that are free or in complexes that dissociate in the column. The second column is packed with AG MP-1 anion resin and retains negatively charged Cu(II) complexes. The retained Cu(II) species are then eluted from the columns and determined with an on-line flame atomic absorption spectrophotometer. It is necessary to correct for a small fraction of free Cu$^{2+}$ ions that pass through the first column and are retained by the second column. The Cu(II) complexing capacity and conditional stability constants of ligands in sample solutions are determined from plots of the ratio of the concentration of free Cu$^{2+}$ ions to the concentration of Cu(II) complexes vs. the concentration of free Cu$^{2+}$ ions. The complexing capacity of sample solutions can also be determined rapidly by measuring the complexed Cu(II) concentration after spiking the sample with an excess amount of Cu$^{2+}$ ion. The sample solutions tested were 4.0 µM NTA, 4.0 mg/L humic acid, and a river water.
The determination of trace metal complexing capacity and conditional stability constants of ligands in natural waters is an important study. The complexing capacity is usually interpreted as the total concentration of ligands capable of binding the metal under study. A number of techniques, including anodic stripping voltammetry (ASV), ion selective electrode (ISE) potentiometry, ion exchange methods, ultrafiltration, dialysis, solubilization, and bioassay have been used to determine the complexing capacity of natural waters. Several recent reviews of these methods are available (1-3).

Most methods for determination of complexing capacity are based on measurement of the concentration of a particular fraction of Cu(II) species in a sample solution spiked with free Cu\(^{2+}\) ion. Normally, a titration curve is constructed by plotting the concentration of the fraction of Cu(II) species measured against the total Cu(II) concentration added. The "break point" in the titration curve is related to the Cu(II) complexing capacity. The measurement technique for monitoring the titration process is often ISE potentiometry which responds directly to free Cu\(^{2+}\) ions or ASV which detects free Cu\(^{2+}\) ions plus so called "ASV-labile" Cu(II) (i.e., Cu\(^{2+}\) from dissociation of labile complexes). Another common approach is to separate the free Cu\(^{2+}\) ions from Cu(II) complexes with ion exchange techniques. Then the concentration of free Cu\(^{2+}\) ion or Cu(II)
complexes can be determined with techniques such as atomic spectrometry.

Several ion exchange separation methods have been used to separate free metal ions from metal complexes. Crosser and Allen (4) developed a method in which a sample solution spiked with a known amount of Cu$^{2+}$ ion was equilibrated with Dowex 50W X-8 strong cation exchange resin for 24 hours. The concentration of Cu(II) in the solution was then measured with flame atomic absorption (AA) spectrophotometry. From the titration plot of the amount of Cu(II) in the solution vs. the amount of Cu(II) retained by the resin, the ligand concentration and the conditional stability constant were calculated from the relationship they proposed. They demonstrated the use of the method with sample solutions of glycine, EDTA and peat extract but did not report its application to natural water samples.

Stolzberg and Rosin (5) reported the use of Chelex-100 chelating resin in a one-point determination of complexing capacity of phytoplankton media. An excess amount of Cu$^{2+}$ ion was added to a sample solution. The sample solution was then passed through a Chelex-100 column which retains free Cu$^{2+}$ ion and Cu$^{2+}$ ion dissociated from weak complexes. The concentration of complexed Cu(II) species in the column eluent was determined with flame AA detection and was reported as the measure of complexing capacity of strong ligands in the sample. Stolzberg (6) and Wood et al. (7) applied similar methods to the determination of the copper complexing capacity in lake water and seawater. However, these methods did not provide values for conditional stability constants for Cu(II)
complexes.

Ven den Berg and Kramer (8) developed a method based on the absorption of free Cu$^{2+}$ ion on the inorganic ion exchanger MnO$_2$. The sample solution was mixed with a fine dispersion of MnO$_2$, to which was then added free Cu$^{2+}$ ion. The concentration of Cu(II) complexes and Cu$^{2+}$ ions in the solution was measured with ASV. Their method is capable of determining both Cu(II) complexing capacity and the related conditional stability constants of ligands in river water and lake water samples. Ven den Berg (9, 10) also reported the use of the MnO$_2$ absorption technique in seawater.

In this paper, we present an ion exchange method based on an automated two-column ion exchange system developed recently (11). The application of the method is demonstrated by determining the Cu(II) complexing capacity and conditional stability constants of ligands in the solutions of 4 μM NTA, 4 mg/L humic acid, and a river water sample.
EXPERIMENTAL

Reagents and Solutions

All reagents used were of analytical grade and used as received unless otherwise specified. Deionized water from a Millipore Milli-Q water system was used to prepare all aqueous solutions. A 1000 mg/L Cu(II) stock solution was made from dissolution of copper (II) sulfate pentahydrate in 0.1% HNO₃. Solutions of lower Cu(II) concentrations were prepared just before use.

Chelex-100 resin of 100-200 mesh in NH₄⁺ form and AG MP-1 macroporous anion resin of 100-200 mesh in OH⁻ form were prepared as previously described (11). A solution of 0.025 M cysteine/0.5 M NH₄OH/2.0 M NH₄NO₃ was used as the stripping reagent. A solution of 2.5 M NH₄Ac at pH 6.8 was prepared from NH₄Ac and used as the pH buffer.

A 0.01 M NTA solution was prepared from the sodium salt of NTA (Aldrich Chemical Co., Cat. No. 10,629-1). A 100 mg/L humic acid (HA) solution was prepared from the sodium salt of HA (Aldrich Chemical Co., Cat. No. H1,675-2) and filtered through an acid-cleaned 0.4 μm Nuclepore polycarbonate filter.

River water samples were collected from the Willamette River (Corvallis, Oregon) with 1-L polyethylene bottles which had been carefully cleaned with concentrated nitric acid and deionized water. The bottles were conditioned with the same water sample before the
sampling for analysis. The river water samples were filtered through acid-cleaned 0.4 μm Nuclepore polycarbonate filters immediately after sampling. The filtered water samples were used in the determination of Cu(II) complexing capacity.

**Preparation of Ion Exchange Columns**

Two modified 3 mm i.d. x 50 mm Altex microbore glass columns (12) were used in the experiments. One column, which will be referred to as the Chelex-100 column, was packed with the water-slurry of Chelex-100 resin. Another column, which will be referred to as the AG MP-1 column, was packed with the water-slurry of AG MP-1 resin up to about 90% of the column bed volume and the top 10% of the column bed volume was then packed with Chelex-100 resin.

**Apparatus**

Detailed descriptions of the automated two-column ion exchange system have been reported previously (11). A fixed volume (e.g., 1.0 mL) of the sample solution is passed sequentially through the Chelex-100 column and the AG MP-1 column by the NH₄Ac carrier buffer stream from a constant flow rate pump. The Chelex-100 column retains metal ions that are free or in complexes that dissociate in the column. The undissociated anionic metal complexes are retained by the AG MP-1 column.

Then the metal species are stripped off the Chelex-100 column and the AG MP-1 column separately with the stripping reagent and detected with an on-line flame AA spectrophotometer with an air-acetylene
The absorption of Cu at 324.7 nm was monitored for all studies in this paper. All the operations (e.g., loading sample, eluting columns, data acquisition, etc.) are controlled by a microcomputer.

Instrumental parameter values are similar to those used previously (11) except those noted below. The column washing time during the elution step is increased from 40 s to 70 s for the Chelex-100 column to ensure complete removal of eluted Cu(II) in the flow path. After each sample run, the column conditioning time was increased from 90 s to 150 s. The sample loop volume was 1.0 mL. The carrier buffer solution was 0.02 M NH$_4$Ac. The flow rate of the carrier buffer stream was 5.0 mL/min. The sample throughput rate of the system is about 10 minutes per sample.

**Procedures**

Solutions of 1.26, 2.52, 5.04, 10.1, 18.9, 31.5, 63.0, 126, 252, 441, and 630 μM Cu(II) were prepared in each complexing medium tested and 0.01 M NH$_4$Ac and were adjusted to pH 6.8. These solutions were analyzed with the two-column ion exchange system. The concentration of Cu(II) species retained on the AG MP-1 column was measured for each of the above solutions and treated as a titration data point.

For each complexing medium tested, freshly-packed Chelex-100 and AG MP-1 columns were used. To determine the retention efficiency of Cu$^{2+}$ ion by the Chelex-100 column used, solutions of 126, 252, 441, and 630 μM of Cu$^{2+}$ ions were prepared in 0.01 M NH$_4$Ac and adjusted to pH 6.8. These solution were analyzed with the two-column system. The concentration of free Cu$^{2+}$ retained on the AG MP-1 column was
measured for each solution.

The complexing media tested were 4.0 μM NTA, 4.0 mg/L HA, and the Willamette River water sample. All solutions were prepared about 8-12 h before measurement to allow equilibration of Cu(II) spikes with the sample solution.
RESULTS AND DISCUSSION

**Two-column Measurement Scheme**

Chelex-100 resin is a chelating resin with iminodiacetate functional group, which binds most transition metal ions strongly. Therefore, Chelex-100 resin can compete with other organic ligands for trace metal ions; some weakly complexed metal species can dissociate and contribute to the fraction of metal species retained by the resin. AG MP-1 resin is a macroporous strongly basic anion exchange resin with large pore size. It has been shown that the resin retains anionic metal complexes and some metal strongly associated with negatively charged organic colloidal matter such as humic acid (11). The use of these two resins in a two-column ion exchange measurement scheme for determination of the speciation of trace metals in natural waters has been discussed previously (11). A similar two-column arrangement used in this work is shown in Figure 5.1.

The interaction between Cu$^{2+}$ ion in solution and the iminodiacetate chelating group on the Chelex-100 resin can be described by the following complexation reaction:

$$\text{Cu}^{2+} + R \rightleftharpoons \text{CuR}$$

For a batch experiment, Equation 1 applies when the complexation reaction has reached equilibrium.

$$K_{\text{CuR}} = \frac{[\text{CuR}]}{[\text{Cu}^{2+}]c_R - [\text{CuR}]})$$

(1)
Figure 5.1. Two-column Ion Exchange Measurement Scheme.
where $K'_{CuR}$ is the conditional stability constant of Cu(II) with the resin, $[CuR]$ is the concentration of the Cu retained by the resin, $[Cu^{2+}]$ is the concentration of Cu$^{2+}$ ion left in the solution, and $c_R$ is the total concentration of the chelating groups on the resin.

If $[CuR]$ is much smaller than $c_R$, Equation 1 is rearranged into:

$$K'_{CuR} \times c_R = \frac{[CuR]}{[Cu^{2+}]} \quad (2)$$

In a column experiment, a plug of Cu$^{2+}$ ion solution is passed through the Chelex-100 column at a given flow rate. For experiments in this study, the sample volume (1 mL) is greater than the void volume of the packed column ($\approx 0.12$ mL). Most of Cu$^{2+}$ ions in the solution are retained by the Chelex-100 column, but a small fraction of Cu$^{2+}$ ions is not retained by the column. The amount of Cu$^{2+}$ ion in the column effluent is much greater than that predicted by the batch model as described by Equation 2 and estimates of $K'_{CuR}$ and $c_R$ (about $10^{10}$ and 0.3 meq/mL resin bed). Possibly, some of Cu$^{2+}$ ions follow a flow path through the column such that they do not contact the chelating functional groups on the resin beads. Hence the kinetics of the transport rather than the kinetics of complexation by the resin may limit the amount of Cu$^{2+}$ ion retained.

Although the batch model cannot be applied to the column experiment, the efficiency of the retention of Cu$^{2+}$ ion by the Chelex-100 resin in the column can be described empirically by:

$$E = \frac{N_1}{N_2} = \frac{[Cu^{2+}]_1}{[Cu^{2+}]_2} = \frac{c_{Cu} - [Cu^{2+}]_2}{[Cu^{2+}]_2} \quad (3)$$
where $E$ is termed as the retention efficiency of Cu$^{2+}$ ion by the Chelex-100 column, $N_1$ is the number of moles of Cu$^{2+}$ ions retained by the Chelex-100 column, and $N_2$ is the number of moles of Cu$^{2+}$ ions that pass through of the Chelex-100 column and that are retained by the AG MP-1 column. Most of the Cu$^{2+}$ ions not retained by the Chelex-100 column are retained by the Chelex-100 resin and the AG MP-1 resin in the AG MP-1 column (11).

The second form of Equation 3 is written in terms of effective concentration of Cu(II). The effective concentration of Cu(II) is defined as the moles of Cu(II) retained by the Chelex-100 column or the AG MP-1 column divided by the sample volume. $[\text{Cu}^{2+}]_1$ is the effective concentration of Cu(II) in the Chelex-100 column and $[\text{Cu}^{2+}]_2$ is the effective concentration of Cu(II) in the AG MP-1 column. Effective concentrations are used in the further discussions and are determined by comparing the area of elution peak of each column for a test solution to that for a Cu$^{2+}$ ion standard solution where the sample volume of the test and standard solutions are identical.

To determine $E$, the third form of Equation 3 is employed. In the experiment, the retention of Cu ion by the Chelex-100 column is tested with a series of Cu$^{2+}$ ion standard solutions. The total concentrations of Cu$^{2+}$ ion standard solutions have to be relative high to ensure the amount of Cu$^{2+}$ retained by the AG MP-1 column can be detected. Under these conditions, the absorbance of the elution peak for the Chelex-100 column is outside the linear range of response of the AA spectrophotometer. Hence $[\text{Cu}^{2+}]_1$ is determined by
difference between the total Cu$^{2+}$ concentration in the original sample solution, $c_{Cu}$, and $[Cu^{2+}]_2$. A plot of $(c_{Cu} - [Cu^{2+}]_2)$ against $[Cu^{2+}]_2$ is constructed with a series of Cu$^{2+}$ ion standard solutions. The retention efficiency, $E$, is determined from the slope of the plot. It is typically 200-300. The value of $E$ is affected by variables such as pH, flow rate, Chelex-100 resin particle size, column length and column packing. However, the retention efficiency becomes a constant if all variables identified above are kept constant and the amount of Cu$^{2+}$ ion retained by the column is much smaller than the resin capacity.

In a sample solution containing Cu$^{2+}$ ion and ligand, L, the complexation equilibrium can be described by the following reaction and Equation 4:

$$Cu^{2+} + L \rightleftharpoons CuL$$

$$K'_{CuL} = \frac{[CuL]}{[Cu^{2+}][L]} = \frac{[CuL]}{[Cu^{2+}](c_L - [CuL])}$$ (4)

where $K'_{CuL}$ is the conditional stability constant of Cu(II) with the ligand, $[CuL]$ is the concentration of the Cu(II) complex in the solution, $[L]$ is the concentration of the free ligand in the solution, and $c_L$ is the total concentration or the complexing capacity of the ligand.

As a plug of sample solution moves through the Chelex-100 column, the concentration of Cu$^{2+}$ ion in the solution decreases along the length of the column to a small fraction of the initial concentration. This can result in some dissociation of the CuL
complex as the sample plug proceeds through the column. Thus the concentration of CuL in the solution exiting the Chelex-100 column may be lower than the initial concentration of CuL entering the column. It is assumed in further discussions that the dissociation rate constant of the CuL complex is sufficiently large such that Cu$^{2+}$, L, and CuL are in equilibrium at all points in the Chelex-100 column. Then the conditional stability constant of the CuL complex can be evaluated from the effective concentrations of Cu$^{2+}$, L, and CuL in the solution exiting the Chelex-100 column (i.e., [Cu$^{2+}$]$_2$, [L]$_2$, and [CuL]$_2$, respectively).

The sample solution that exits the Chelex-100 column contains both Cu$^{2+}$ and CuL. They are retained by the Chelex-100 resin and the AG MP-1 anion resin in the AG MP-1 column (assuming that CuL is a negatively charged complex). Then the total amount of Cu(II) retained by the AG MP-1 column expressed in terms of effective concentrations of Cu$^{2+}$ and CuL is given by:

$$[\text{Cu}]_2 = [\text{Cu}^{2+}]_2 + [\text{CuL}]_2$$

(5)

In the two-column measurement scheme, the concentration, [Cu]$_2$, is determined from the area of the elution peak for the AG MP-1 column. The effective concentration of the Cu$^{2+}$ ion in the column effluent, [Cu$^{2+}$]$_2$, is calculated for a given total Cu(II) concentration using the determined retention efficiency, E, of the Chelex-100 column used ([Cu$^{2+}$]$_2 = (c_{\text{Cu}} - [\text{Cu}]_2)/E$). The effective concentration, [CuL], is obtained from the difference between [Cu]$_2$ and [Cu$^{2+}$]$_2$. Here it is assumed that any Cu$^{2+}$ ions released from dissociation of the Cu(II) complexes in the column
are retained with the same efficiency as the Cu\(^{2+}\) ions in the original sample solution passing through the Chelex-100 column. The validity of this assumption was not tested. It might be expected that the Cu\(^{2+}\) ions released from the dissociation of Cu(II) complexes further down the column would be retained with a smaller efficiency. Other studies (11) showed that, when a Cu(II)-glycinate solution in which all the Cu(II) was initially complexed was passed through the Chelex-100 column, over 99% of the Cu(II) was retained by the column. This demonstrates that the retention efficiency for Cu\(^{2+}\) ions released from dissociation of the Cu(II) complexed is at least about 100. As a worse case (i.e., complete dissociation of the Cu(II) complexes in the Chelex-100 column), the value of \([\text{Cu}^{2+}]_2\) calculated would be a factor of 2 too low if the retention efficiency for Cu\(^{2+}\) ions from dissociation of the Cu(II) complexes is a factor of 2 smaller. For titration points with \(c_{\text{Cu}} > [\text{Cu}]_2\), the contribution from dissociation of Cu(II) complexes would be expected to be less (i.e., the degree of dissociation is much less than 100%).

Equation 4 can be rearranged to Equation 6 to determine the Cu(II) complexing capacity and the conditional stability constant of Cu(II) with the ligand.

\[
\frac{[\text{Cu}^{2+}]_2}{[\text{CuL}]_2} = \frac{[\text{Cu}^{2+}]_2}{c_L} + \frac{1}{K'_{\text{CuL}} x c_L} \tag{6}
\]

The experimental data are fitted to Equation 6 by constructing a plot of \([\text{Cu}^{2+}]_2/[\text{CuL}]_2\) vs. \([\text{Cu}^{2+}]_2\) for a series of solutions of known amounts of Cu(II) prepared in a sample complexing medium. The
plot gives a straight line with a slope equal to $1/c_L$. The conditional stability constant, $K'_{CuL}$, can be obtained by dividing the slope by the intercept.

If there are two different ligands, $L_1$ and $L_2$, in the sample solution, the effective concentration of the Cu complexes retained on the AG MP-1 column becomes:

$$[CuL]_2 = [CuL_1]_2 + [CuL_2]_2$$

(7)

where $[CuL_1]_2$ and $[CuL_2]_2$ are the effective concentrations of the Cu(II) complexes with ligands, $L_1$ and $L_2$, respectively. In this case, a plot of $[Cu^{2+}]_2/[CuL]_2$ vs. $[Cu^{2+}]_2$ is not linear. To determine the Cu(II) complexing capacity and conditional stability constant of Cu(II) with each ligand, an estimation procedure similar to the one reported by Ven den Berg (13) is used.

When the total Cu(II) concentration in the solution is low, the formation of the Cu(II) complex with the stronger ligand, Cu$L_1$, is predominate (i.e., $[CuL]_2 = [CuL_1]_2$). A plot of $[Cu^{2+}]_2/[CuL]_2$ vs. $[Cu^{2+}]_2$ is approximately linear and is used to obtain the initial estimates for $c_{L_1}$ and $K'_{CuL_1}$, the Cu(II) complexing capacity and the conditional stability constant of Cu(II) with the stronger ligand. The estimated value of $[CuL_1]_2$ is calculated for each titration point from Equation 8:

$$[CuL_1]_2 = \frac{K'_{CuL_1} \times [Cu^{2+}]_2 \times c_{L_1}}{1 + K'_{CuL_1}[Cu^{2+}]_2}$$

(8)

The calculated value of $[CuL_1]_2$ is used to estimate the effective concentration of the Cu(II) complex with the weaker ligand, Cu$L_2$, at higher total Cu(II) concentrations using Equation 7.
\([\text{CuL}_2]^2 - \text{CuL}_2\). A plot of \([\text{Cu}^{2+}]_2/[\text{CuL}_2]^2\) vs. \([\text{Cu}^{2+}]_2\) is constructed following Equation 6 to obtain the first estimates of \(c_{\text{L2}}\) and \(K'_{\text{CuL2}}\), the Cu(II) complexing capacity and the conditional stability constant of Cu(II) with the weaker ligand. These estimated values are then used to correct the contribution of \([\text{CuL}_2]^2\) to \([\text{CuL}_2]\) using an equation similar to Equation 8 (i.e., \(L_2\) substituted for \(L_1\)) to get better estimates of \([\text{CuL}_1]^2\) at lower total Cu(II) concentrations. A plot of \([\text{Cu}^{2+}]_2/[\text{CuL}_1]^2\) vs. \([\text{Cu}^{2+}]_2\) is constructed to obtain the second estimates of \(c_{\text{L1}}\) and \(K'_{\text{CuL1}}\). Therefore, the values of \(c_{\text{L1}}, K'_{\text{CuL1}}, c_{\text{L2}},\) and \(K'_{\text{CuL2}}\) can be determined after several such iterations with this linear procedure.

Application to Water Samples

An example of plot of \((c_{\text{Cu}} - [\text{Cu}^{2+}]_2)\) vs. \([\text{Cu}^{2+}]_2\) for the determination of the retention efficiency of Cu\(^{2+}\) ion by the Chelex-100 column used is shown in Figure 5.2. For each complexing medium, \(E\) was determined for the Chelex-100 column used. The values of \(E\) were found to be 190, 259, and 235 for the Chelex-100 column used for the 4.0 \(\mu\)M NTA, 4.0 mg/L HA, and Willamette River water sample solutions, respectively. The variation in the retention efficiency determined is possibly due to fact that the packing of the Chelex-100 column is slightly different for each column. Therefore, it is necessary to determine the retention efficiency for each Chelex-100 column used in the experiment.

The titration curve for the NTA solution obtained with the
Figure 5.2. Plot of (c_{Cu} - [Cu^{2+}]_2) vs. [Cu^{2+}]_2 for the Chelex-100 column used for the 4.0 μM NTA solution.
two-column measurement scheme is shown as the curve (a) in Figure 5.3. During the early stage of the titration, the effective concentration of Cu(II) species retained by the AG MP-1 column, \([\text{Cu}]_2\), increases rapidly with the total Cu(II) concentration because a major fraction of the Cu(II) added to the sample solution is complexed. The rate of increase of \([\text{Cu}]_2\) decreases as the titration proceeds. After the complexing capacity is exceeded, the titration curve shows a constant slope and the increase of \([\text{Cu}]_2\) is totally due to the contribution of \(\text{Cu}^{2+}\) ion unretained by the Chelex-100 column. The effective concentration of \(\text{Cu}^{2+}\) ion calculated with the retention efficiency, \(E\), for each titration point is shown as the curve (b) in Figure 5.3.

The effective concentration of the complexed Cu(II) species, \([\text{CuL}]_2\), for each titration point is obtained from the difference between \([\text{Cu}]_2\) and \([\text{Cu}^{2+}]_2\). The resulting corrected titration curve ([CuL]₂ vs. c_{Cu}) for the NTA solution is shown in Figure 5.4. The corrected titration curves for the other two complexing media tested are shown in Figures 5.5 and 5.6. The magnitude of \([\text{CuL}]_2\) increases and reaches a plateau as the total Cu(II) concentration increases. The Cu(II) complexes in the sample solution dissociate to some degree due to the decrease in the free \(\text{Cu}^{2+}\) concentration as the sample solution passes through the Chelex-100 column. The degree of dissociation of Cu(II) complexes in the Chelex-100 column and therefore the shape of corrected titration curve depend on the retention efficiency of the column, the stability constants of Cu(II) complexes, and the complexing capacity of
Figure 5.3. Typical Titration Curves of the Two-column Method.

Sample: 4.0 µM NTA at pH 6.8.
Figure 5.4. Corrected Titration Curve for the 4.0 $\mu$M NTA Solution.
Figure 5.5. Corrected Titration Curve for the 4.0 mg/L HA Solution.
Figure 5.6. Corrected Titration Curve for the Willamette River Water Sample.
ligands. For a given complexing capacity (i.e., the total ligand concentration), the plateau of the corrected titration curve is reached at lower total Cu(II) concentrations for stronger ligands. With weaker ligands, the plateau of corrected titration curve is reached at higher total Cu(II) concentrations because a higher Cu$^{2+}$ concentration is required to push the equilibrium to ensure that all the ligands are complexed. If the stability constants of Cu(II) complexes are too small, the plateau of the corrected titration curve may not be reached at a reasonable total Cu(II) concentration.

The CuL concentration at the plateau of the titration curve is an indication of the Cu(II) complexing capacity of the sample solution since all the ligands in the sample solution are saturated when the concentration of added Cu$^{2+}$ ion is high enough. It is interesting to note that these three complexing media have similar Cu(II) complexing capacity. However, the ligands in the humic acid and the river water sample solutions form weaker Cu(II) complexes than NTA since the plateau in their titration curves is reached at higher Cu(II) concentrations.

Plots of $[\text{Cu}^{2+}]_2/[\text{CuL}]_2$ vs. $[\text{Cu}^{2+}]_2$ were constructed for the three complexing media as shown in Figures 5.7, 5.8, and 5.9. For the NTA solution, the curve of $[\text{Cu}^{2+}]_2/[\text{CuL}]_2$ vs. $[\text{Cu}^{2+}]_2$ is linear as expected for the 1:1 Cu(II)-NTA complex. For the humic acid and river water sample solutions, the curves are nonlinear. To determine the Cu(II) complexing capacity and conditional stability constants, the titration data for the NTA solution were analyzed with the one-ligand model. The titration data
Figure 5.7. Plot of $[\text{Cu}^{2+}]_2/\text{[CuL]}_2$ vs. $[\text{Cu}^{2+}]_2$ for the 4.0 µM NTA Solution.
Figure 5.8. Plot of $[\text{Cu}^{2+}]_2/\text{[CuL]}_2$ vs. $[\text{Cu}^{2+}]_2$ for the 4.0 mg/L HA Solution.
Figure 5.9. Plot of $[\text{Cu}^{2+}]_2/[\text{CuL}]_2$ vs. $[\text{Cu}^{2+}]_2$ for the Willamette River Water Sample.
for the humic acid solution and the river water sample were analyzed with both the one- and two-ligand models. When using the two-ligand model, the first four titration data points for humic acid solution and the first five titration data for the river water sample solution were used to obtain the estimates of $c_{L1}$ and $K'_{CuL1}$. Other titration data points were used to obtain the estimates of $c_{L2}$ and $K'_{CuL2}$.

Table 5.1 summarizes the values determined for the Cu(II) complexing capacity and conditional stability constants based on one- and two-ligand models. It is noted that the total complexing capacity obtained from both the one- and two-ligand models agrees well with $[CuL]_2$ value at the plateau of the titration curve. This indicates that the total complexing capacity of the sample solution can also be determined rapidly with only one titration point at the plateau region of the titration curve with the two-column system. For example, the results suggest that the Cu(II) complexing capacity of a sample solution with $c_L$ on the order of 1 µM and log $K'_{CuL} = 7.8$ can be rapidly determined with a one-point addition yielding a total Cu(II) concentration of 630 µM.

NTA forms 1:1 complex with Cu$^{2+}$ ion under the experimental condition and the Cu(II)-NTA complex is an anion at neutral pH. Thus, NTA was chosen as a model ligand to test the two-column measurement method. The conditional stability constant of the Cu(II)-NTA complex determined with the two-column system is log $K = 8.64$ which agrees reasonably well with the literature value (14) of log $K = 9.26$ (the value is adjusted to pH 6.8 and ionic strength of 0.01 M used in the
**Table 5.1. Cu(II) Complexing Capacity and Conditional Stability Constants of Ligands in Three Complexing Media**

<table>
<thead>
<tr>
<th>Sample</th>
<th>([\text{CuL}]_{\text{max}}, \mu M)</th>
<th>(c_L, \mu M)</th>
<th>(\log K'_{\text{CuL}})</th>
<th>(c_{L1}, \mu M)</th>
<th>(\log K'_{\text{CuL1}})</th>
<th>(c_{L2}, \mu M)</th>
<th>(\log K'_{\text{CuL2}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTA</td>
<td>3.51 ± 0.05</td>
<td>3.64 ± 0.01</td>
<td>8.64 ± 0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HA</td>
<td>3.61 ± 0.03</td>
<td>3.70 ± 0.06</td>
<td>7.45 ± 0.21</td>
<td>1.30 ± 0.09</td>
<td>8.42 ± 0.95</td>
<td>2.46 ± 0.11</td>
<td>7.00 ± 0.23</td>
</tr>
<tr>
<td>River</td>
<td>3.56 ± 0.05</td>
<td>3.68 ± 0.16</td>
<td>6.78 ± 0.13</td>
<td>0.72 ± 0.08</td>
<td>8.03 ± 0.91</td>
<td>3.28 ± 0.32</td>
<td>6.28 ± 0.12</td>
</tr>
</tbody>
</table>

\(^a\) The standard deviations in values of \(c_L\) and \(\log K'_{\text{CuL}}\) are calculated from the standard deviations of the slope and intercept of the linear least square fit of titration data to Equation 6. The standard deviations in \([\text{CuL}]_{\text{max}}\) are the standard deviations of data in the plateau of titration curve of \([\text{CuL}]_2\) vs. \(c_{\text{Cu}}\).

\(^b\) NTA = 4.0 \(\mu M\) NTA solution, HA = 4.0 mg/L humic acid solution, and River = Willamette River water sample. All solutions were buffered at pH 6.8.
experiment). However, the total concentration of NTA in the solution determined with the two-column method is lower than expected, which is possibly due to a small fraction of Cu(II)-NTA complex was not retained by the AG MP-1 column.

The results obtained for the humic acid and river water sample solutions generally agree with those for similar samples reported by other researchers. Van den Berg and Kramer (8) reported that the Cu(II) complexing capacity of a fulvic solution and a river water sample to be 2.2 and 2.5 μM with log K = 7.8 and 8.5 at pH 7.6. Hart and Jones (15) found that the Cu(II) complexing capacity of a creek water sample was about 0.2 μM with a conditional stability constant about 10^8 at pH 6.0 with an ASV method. Using an ISE method, McKnight et al. (16) showed that aquatic humic substances could be modeled as having two types of Cu(II)-binding sites: one with log K'_{CuL1} = 6 and c_{L1} = 1.0 ± 0.4 μM (mg C)^{-1} and another with log K'_{CuL2} = 8 and c_{L2} = 2.6 ± 1.6 μM (mg C)^{-1}, pH 6.25.

The complexation of Cu(II) by ligands in the humic acid solution or in natural waters is very complex and can not be described fully with the one- or two-ligand models. Ligands in such samples are actually composed of a multitude of different species that contain complexing sites of different strengths. Thus the conditional stability constants determined represent average weighted values of a distribution. Recently, there have been several reports on the discrete and continuous multiligand metal binding models and their applications (17-19).

It is noted that the values of log K'_{CuL1} obtained for the
humic acid and river water sample solutions have relatively large standard deviations possibly because only a few data points were obtained at the initial portion of the titration curve. To obtain better estimates for log $K'_{\text{CuL}}$ and $c_{\text{CuL}}$, the sample solution could be spiked with very small amounts of Cu$^{2+}$ ions. A larger sample loop (e.g., 10 mL) could be used to increase the preconcentration factor to allow small concentrations of complexed Cu(II) to be determined with better accuracy.

One potential problem associated with the two-column measurement scheme is that the non-labile neutral metal complexes and metal strongly associated with very large colloidal matter may not be retained by AG MP-1 resin (11). It was found that this fraction was about 4% for 0.32 mg/L Cu(II) in the 4.0 mg/L HA solution. Thus, the Cu(II) complexing capacity determined by the method is operationally defined and may be slightly negatively-biased.

The data treatment scheme used in this study assumes that species Cu$^{2+}$, L, and CuL are in equilibrium when they exit the Chelex-100 column. However, if CuL is a weak and non-labile complex and dissociates very slowly, the equilibria may not be reached in the time scale of the experiment (the contact time of the sample solution with the resin in the Chelex-100 column is 1.4 s). In the limiting case that the complex CuL does not dissociate at all because of the slow dissociation kinetics, the measured concentration of CuL would be equal to the concentration of CuL in the original sample solution even at the titration points corresponding to low total Cu(II) concentrations. The conditional stability constant determined using
Equation 6 would be larger than the true value by a factor of $E$ (retention efficiency) but the complexing capacity determined would still be the correct value. For very strong complexes (e.g., CuEDTA$^{-2}$), the dissociation of the complexes in the Chelex-100 column is insignificant and the lability of the complexes is not of concern in the two-column measurement scheme when the ligand concentration is at the level of micromolar.

For the three complexing media tested, the significant curvature of the corrected titration curves shows that significant dissociation of Cu(II) complexes occurs in the column. The measured concentrations of CuL for the titration points corresponding to low total Cu(II) concentrations are reasonably close to equilibrium concentrations in the solution exiting the Chelex-100 column estimated with the determined values of $E$, $\log K'_{\text{CuL}}$, and $c_L$ and smaller than the calculated concentrations of CuL in the original sample solution. This demonstrates that the assumption of equilibria among Cu$^{2+}$, L and CuL in the solution exiting the Chelex-100 column is a reasonable approximation for the ligands studied.
CONCLUSIONS

The automated two-column ion exchange system provides a new rapid method to determine trace metal complexing capacity and conditional stability constants of ligands in natural waters. Compared to other ion exchange methods, the two-column method is simple and rapid due to automation of measurement process. One distinct characteristic of the method is that the complexed metal species are preconcentrated and measured directly. In most methods, the uncomplexed Cu fraction is measured and used to calculate the complexed fraction. The ability to measure the complexed fraction directly allows a rapid (10 min), one-point determination of complexing capacity. Therefore, the method can potentially be used to determine trace amounts of organic ligands in the water samples.
REFERENCES


CHAPTER 6

STUDY OF THE DISSOCIATION KINETICS OF TRACE METAL COMPLEXES

WITH AN AUTOMATED TWO-COLUMN ION EXCHANGE SYSTEM

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ABSTRACT

An ion exchange method to study the dissociation kinetics of trace metal complexes is presented. The sample solution is passed sequentially through a column of Chelex-100 resin and a column of AG MP-1 anion resin. The concentrations of metal species retained by each column are determined at different flow rates. The degree of dissociation of trace metal complexes such as CdNTA\(^{-1}\), CuIDA, and Cu(II)-humate complexes in the Chelex-100 column are shown to be dependent on the sample loading flow rate. The results of flow rate studies are analyzed to determine lower limits for the 1st-order dissociation rate constants of metal complexes and the speciation of trace metals in the sample solution. The apparent dissociation rate constant of Cu(II)-humate complexes is estimated to be about 0.3 s\(^{-1}\).
INTRODUCTION

The complexation of trace metals with ligands in natural waters such as aquatic humic substances is highly complex due to the unknown characteristics of ligands and the large number of possible interactions. Most research effort has been focused on the study of the thermodynamic aspect of complexation of trace metals with natural ligands (e.g., the determination of trace metal speciation, trace metal complexing capacity, and conditional stability constants). The kinetics of complexation reactions of trace metals with natural ligands is also very important. The process of metal accumulation in an organism and thus the metal toxicity may be regulated by the dissociation rate of metal complexes surrounding a biomembrane as well as by the stability of metal complexes (1-2). The formation and dissociation kinetics of metal complexes also affect the results obtained with many experimental techniques for determining the speciation of trace metals in natural waters.

There have been only a few reports on the study of dissociation kinetics of complexes of trace metals with ligands in natural waters. Shuman and Michael (3, 4) reported a method based on anodic stripping voltammetry using a rotating disk electrode. The deposition current was measured at different electrode rotation rates to obtain the dissociation rate constants of trace metal complexes in the sample solution. The dissociation rate constant of Cu(II) complexes
in coastal seawater samples was estimated to be on the order of 2 s\(^{-1}\) (4). Olson and Shuman (5, 6) developed a photometric method based on monitoring the formation of a colored complex of Cu(II) with 4-(2-pyridylazo)-resorcinol (PAR), Cu(PAR)\(_2\). The rate of formation of Cu(PAR)\(_2\) was related to the rate of dissociation of Cu(II)-humate complexes. A kinetic spectrum model was used to obtain the distribution of dissociation rate constants of Cu(II) complexes. The dissociation rate constants of Cu(II)-humate complexes were found in the range of 0.001-40 s\(^{-1}\) and about 64% of Cu(II)-humate complexes with dissociation rate constants greater than 1 s\(^{-1}\) (6). Figura and McDuffie (7, 8) used ion exchange techniques to study the dissociation kinetics of trace metal complexes. The uptake of trace metal species such as Cd(II)-NTA by Chelex-100 chelating resin was found to be dependent upon the contact time between the sample solution and the Chelex-100 resin. The contact time was changed by varying the sample loading flow rate in a column procedure or the equilibration time in a batch procedure. They estimated the dissociation rate constant of Cu(II)-humate complexes to be about 0.1 s\(^{-1}\) (8).

In this paper, we present a method based on an automated two-column ion exchange system developed recently (9). The dissociation kinetics of trace metal complexes are studied by varying the flow rate that the sample solution is passed through a column of Chelex-100 resin and a column AG MP-1 anion resin. The method is used to estimate the lower limits of 1st-order dissociation rate constants of some Cu(II) and Cd(II) complexes and the speciation of these metals in synthetic and natural water sample solutions.
EXPERIMENTAL

Reagents and Solutions

All reagents were of analytical grade and used as received unless otherwise specified. Deionized water from a Millipore Milli-Q water system was used to prepare all solutions. Solutions of 1000 mg/L Cu(II) and Cd(II) were prepared from dissolution of metal salts in 0.1% (w/w) HNO₃. Metal solutions of lower concentrations were prepared as needed.

Solutions of 1.00 x 10⁻² M EDTA and 1.00 x 10⁻² M NTA were prepared from the sodium salts of EDTA and NTA, respectively. A solution of 0.500 M iminodiacetic acid (IDA) was prepared by adding enough 2.0 M NH₄OH to dissolve IDA in water. A 100 mg/L humic acid solution was prepared by dissolving 0.1000 g of the sodium salt of humic acid (Aldrich Chemical Co.) in 1 L water and filtering through an acid-cleaned 0.4 μm Nuclepore polycarbonate filter. River water samples were collected from the Willamette River (near Corvallis, Oregon) and filtered with 0.4 μm Nucleopore polycarbonate filters using a procedure described previously (9).

A solution of 5.0 x 10⁻³ M NH₄Ac/1.0 x 10⁻³ M HAc was used as the carrier buffer solution. For Cu(II), a solution of 0.025 M cysteine/0.5 M NH₄OH/2.0 M NH₄NO₃ was used as the stripping reagent, and for Cd(II), a solution of 0.1 M EDTA/0.5 M NH₄OH/2.0 M NH₄NO₃ was the stripping reagent.
Two modified 3 mm i.d. x 50 mm Altex microbore glass columns (10) were used in the experiments. One column was packed with the Chelex-100 resin of 100-200 mesh in NH$_4^+$ form. Another column was packed with the AG MP-1 macroporous anion resin of 100-200 mesh in OH$^-$ form. These two columns will be referred to as the Chelex-100 column and the AG MP-1 column, respectively.

**Procedures**

The automated two-column ion exchange system reported previously (9) was used in the experiments. Instrumental conditions of the two-column system were similar to those used previously unless otherwise specified.

To study the dissociation kinetics of trace metal complexes, sample solutions were passed sequentially through the Chelex-100 column and the AG MP-1 column by the NH$_4$Ac carrier buffer solution from constant flow rate pumps at the flow rates of 1.0, 2.5, 5.0, 10, and 15 mL/min. The concentration of metal species retained by each column was determined by elution into the nebulizer of an on-line flame atomic absorption spectrophotometer. The column elution flow rate was set at 5.0 mL/min. A sample loop volume of 2.0 mL was used.

The contact time of the sample solution with the resin in the Chelex-100 column is determined by the void volume of the column and the sample loading flow rate. The void volume of the Chelex-100 column was determined using the following procedure. The Chelex-100 column and the AG MP-1 column were replaced with two couplers (zero void volume). A solution of 100 mg/L HA was loaded into a sample loop
of 0.1 mL and injected at a flow rate of 1.0 mL/min. The injection peak was monitored with an on-line UV monitor of the two-column system at 254 nm. The retention time (relative to the time of initiating the injection) of the injection peak, \( t_1 \), was measured. Then the Chelex-100 column was placed back in the flow system and a similar injection of the HA solution was made. The retention time of the injection peak, \( t_2 \), was measured. The difference between \( t_2 \) and \( t_1 \) is the contact time of the sample solution in the Chelex-100 column. Since humic acid is not retained by the Chelex-100 column, the void volume of the Chelex-100 column is calculated from the difference between \( t_2 \) and \( t_1 \) and the flow rate. The void volume of the Chelex-100 column was found to be 0.12 mL (compared to the column bed volume of 0.35 mL). The contact times of sample solution in the Chelex-100 column at flow rates of 1.0, 2.5, 5.0, 10, and 15 mL/min were calculated to be 7.2, 2.9, 1.4, 0.72, and 0.48 s, respectively.
Kinetics Measurement Scheme

The arrangement of the two ion exchange columns for studying the dissociation kinetics of trace metal complexes is shown in Figure 6.1. The Chelex-100 column contains the Chelex-100 chelating resin with iminodiacetate functional groups that bind most trace transition metal ions strongly. Thus the Chelex-100 resin can compete with other organic ligands for trace metal ions. The AG MP-1 column contains the AG MP-1 macroporous strongly basic anion resin. It has been shown that the AG MP-1 resin retains anionic metal complexes and some metal strongly associated with negatively charged organic colloidal matter such as humic acid (9).

To illustrate the measurement scheme, consider that a sample solution of a divalent trace metal, M, contains two fractions of metal species: M1 and M2. The M1 fraction includes the free metal ions and weak or very labile metal complexes that dissociate completely when passing through the Chelex-100 column at all flow rates. This fraction of metal species is retained by the Chelex-100 column. The M2 fraction will be denoted as ML to indicate the association of the metal with a particular ligand. The ML species are anionic metal complexes that are non-labile or moderately labile such that they dissociate to some degree when passing through the Chelex-100 column. The anionic ML species are retained by the AG MP-1 column.
Figure 6.1. Two-Column Kinetics Measurement Scheme.
The dissociation of a ML complex is described by the following reaction:

\[
\begin{align*}
\text{ML} & \to \frac{k_d}{k_f} \text{M}^{2+} + \text{L} \\
\end{align*}
\]

where \(M^{2+}\) is the free metal ion, \(L\) is the ligand, and \(k_d\) and \(k_f\) are the dissociation and formation rate constants of the complex, ML, respectively.

When a sample plug containing ML, \(M^{2+}\), and L passes through the Chelex-100 column, the majority of the \(M^{2+}\) ions are retained by the Chelex-100 resin. The reduction in the \(M^{2+}\) concentration results in some dissociation of the ML complex if the contact time of the sample solution with the resin in the Chelex-100 column, \(t_c\) (in s), is of the order or greater than \(k_d\) (in \(s^{-1}\)). To model the degree of dissociation of the ML complex that occurs in the Chelex-100 column, it is first assumed that essentially all of the ML species are retained in the very first part of the column and that essentially all \(M^{2+}\) ions released due to dissociation of ML complexes during passage through the column are also retained by the Chelex-100 resin. Under these conditions, the dissociation of ML can be described by simple first order dissociation \((d[ML]/dt = -k_d[ML])\). The degree of dissociation depends on \(k_d\) and \(t_c\). The contribution of the formation reaction of ML is negligible because the \(M^{2+}\) concentration is assumed to be negligibly small in the column.

Above it was assumed that the formation reaction of the ML complex in Equation 1 is negligible in the column because the chelating functional groups on the Chelex-100 resin compete...
successfully and rapidly for all the free Cu\textsuperscript{2+} ions in the solution. However even if the reaction between M\textsuperscript{2+} and chelating functional group, R, on the Chelex-100 resin:

\[
M^{2+} + R \xrightarrow[]{} MR
\]  

was in complete equilibrium throughout the column, the concentration of M\textsuperscript{2+} ion would not be zero but some finite value determined by the conditional stability constant of the MR complex and the concentration of R. Hence the dissociation of the ML complex would not be complete even if the contact time was infinite. In reality, other studies (11) have shown that a small fraction (about 0.5\%) of Cu\textsuperscript{2+} ions in a sample plug passed through a Chelex-100 column is unretained.

To model the experiment results observed, it is assumed that the retention of M\textsubscript{1} species by the Chelex-100 column and the retention of anionic ML species by the AG MP-1 column are independent of the contact time of the sample solution in the two columns. The concentration of trace metal species retained on the Chelex-100 column (c\textsubscript{1}) is fitted empirically to simple exponential functions:

\[
c_1 = c_{M1} + (c_{ML} - c^*)(1 - e^{-k'dt_c})
\]  

and the concentration of trace metal species retained by the AG MP-1 column (c\textsubscript{2}) is fitted empirically to:

\[
c_2 = (c_{ML} - c^*) e^{-k'dt_c} + c^*
\]  

where k\textsubscript{d}' is the apparent 1st-order dissociation rate constant, c\textsubscript{M1} and c\textsubscript{ML} are the concentrations of M\textsubscript{1} and ML species in the
original sample solution, respectively, and $c^*$ is a fitting parameter that represents the concentration of ML that would exit the column for $t_c = \infty$.

The concentrations, $c_1$ and $c_2$, are measured with the two-column ion exchange system at different sample loading flow rates (different contact times). The results of the flow rate studies (i.e., $c_1$, $c_2$, and $t_c$) are fitted to Equation 3 and 4 to estimate the values of concentrations of ML and ML in the original sample solution, the apparent 1st-order dissociation rate constant of the ML complex and $c^*$.

The apparent dissociation rate constant is a fitting parameter and is not the true 1st-order dissociation rate constant. If Equations 3 and 4 provide a good fit to the data, the relationship of $k'_d$ to the true dissociation rate constant is not critical. Equations 3 and 4 can be used to estimate the values of $c_1$ and $c_2$ at $t_c = 0$ and $t_c = \infty$ and hence to estimate $c_{ML}$, $c_{M2+}$, and $c^*$. The value of $k'_d$ does however provide an estimate of the lower limit of the true dissociation rate constant. Because the $M^{2+}$ concentration is finite and varies throughout the length of the column, the degree of dissociation at any point in the column is less than if the $M^{2+}$ concentration were negligibly small throughout the column. Hence the degree of dissociation will be less due to the finite contribution from the forward reaction. In this case, the apparent dissociation rate constant determined is less than the true 1st-order dissociation rate constant of the ML complex. For some moderately stable metal complexes with a relatively large dissociation
rate constant, the degree of dissociation can be high at longer contact times \((c_1 = c_{M1} + c_{ML} \text{ and } c_2 = 0)\). For such cases, \(k'_d\) is a better estimate of \(k_d\).

**Applications to Model Ligands and Water samples**

The retention of free Cu\(^{2+}\) ions by the Chelex-100 column has been shown to be independent of the contact time down to 0.48 s (a sample loading flow rate of 15 mL/min) (10). The effect of the contact time on the retention of anionic metal complexes by the AG MP-1 column was studied with the CuEDTA\(^{-2}\) complex. Figure 6.2 shows the results of flow rate studies for a solution of 3.2 \(\mu\)M Cu(II) and 2.0 \(\mu\)M EDTA at pH 7.0. Similar results were also obtained for a solution of Cd\(^{2+}\) and CdEDTA\(^{-2}\). These results indicate that the retention of M\(_1\) species such as Cu\(^{2+}\) ion by the Chelex-100 column and the retention of anionic complexes such as (CuEDTA)\(^{-2}\) by the AG MP-1 column are independent of the contact time used in the experiments (0.48 to 7.2 s). The stability constants for these complexes are large enough that insignificant dissociation occurs (i.e., \(c_1 = c_{M1}\) and \(c_2 = c_{ML} - c^*\)).

The dissociation kinetics of the 1:1 Cd(II)-NTA and Cu(II)-IDA complexes were studied under selected experimental conditions and the results are shown in Figures 6.3 and 6.4. Clearly the dissociation of these two complexes does occur. The results of flow rate studies were fitted to Equations 3 and 4 and the values of \(k'_d\) and the percentage distribution of M\(_1\) and ML species in the original sample solutions obtained are presented in Table 6.1. The apparent
Figure 6.2. Effect of the Column Contact Time on the Dissociation of the CuEDTA\(^{-2}\) complex.

Sample solution: 200 \(\mu g/L\) Cu(II) in 2.0 \(\mu M\) EDTA at pH 7.0.
Figure 6.3. Effect of the Column Contact Time on the Dissociation of the CdNTA$^{-1}$ complex.

Sample solution: 100 µg/L Cd(II) in 2.0 µM NTA at pH 5.4.
Figure 6.4. Effect of the Column Contact Time on the Dissociation of the CuIDA complex.

Sample solution: 200 μg/L Cu(II) in 0.01 M IDA at pH 8.1.
Table 6.1. Results of Kinetic Measurements\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sample</th>
<th>$k_d$</th>
<th>$%$ M1</th>
<th>$%$ ML</th>
<th>$%$ c\textsuperscript{*}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd(II)-NTA</td>
<td>0.43 ± 0.09 (0.32 ± 0.04)</td>
<td>61 ± 2 [63]</td>
<td>42 ± 2 (41 ± 2) [36]</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Cu(II)-IDA</td>
<td>1.26 ± 0.03 (1.12 ± 0.06)</td>
<td>---</td>
<td>98 ± 1 (89 ± 4) [100]</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Cu(II)-NTA</td>
<td>0.26 ± 0.06 (0.12 ± 0.03)</td>
<td>38 ± 2</td>
<td>59 ± 2 (59 ± 3) [63]</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>Cu(II)-HM</td>
<td>0.32 ± 0.03 (0.29 ± 0.10)</td>
<td>33 ± 1</td>
<td>64 ± 1 (59 ± 2)</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Cu(II)-River</td>
<td>0.67 ± 0.09 (0.49 ± 0.08)</td>
<td>80 ± 1</td>
<td>18 ± 1 (16 ± 1)</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}$\%$ M1 and $\%$ ML are the percentage of M1 and ML species in the sample solution, respectively. $\%$ c\textsuperscript{*} is the percentage of ML species at $t_c - \infty$. The values of $k_d$, $\%$ M1, $\%$ ML and $\%$ c\textsuperscript{*} and the standard deviations were determined by fitting the data from the Chelex-100 column in flow rate studies to Equations 3 and 4. The values in the parenthesis are obtained using the data from the AG MP-1 column. The values of $\%$ ML in the brackets are results from equilibrium calculations.

\textsuperscript{b}Detailed information about Sample 1 to 5 is given in Figures 6.3, 6.4, 6.5, 6.6, and 6.7, respectively.
dissociation rate constant is found to be about 0.5 s^{-1} for CdNTA^{-1}. The dissociation rate constant of CdNTA^{-1} has been determined to be on the order of 1-3 s^{-1} at pH 7-8 by several researchers using polarographic techniques (12-14). The apparent dissociation rate constant of CdNTA^{-1} determined by the two-column method is reasonably close to the literature values of the 1st-order dissociation rate.

The apparent dissociation rate constant for the Cu-IDA complex is found to be about 1 s^{-1}. Under the conditions used, the Cu(II)-IDA complex is primarily in form of Cu(IDA)_{2}^{-2}. The dissociation from the 1:2 complex Cu(IDA)_{2}^{-2} to the 1:1 complex CuIDA is assumed to be much more rapid compared to the dissociation from CuIDA to Cu^{2+} and IDA as is true for similar 1:2 metal complexes such as Zn(NTA)_{2}^{4-} (15). Thus the rate of dissociation of the Cu(IDA)_{2}^{-2} complex is determined by the rate of dissociation of the CuIDA complex. The formation rate constant of CuIDA has been reported to be 3.0 \times 10^{9} M^{-1}s^{-1} (16). The dissociation rate constant of CuIDA is calculated to be 0.08 s^{-1} from the reported formation rate constant and the stability constant (10^{10.57}) of the CuIDA complex. The value of k'_d determined by the two-column method for the Cu-IDA complex is considerably greater than the value of k_d calculated above. The degree of dissociation of the CuIDA complex determined by the two-column method may appear to be greater than expected due to the possible formation of the mixed ligand complex of R-Cu-IDA.

The results of the flow rate studies show that about 62% and 42%
of Cd(II) in the solution are M1 species (i.e., free Cd\(^{2+}\) ions) and ML species (i.e., the CdNTA\(^{-1}\) complexes), respectively. These agree well with the results of equilibrium calculations which indicate that 63% of Cd(II) is uncomplexed and 37% of Cd(II) is complexed in the original Cd-NTA solution. The equilibrium calculations indicate that all the Cu(II) in the IDA solution is complexed. The ML fraction (i.e., the CuIDA complex) determined for the Cu-IDA solution is about 94% of the total Cu(II) and agrees reasonably well with 100% of the total Cu(II) predicted by the equilibrium calculations.

Flow rate studies were also conducted for solutions of 3.2 \(\mu\)M Cu(II) prepared in 2.0 \(\mu\)M NTA, 8 mg/L humic acid and the Willamette River water sample. The results are shown in Figures 6.5, 6.6, and 6.7, respectively. The determined values of \(k'_d\) and the percentage distribution of M1 and ML are presented in Table 6.1. The apparent dissociation rate constants of Cu(II) complexes with ligands in the humic acid solution and the river water sample are found to be about 0.3 and 0.6 s\(^{-1}\), respectively. These results are consistent with those reported by other researchers. Figura and McDuffie estimated the dissociation rate constant of Cu-humate complexes to be about 0.1 s\(^{-1}\) (8). Shuman and Michael reported that the dissociation rate constant of Cu(II) complexes in the coastal water samples to be about 2 s\(^{-1}\) (4). The results also show that 59% of the Cu(II) in the humic acid solution is a ML species and 18% of the Cu(II) added to the river water sample is a ML species. These results indicate that the dissociation kinetics of Cu(II) complexes in the humic acid solution are similar to that of the Cu(II) complexes in the
Figure 6.5. Effect of the Column Contact Time on the Dissociation of the CuNTA⁻¹ complex.

Sample solution: 200 μg/L Cu(II) in 2.0 μM NTA at pH 7.0.
Figure 6.6. Effect of the Column Contact Time on the Dissociation of Cu(II)-humate complexes.

Sample solution: 200 μg/L Cu(II) in 8 mg/L humic acid at pH 6.5.
Figure 6.7. Effect of the Column Contact Time on the Dissociation of Cu(II) complexes in the Willamette River Water Sample.

Sample solution: 400 μg/L Cu(II) in the Willamette River water sample at pH 7.0.
river water sample and the Cu(II) complexes in the humic acid solution are more stable (i.e., the ratio of % ML to % Ml is greater).
CONCLUSIONS

The two-column kinetics measurement scheme presented here provides a new method for studying the dissociation kinetics of trace metal complexes in natural waters. The results of flow rate studies indicate that the fraction of metal species retained by the Chelex-100 column depends on the contact time of the sample solution in the column. The concentrations of M1 and ML species obtained with the kinetics measurement scheme are the values extrapolated to the zero contact time. They provide a better estimate of the speciation of trace metals in the original sample solution than the results obtained with the two-column method at a constant flow rate (9) or other measurement schemes using Chelex-100 resin since the dissociation of some metal complexes in the Chelex-100 column is corrected. The kinetics measurement scheme can be employed to study the dissociation kinetics of trace metal complexes at concentrations lower than reported here by using a larger sample loop to increase preconcentration factor.

The results of the flow rate studies are used to determine the apparent 1st-order dissociation rate constants of trace metal complexes. This provides an estimate of the lower limit of the true dissociation rate constant. The range of the dissociation rate constants that can be determined depends on the range of the contact time of the sample solution with the Chelex-100 column. There should
be at least 10-20% of change in the degree of dissociation of metal complexes over the contact time range used to obtain reasonable estimates of the apparent 1st-order dissociation rate constants. With the range of contact time from 0.48 to 7.2 s used in this study, the range of apparent 1st-order dissociation rate constant than can be determined is limited to about 0.05 to 2 s⁻¹. Smaller apparent dissociation rate constants could be determined with flow rates lower than 1 mL/min or a Chelex-100 column with a larger diameter and length.
REFERENCES


CHAPTER 7
CONCLUSIONS

The determination of the speciation of trace metals in natural waters is a very challenging problem because of the complex nature of the trace metal speciation and very low concentrations of trace metals. The automated on-line ion exchange trace enrichment system developed in this research allows the readily available technique of flame AA spectrophotometry to determine accurately low concentrations of trace metal ions in the water samples. Studies have shown that variables such as column dimensions, resin particle sizes, sample loading flow rates, and elution flow rates are critical to the performance of the on-line ion exchange trace enrichment system. Complexing reagents such as cysteine and EDTA have been successfully used to elute trace metal ions off the Chelex-100 resin and shown to be advantageous compared to elution with strong acids such as nitric acid in the on-line ion exchange system. The detection limit of the trace enrichment system with on-line flame AA detection is about 0.1 µg/L for Cu, Cd, Zn, and Mn, about a factor of 60 lower than that of the conventional flame AA spectrophotometry. The sample throughput rate of the system is about 2-3 min per sample with a 1-mL sample loop and 5-6 min per sample with a 10-mL sample loop.

The automated two-column ion exchange system developed has been shown to provide a rapid, sensitive method to determine the speciation...
of trace metals in natural waters. The measurement scheme based on the two-column system classifies the dissolved metal species into three fractions: M1, M2 and M3. The studies with the two-column system have shown that the M1 fraction (retained by the Chelex-100 column) includes free metal ions and labile metal complexes that dissociate in the Chelex-100 column. The M2 metal species (retained by the AG MP-1 column) are anionic moderately labile and non-labile metal complexes and metals strongly associated with negatively charged organic matter such as humic acid. The M3 fraction includes neutral non-labile metal complexes and metals strongly associated with very large organic and inorganic colloidal particles. The studies have also shown that variables such as the sample loading flow rate, pH, and ionic strength affect the results of the measurements with the two-column system. In contrast to other measurement schemes, the complexed metal fraction is determined directly rather by difference.

The two-column measurement scheme has been applied to determine the speciation of Cu(II), Cd(II) and Zn(II) in natural waters samples at concentrations near 1 µg/L. For the two water samples tested, the results show that most of the Cu(II) is the M2 species and most of the Cd(II) and Zn(II) are M1 species.

The two-column system is a useful tool to study the complexation of trace metals with organic ligands in natural waters. The two-column system has been used to determine the trace metal complexing capacity and the conditional stability constants of ligands in natural waters. The results show that the average Cu(II) complexing capacity of a Willamette River sample is about 4 µM and the
conditional stability constant of Cu(II) complexes is about $10^7$ at pH 7. A one-point determination of trace metal complexing capacity was developed which is much more rapid than other existing procedures.

It has also been demonstrated that the two-column system can be used to study the dissociation kinetics of trace metal complexes in natural water by varying the sample loading flow rate. The apparent 1st-order dissociation rate constant of Cu(II)-humate complexes is determined to be about 0.3 s\(^{-1}\) at pH 6.5. The range of apparent 1st-order dissociation rate constants that can be determined by the two-column system is about 0.05 to 2 s\(^{-1}\). Sample loading flow rate data can also be used to estimate the concentration of M1 and M2 (ML) species in the original sample solutions in cases where some dissociation of metal complexes occurs during passage through the Chelex-100 column.

For possible future studies, the two-column system could be interfaced with an electrothermal atomizer in an AA spectrophotometer to further improve the ability to determine trace metals in natural waters at even lower concentrations. A small plug (e.g., 20 $\mu$L) of the eluted metal species at the maximum of elution peak could be split out and delivered to an electrothermal atomizer using additional flow and mechanical components. It would also be possible to nebulize and deposit the solution into the electrothermal atomizer during an elution peak.

Multielement atomic spectrometric techniques such as inductively coupled plasma emission spectrometry could also be coupled with the two-column system. This detection system would improve detection
limits for some trace metals and allow to the determination of the speciation of a number of trace metals in one sample run, compared to one metal at a time with the flame AA detection.

The two-column system can potentially be used in a number of other applications. A preliminary study has shown that the two-column system can be used to determine the speciation of Cr(III) and Cr(VI) in water samples. In that study, the Chelex-100 column was used to retain Cr$^{3+}$ ions and the AG MP-1 column was used to retain CrO$_4^{2-}$ ions. A solution of 2 M HNO$_3$ was used to elute the metal species off the columns. It is also possible to use the system to determine the speciation of other trace metals existing in two different oxidation states with different charges.

One or both of the ion exchange columns could be replaced with two electrochemical flow cells operated in a coulometric mode. One cell could be held at a potential which is just negative enough to reduce free metal ions and ASV labile metal species. The AG MP-1 column would retain the ASV non-labile metal species. For a two-cell system, the second cell could be held at a more negative potential to reduce some non-labile metal complexes. A large volume of the sample solution could be passed through a flow-cell system to achieve trace enrichment of metal species. After the deposition step, the reduced metals could be stripped off the electrodes by reversing the cell potentials and be sent directly into the nebulizer of the flame AA detector for measurement. The flow system developed for the two-column system could also be used in other flow injection analysis applications.
The AIM-65 microcomputer system used to control the operations of the two-column could be replaced with a more powerful microcomputer such as a PC. Elution peak data could be directly displayed on the monitor and data files could be saved on floppy disks. More sophisticated software could be developed to enhance data manipulation capabilities.
BIBLIOGRAPHY


Appendices
APPENDIX I

MICROCOMPUTER HARDWARE AND SOFTWARE OF THE

AUTOMATED TWO-COLUMN ION EXCHANGE SYSTEM
An AIM-65 microcomputer and its peripheral I/O devices are used to control the operation of the automated two-column ion exchange system. The microcomputer system is modified from a similar system developed previously by Ambrose (1). The major hardware components and their functions have been described by Ambrose in detail. An overview of the microcomputer system hardware is given here.

The AIM-65 microcomputer is a single board microcomputer based on a 6502 central processing unit. It has limited memory (4K RAM) and I/O capabilities on board. The computer is expanded by connection to a mother board into which is plugged a 16K RAM board and a variable address I/O board to provide additional memory and I/O capabilities. The mother board and the memory and I/O expansion boards are housed in a blue aluminum and plexiglass box with a cooling box fan (denoted as the expansion box). The mother board is connected to a 44-pin expansion edge connector on the right rear section of the computer. The mother board is powered by an unregulated +8 V output from a dual output power supply. A regulated +24 V output from the same power supply is used to power the thermal printer of the computer. The microcomputer itself is powered by a regulated +5 V from the mother board. On the left rear section of the computer is a 44-pin application edge connector. It provides the connections to an ADC/DAC module, a cassette tape recorder (for loading and saving computer programs), and a 20-mA-loop teletype (for hard copy program listing).
The ADC/DAC module consists of a 12-bit ADC board, a 12-bit DAC board, and an auxiliary power supply which provides +15, -15, and +5 V. The ADC/DAC module is housed in a black aluminum box (denoted as ADC/DAC box). This module was moved out of the expansion box of the original system.

The output from the Varian AA spectrophotometer (100 mV = 1 AU) is first fed into a Spectrum active noise filter. The filter serves to reduce the noise in the AA signal (cut-off frequency is usually at 0.1 s) and to amplify the AA signal by a factor of 10. The output of the active noise filter is further amplified by another factor of 10 with an instrumentation amplifier making 10 V = 1 AU. The amplifier circuit (see Figure I.1) is set-up on a breadboard mounted on the top side of the ADC/DAC box. It replaces the op amp voltage amplifier in the original system. The output of the active noise filter is also connected to the chart recorder for a hard copy of the elution peaks.

The output of the instrumentation amplifier is brought to the input of the ADC board in the ADC/DAC module. The ADC board is used to digitize the amplified AA signal by the microcomputer. As the AA signal is digitized, the DAC board in the ADC/DAC module converts the digital data to an analog voltage for a nearly real-time display on the oscilloscope. The output of the DAC goes to channel 1 of the scope. A digital cursor signal generated by software is sent to channel 2 of the scope from PA1 of the 6522 chip on the microcomputer board via a banana plug connector (labeled as "c") on the ADC/DAC box. The scope is triggered by another digital signal from PA0 of the same chip via a banana plug connector (labeled as "b") on the ADC/DAC
Figure I.1. Circuit Diagram of the Instrumentation Amplifier.
box. The offset null potentiometer of the instrumentation amplifier is set so that the ADC observes 0 V or a slightly positive voltage when the AA is zeroed.

The variable address I/O board in the expansion box has two 6522 versatile interface chips. By means of two 8 pin switches, any address can be assigned to the chips and the board occupies 1 K bytes memory. One 6522 chip is located in the "upper" 1/2 K of the memory block and the other is in the "lower" 1/2 K of the block. The I/O board provides 40 additional I/O lines for the microcomputer. However only 16 I/O lines from the ports B of the two 6522 chips are used by the microcomputer. These I/O lines are brought out the I/O board with a 44-pin edge connector. The PB7 and PB6 pins of the "upper" 6522 chip are connected together with a wire jumper on the edge connector to make the timers of the 6522 chip available to the microcomputer. The PB0-PB5 pins of the "upper" 6522 chip and PB0-PB7 pins of the "lower" 6522 chip are connected to banana plug connectors mounted on a small aluminum box to make them accessible by external devices.

These I/O lines are used to turn ac power on and off for devices such as peristaltic pumps, HPLC pumps, and 3-way solenoid valves via solid state relays. A logic "1" signal on the I/O line turns the solid state relay on which in turn causes the ac power applied to the external device. A logic "0" signal turns the solid state relay off and thus turns the external device off. The solid state relays and the control circuit are housed in a aluminum box mounted on the exterior rear side of the flow system box in which are housed all the solenoid valves, 4-way slider valves and peristaltic pumps. The
control lines of the solid state relays are connected to the banana plug connectors mounted on the side panel of the aluminum box for connections to those I/O lines. The two solid state relays for controlling the HPLC pumps are housed in a separate box. The assignments of the I/O lines are listed in Table I.1.

MICROCOMPUTER SOFTWARE

The computer program developed for the automated two-column ion exchange system is a BASIC language program with assembly language subroutines. The main BASIC language program is used to control the operation of the flow system and to perform the data analysis. The assembly language subroutines are called by the BASIC program to perform data acquisition and display as well as system timing. Some of the BASIC language routines and the assembly language subroutines are modified from a similar program developed by Ambrose (1).

The memory map of the microcomputer system is shown in Figure I.2. The BASIC and the assembly language programs are saved on a cassette tape as one file under the name of "COLLECT" by recording machine codes of the memory locations from 0000 to 00FF, 0200 to 3900, and 4000 to 4400 (all numbers are hexadecimal numbers). The commands for loading and saving programs with the cassette tape recorder are discussed in the user's guide of the AIM-65 microcomputer. After the programs are loaded into the computer, one enters the BASIC program by typing "6" from the keyboard and start running the program by typing
Table I.1. The Assignments of the I/O Lines.

<table>
<thead>
<tr>
<th>6522 Chip</th>
<th>I/O line</th>
<th>Devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower</td>
<td>PB0</td>
<td>HPLC Pump A</td>
</tr>
<tr>
<td></td>
<td>PB1</td>
<td>V1, V2, V3, V8, V9</td>
</tr>
<tr>
<td></td>
<td>PB2</td>
<td>V4, V4'</td>
</tr>
<tr>
<td></td>
<td>PB3</td>
<td>Peristaltic Pump I</td>
</tr>
<tr>
<td></td>
<td>PB4</td>
<td>Peristaltic Pump II</td>
</tr>
<tr>
<td></td>
<td>PB5</td>
<td>Peristaltic Pump III</td>
</tr>
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<td></td>
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<td>V6</td>
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<td>V7</td>
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<tr>
<td>Upper</td>
<td>PB0</td>
<td>V5</td>
</tr>
<tr>
<td></td>
<td>PB1</td>
<td>HPLC Pump B</td>
</tr>
<tr>
<td></td>
<td>PB2</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>PB3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>PB4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>PB5</td>
<td>None</td>
</tr>
</tbody>
</table>
Figure I.2. The memory map of the AIM-65 microcomputer.
"RUN <CR>".

In the initialization step, the BASIC program prompts the user to enter heading information. It also defines a number of memory locations used in the assembly language subroutines and initializes the I/O lines used to control the various components in the flow system. Then the user is prompted with a master menu "RUN VIEW STAT MAN" which provides four options. The desired option on the master menu (and the other menus discussed in the followings) is usually chosen by entering the first character of the option (e.g., type "R" to get the "RUN" option) unless otherwise specified. The functions of the four options are discussed in the followings.

Once the "RUN" option is chosen, the user gets into the routines that perform an experimental run with the automated two-column ion exchange system. The user is prompted with a menu "TWO OR ONE COLUMN X". The "X" option returns the program to the master menu (this is also true on most other menus). The "ONE COLUMN" option configures the two-column ion exchange system into an on-line one-column ion exchange trace enrichment system. Only the first column (i.e., the Chelex-100 column) is eluted after the sample loading. The "TWO COLUMN" option specifies the normal operation mode of the two-column system. If a "T" or an "O" is typed, the user is prompted with another menu "NEW RERUN LABELING". If the "NEW" option is the choice, the program prompts the user to input a number of the operation parameters for each ion exchange column. This option is chosen if the user starts a new experiment or desires to change the operation parameters. These parameters include the option of the stop-flow
mode, the data acquisition rate, the collection delay time (how long after initiating the stripping step to wait before commencing the data acquisition), the column washing time after stripping the column, and the stripping reagent loading time. Only one set of these parameters is required if the "ONE COLUMN" option is selected. Then the user is prompted to input a label for the sample solution to be tested, the sample injection time, sample loading time, the number of the sample injections (multiple injection option), and the column conditioning time after each sample run. The typical values of these parameters are given in Table I.2. Once these parameters are input, the program conducts the routines for a sample run with the flow system. The details of the operation steps of the flow system have been discussed in Chapter 3 and Chapter 4. The user is continuously prompted with messages informing the occurrence and timing of the operation events. If the "RERUN" option is chosen, the user can repeat experimental runs for a given sample solution without changing the operational parameters. The "LABELING" option is same as the "RERUN" option except it prompts the user to enter the desired sample label if a different sample solution is to be tested.

The user can choose the "VIEW" option on the master menu to examine the elution peaks on the oscilloscope and to perform the data analysis for the elution peak data acquired by the computer after a sample run. Once the "VIEW" option is selected, the user is prompted with a menu "PEAK ONE PEAK TWO X". The elution peak to be analyzed is chosen by typing an "O" for peak one (i.e., the elution peak from the Chelex-100 column) or a "T" for peak two (i.e., the elution peak
Table I.2. Typical Values of the Operation Parameters of the Automated Two-column Ion-Exchange System.

<table>
<thead>
<tr>
<th>Operation Parameter</th>
<th>Input Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop-flow Mode</td>
<td>No (or Yes)</td>
</tr>
<tr>
<td>Data Acquisition Rate</td>
<td>10 points/s</td>
</tr>
<tr>
<td>Collection Delay</td>
<td>2 - 4 s</td>
</tr>
<tr>
<td>Column Washing</td>
<td>40 s</td>
</tr>
<tr>
<td>Stripping Reagent Loading</td>
<td>8 s</td>
</tr>
<tr>
<td>Sample Loading</td>
<td>15 s (1-mL Loop)</td>
</tr>
<tr>
<td></td>
<td>20 s (2-mL Loop)</td>
</tr>
<tr>
<td></td>
<td>60 s (10-mL Loop)</td>
</tr>
<tr>
<td>Sample Injection</td>
<td>50 s (1-mL Loop)</td>
</tr>
<tr>
<td></td>
<td>80 s (2-mL Loop)</td>
</tr>
<tr>
<td></td>
<td>300 s (10-mL loop)</td>
</tr>
<tr>
<td>Number of Injections</td>
<td>1 (or more)</td>
</tr>
<tr>
<td>Column Conditioning</td>
<td>90 s</td>
</tr>
</tbody>
</table>
from the AG MP-1 column). Once the choice is made, the user is prompted with another menu "RLABIX" to perform the calculations of the peak height, peak area, and the peak retention time. The options of this menu and their uses have been discussed by Ambrose (1) in detail and are not addressed here. The typical baseline width used for the calculations is about 100 (equivalent to 10 s). After the peak height, peak area and retention times are calculated, the computer printer provides a print-out of these data as shown in Figure I.3.

The "STAT" option on the master menu is used to perform statistical analysis when enough repetitive measurements of a sample solution have been made (e.g., 3 or 4 measurements, although the program has the data array space for 10 measurements). Once this option is selected, the user is again prompted with the menu "PEAK ONE PEAK TWO X". If an "O" is entered, the program performs the statistical analysis for the peak one data. The user has the option to delete any data before the statistical calculations. The printer of the computer provides a print-out of the mean, the standard deviation (SD) and the relative standard deviation (RSD) for the peak height and the peak area as well as the mean and the standard deviation of the peak retention time as shown in Figure I.3. Similar statistical analysis is performed for the peak two data if a "T" is typed.

Finally, the "MAN" option on the master menu allows the user to change the logic states of the I/O lines from the variable address I/O board and thus to manually control the operations of the valves and
Figure I.3. Typical Print-out of Data and Statistical Summary.
the pumps in the flow system. The assignments of these I/O lines are given in Table I.1. Once the option is selected, the user is prompted with a menu "LO OR UP I/O OR X". The "LO" option is used to access the I/O lines (PBO to PB7) from the port B of the lower 6522 chip. If this option is chosen, the user is prompted with a menu "PBO, 1, 2, 3, 4, 5, 6, 7, X". For example, A "0" is selected to change the logic state of the I/O line from PBO of the lower 6522 chip. The user is then prompted with a menu "HIGH OR LOW". The "HIGH" option sets the I/O line to the logic "1" state and the "LOW" option resets the I/O line to the logic "0" state. Similarly, the "UP" option is used to access the I/O lines (PBO to PB5) from the port B of the upper 6522 chip.

The listings of the BASIC language and assembly language programs are given in Appendix II.

**BACK-FLUSHING VALVE ARRANGEMENT**

The back-flushing valve arrangement is used to reduce the pressure drop across the Chelex-100 column after elution with nitric acid solution by reversing the flow direction of the carrier buffer stream through the column. Otherwise the swelling and shrinking of the Chelex-100 resin due to the change of ionic forms of the resin in the column during the elution step cause the packing of the column becoming progressively tighter because of unidirectional flow. To achieve the back-flushing operation, valves V10 and V11 are added to
the flow system. The normal positions of valves V10 and V11 are shown in Figure 1.4. When the step of loading the sample, SR and NH₄OH solutions is initiated, the valves V10 and V11 are turned to the positions shown in Figure 1.5 so that the flow direction of the carrier buffer stream through the ion exchange columns is reversed. The valves V10 and V11 are controlled by one pneumatic activator. The solenoid valves of the pneumatic activator are controlled by the same I/O line that is used to control the ON/OFF states of the peristaltic pumps II and III. After loading the SR and NH₄OH solutions (about 10 s later), the valves V10 and V11 are turned to their normal positions.
Figure I.4  The Two-column Ion Exchange System with Back-flushing Valve Arrangement: Normal Position.
Figure I.5  The Two-column Ion Exchange System with Back-flushing Valve Arrangement: Back-flushing Position.
REFERENCES

APPENDIX II

COMPUTER PROGRAM LISTINGS
BASIC LANGUAGE PROGRAM

10 REM ****************************************************************
20 REM VALVE CONTROL AND DATA COLLECTION PROGRAM
30 REM FOR THE ON-LINE ION EXCHANGE SYSTEM
40 REM BY YAN LIU
50 REM VERSION TWO
60 REM MARCH 23.1986
90 REM
100 GOSUB 500:REM SET-UP SUBROUTINE
150 PRINT "RUN VIEW STAT MAN"
160 GET R$: IF R$ = "V" THEN GOSUB 500: GOTO 150: REM VIEW SUBROUTINE
170 IF R$ = "R" THEN GOSUB 300: GOTO 150: REM RUN SUBROUTINE
180 IF R$ = "M" THEN GOSUB 400: GOTO 150: REM MANUAL SUBROUTINE
200 IF R$ = "S" THEN 200
230 GOSUB 200: REM STATISTICS SUBROUTINE
300 GOTO 150
500 REM SETUP SUBROUTINE
510 REM GET HEADINGS
520 X = 5: GOSUB 1900
525 INPUT "DATE"; D$
530 PRINT TAB(8) D$
535 INPUT "REFERENCE NUMBER"; D$
540 PRINT TAB(8) D$
545 X = 2: GOSUB 1900
550 INPUT "FLOW RATE(ML/MIN"; F
555 INPUT "PRESSURE"; D
560 PRINT TAB(8) F "ML/MIN"
562 PRINT TAB(8) D " PSI"
565 PRINT "MORE?"
570 GET R$: IF R$ = "N" THEN 600
575 IF R$ = "Y" THEN 570
580 INPUT "WHAT"; D$
585 PRINT TAB(4) D$
590 GOTO 565
600 X = 3: GOSUB 1900
605 REM DEFIN ADDRESS
610 ZL = 16587: SL = 16608: LC = 16634: VL = 16419
615 REM ZL = $40CB; SL = $40E0; LC = $40FA; VL = $4023
620 ZH = 17999: SH = 17120: HC = 17146: VH = 16931
625 REM ZH = $42CB; SH = $42E0; HC = $42FA; VH = $4223
630 B1 = 20480: DM = 20479: KY = 42927: B2 = 20992
635 REM B1 = $5000; DM = $4FFF; KY = $A42B; B2 = $5200
640 CL = 24576: CH = 25008: REM CL = $6000; CH = $6200
645 POKE CL+2, 255: REM MAKE LOWER PB0, 1, 2, 3, 4, 5, 6, 7 OUTPUT
650 POKE CH+2, 63: REM MAKE UPPER PB0, 1, 2, 3, 4, 5 OUTPUT
655 POKE CL+6: POKE CH, 0: REM RESET I/O LINE
665 X = 1: GOSUB 4505: REM TURN ON MAIN PUMP
212

657 X=1:GOSUB4725:REM TURN ON 2ND PUMP
660 T:=194:T2=176:REM T1=#B0C2
670 DIM A(10),P(10),R(10)
675 DIM A1(10),P1(10),R1(10)
680 DIM A2(10),P2(10),R2(10)
685 DEFNH(T)=INT(T/256)
690 DEFFNL(T)=T-DEFNH(T)*256
695 DEFFNV(X)=(10*X/4095-5)/1E-2
700 DEFND(1).-7-161-PEEK(BF+1):INT(PEEK(BF+256+1)/16)
705 DEFFNP(T).:INT(10*T+0.5)/10
730 REM
1000 REM ***** MANUAL PEAK WIDTH SET *****
1005 PRINT"SET PEAK WIDTH?"
1010 GETR$:IFR$="N"THEN1060
1015 INPUT"W(R OR L)";W$
1020 IFR$RIGHT$(W$,0)="L"THEN1070
1025 IFRIGHT$(W$,0)="R"THEN1015
1030 W$=LEFT$(W$,LEN(W$)-1):W=VAL(W$)
1035 B=A+W-(-1):H:IIFK<256:THENPOKE255,B:RETURN
1040 PRINT"8 OFFSCALE-W":255-A:GOSUB1800:POKE255,255:RETURN
1045 A=B-W-1:IFA.15THENPOKE255,A:RETURN
1050 PRINT"A OFFSCALE-W":B-5:GOSUB1800:POKE255,B:RETURN
1060 IFA1THENPOKE255,A+1:RETURN
1065 IFA2THENPOKE255,B-1:RETURN
1070 W$=LEFT$(W$,LEN(W$)-1):W=VAL(W$):GOTO1045
1700 REM ***** TIMER SUBROUTINE; ARRIVE WITH T SEC. ********************
1710 IF T<0THENRETURN
1720 PRINTINT(T/60);"MIN"SPC(2)T-INT(T/60)*60;"SEC LEFT"
1730 POKE4,32:POKES,65:Q=USR(0):REM $4120
1735 T=T-1
1736 IFT=0THENRETURN
1738 PRINTINT(T/60);"MIN"SPC(2)T-INT(T/60)*60;"SEC LEFT"
1740 IFT=0THENRETURN
1750 POKE4,57:Q=USR(0):REM $4139
1760 GOTO1735
1800 REM ***** ONE SECOND PAUSE *****
1810 FORI=0TO500:NEXTI:RETURN
1900 REM ***** BLANK LINE *****
1905 FORJ=1TO4:PRINT""""""""""";NEXTJ:RETURN
2000 REM ***** STATISTICS SUBROUTINE ********************
2010 PRINT"PEAK ONE PEAK TWO X"
2020 GETR$:IFR$="O"THENGOSUB2100:RETURN
2030 IFR$="T"THENGOSUB2200:RETURN
2040 IFR$<"X"THEN2010
2045 RETURN
2100 REM ***** PEAK ONE *****
2105 PRINT"PEAK ONE STATISTICS":GOSUB1800
2110 N1Z=N1Z-1
2115 N2=N2%1
2115 FORI=1TO42
2120 K(I)=A(I); P(I)=P1(I); R(I)=R1(I)
2125 NEXT I
2130 KP=1
2135 GOSUB 2300
2140 N%=0; N1%=0
2150 RETURN
2200 REM ***** PEAK TWO *****
2205 PRINT "PEAK TWO STATISTICS"; GOSUB 1800
2208 N2%=N2%-1
2210 N%=N2%
2215 FOR I=1 TO N%
2220 A(I)=A2(I); P(I)=P2(I); R(I)=R2(I)
2225 NEXT I
2230 VP4,2
2235 GOSUB 2300
2240 4.0; N2%=0
2250 RETURN
2300 REM ***** STATISTICS ****************************
2305 PRINT "ERASE ANY?
2310 GETR$: IF R$="N" THEN 2400
2315 IF R$="Y" THEN 2310
2320 INPUT "WHICH ONE"; J
2325 IF J<0 THEN 2335
2330 IF J>0 THEN 2340
2335 PRINT "ERROR IN "; N$: GOSUB 1800
2340 A(J)=0; P(J)=0; R(J)=0; S1=S1+1: R1=R1+R(I)
2345 FOR I=1 TO N%
2350 S1=S1+A(I); S2=S2+P(I); R1=R1+R(I)
2355 IF N%-2=0 THEN 2490
2360 S1=S1/(N%-1); S2=S2/(N%-1); R1=R1/(N%-1)
2365 REM S1=AVE AREA S2=AVE PK HT ***
2370 IF J=1 THEN 2100
2373 IF A(I)*P(I)=1 THEN 2490
2375 S3=S3+(A(I)-S1)*(A(I)-S1)
2378 S4=S4+(P(I)-S2)*(P(I)-S2)
2380 R2=R2+(R(I)-R1)*(R(I)-R1)
2390 NEXT
2400 IF N%<2 THEN 2500
2404 SR=SQR(R2/(N%*Z-Z-1))
2407 SA=SQR(S3/(N%*Z-Z-1))
2410 SP=SQR(S4/(N%*Z-Z-1))
2411 REM ***** SA=STD DEV(AREA) SP=STD DEV(PK HY)
2412 IF S1=0 THEN 2600
2414 RA=SA/S1+100
2416 IF S2=0 THEN 2600
2418 RP=SP/S2+100
2419 REM ***** RA=RSD(AREA) RP=RSD(PK HY) ***
2420 IF Z%=0 THEN PRINT "SUMMARY: ALL POINTS"; GOTO 2526
2425 IF N%=0 THEN PRINT "SUMMARY: NO POINTS"; GOTO 2526
2430 IF Z%<0 THEN 2500
2435 IF Z%>0 THEN 2500
2440 IF A(I)>0 THEN 2445
2445 A(I)=0; P(I)=0; R(I)=0
2448 A(I)=0; P(I)=0; R(I)=0
2450 IF A(I)>0 THEN 2455
2455 A(I)=0; P(I)=0; R(I)=0
2460 IF A(I)>0 THEN 2465
2465 A(I)=0; P(I)=0; R(I)=0
2470 IF A(I)>0 THEN 2475
2475 A(I)=0; P(I)=0; R(I)=0
2480 IF A(I)>0 THEN 2485
2485 A(I)=0; P(I)=0; R(I)=0
2490 REM **** S1=S1/S1+1: R1=R1+R(I)
2495 NEXT I
2500 IF N%<2 THEN 2500
2504 SR=SQR(R2/(N%*Z-Z-1))
2507 SA=SQR(S3/(N%*Z-Z-1))
2510 SP=SQR(S4/(N%*Z-Z-1))
2511 REM ***** SA=STD DEV(AREA) SP=STD DEV(PK HY)
2512 IF S1=0 THEN 2600
2514 RA=SA/S1+100
2516 IF S2=0 THEN 2600
2518 RP=SP/S2+100
2519 REM ***** RA=RSD(AREA) RP=RSD(PK HY) ***
2520 IF Z%=0 THEN PRINT "SUMMARY: ALL POINTS"; GOTO 2526
214  PRINT"SUMMARY: ALL BUT 1"
2526  IFKP=1 THEN PRINT" PEAK ONE"
2528  IFKP=2 THEN PRINT" PEAK TWO"
2530  X=1: GOSUB 1900
2532  PRINT"SPC(5) "PK HT"SPC(2) "AREA"
2535  PRINT"MEAN" SPC(1) FNP(S2) SPC(2) INT(S1+.5)
2540  PRINT SPC(2) "SD" SPC(1) FNP(SP) SPC(2) INT(SA+.5)
2545  PRINT SPC(1) "RSD" SPC(1) INT(RP+.5) SPC(2) INT(RA+.5)
2550  X=1: GOSUB 1900
2555  PRINT "RET TIME= INT(10*R1 + .5)/10 "SEC"
2560  PRINT SPC(5) "SD= INT(10*SR+.5)/10"
2565  X=3: GOSUB 1900
2570  PRINT "AGAIN?"
2575  GETR$: IFR$="Y" THEN 2305
2580  IFR$="N" THEN 2575
2585  RETURN
3000  REM ***** RUN SUBROUTINE *****************************************
3005  PRINT" TWO OR ONE COLUMN X"
3007  W3=1
3010  GETR$: IFR$="O" THEN W3=0: GOTO 3020
3015  IFR$="T" THEN 3020
3017  IFR$<"X" THEN 3005
3018  RETURN
3020  PRINT "NEW RERUN LABELING"
3025  GETR$: IFR$="R" THEN RT=1: GOSUB 3960: GOTO 3080
3028  IFR$="L" THEN GOSUB 3500: RT=1: GOSUB 3960: GOTO 3080
3030  IFR$<"N" THEN 3025
3035  GOSUB 3500
3040  PRINT "INPUT FOR COLUMN 1": GOSUB 1800
3042  SF=0: PS=0: TS=0: X=0: Y=0: I=0: O=0: R=0
3043  CD=0: WT=0: LT=0: REM RESET FOR COLUMN ONE INPUT
3045  GOSUB 3800
3050  S1=SF: P1=PS: T1=TS: X1=X: Y1=Y
3055  I1=I: O1=O: R1=R: D1=CD
3056  W1=WT: L1=LT
3057  W1=W4: REM STOP-FLOW FLAG
3058  IFW3=0 THEN 3080
3060  PRINT "INPUT FOR COLUMN II": GOSUB 1800
3062  SF=0: PS=0: TS=0: X=0: Y=0: I=0: O=0: R=0
3063  CD=0: WT=0: LT=0: REM RESET
3065  GOSUB 3800
3070  S2=SF: P2=PS: T2=TS: X2=X: Y2=Y
3075  I2=I: O2=O: R2=R: D2=CD: W2=WT: L2=LT
3077  W2=W4: REM STOP-FLOW FLAG
3080  PRINT "INJTIME="; IT; "SEC.NEW?"
3085  GETR$: IFR$="N" THEN 3100
3090  IFR$<"Y" THEN 3085
3095  INPUT "INJTIME (SEC)="; IT
3100  PRINT "SAMPLING?"
3105  GETR$: IFR$="N" THEN WNS=1: GOSUB 3160
3110  IFR$<"Y" THEN 3105
REM LOAD SAMPLE
PRINT "LOAD SAMPLE
X=1:GOSUB4565
T=ST:GOSUB1700
X=0:GOSUB4565
PRINT "INJECT SAMPLE
X=1:GOSUB4525
T=IT:GOSUB1700
X=0:GOSUB4525
V%=V%-1:IFV%=0 THEN 3207
IFNS=0 THEN 3242
MS=0:X=1:GOSUB4585
IFW3=0 THEN 3236
X=1:GOSUB4605
T=L1:GOSUB1700
X=0:GOSUB4605
PRINT "LOAD SAMPLE,SRI,SRII";GOSUB1800
X=1:GOSUB4565
T=IT:GOSUB1700
X=0:GOSUB4605
PRINT "INJECT SAMPLE";GOSUB1800
X=1:GOSUB4525
T=IT:GOSUB1700
X=0:GOSUB4525
 PRINT "INJECT SR I.........";GOSUB1800
X=1:GOSUB4525
T=L1:GOSUB1700
PRINT "INJECT SR II.......";GOSUB1800
X=1:GOSUB4525
T=IT:GOSUB1700
X=0:GOSUB4605
PRINT "REH STOP FLOW"
216

3345 PRINT "HIT KBD TO ABORT"
3350 POKE 4, 8: POKE 5, 64: Q = USR(0)
3352 PRINT "WASHING COLUMN I...": GOSUB 1800
3354 T = W1: GOSUB 1700
3360 X = 0: GOSUB 445
3365 IF W3 = 0 THEN 3410
3368 PRINT "INJECT SR II....": GOSUB 1800
3375 X = 1: GOSUB 4795
3380 T = W2: GOSUB 1700
3385 IF N2 = 0 THEN GOSUB 3740: REM STOP FLOW
3388 PRINT "HIT KBD TO ABORT"
3390 POKE 4, 8: POKE 5, 64: Q = USR(0)
3395 PRINT "WASHING COLUMN II...": GOSUB 1800
3400 T = W2: GOSUB 1700
3405 X = 1: GOSUB 4795
3410 X = 0: GOSUB 4525
3415 X = 0: GOSUB 4625
3420 X = 0: GOSUB 4525
3430 PRINT "AFTER WASHING......": GOSUB 1800
3440 T = W2: GOSUB 1700
3450 GOTO 3005
3500 REM *** SAMPLE LABELING ***
3510 INPUT "SAMPLE ID": A$
3520 L$ = "SAMPLE:"
3530 PRINT !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
3540 PRINT !TAB(63)L$ SPACING A$
3550 X = 3: GOSUB 1900: RETURN
3700 REM STOP-FLOW SUBROUTINE
3705 PRINT "STOP FLOW............": GOSUB 1800
3710 X = 0: GOSUB 4505
3720 X = 1: GOSUB 4505: RETURN
3730 X = 1: GOSUB 4505: RETURN
3740 PRINT "STOP FLOW............": GOSUB 1800
3750 X = 0: GOSUB 4505
3760 T = W2: GOSUB 1700
3770 X = 1: GOSUB 4505: RETURN
3800 REM ***** INPUT PARAMETERS FOR SYSTEM OPERATION************
3805 PRINT " ALL INPUT>=1 " : GOSUB 1800
3810 PRINT "STOP FLOW?"
3815 GETR4: IF R4 = "N" THEN W4 = 1: GOTO 3845
3816 IF R4 <> "Y" THEN 3815
3817 GETR4: IF R4 = "N" THEN W4 = 1: GOTO 3845
3818 W4 = 0
3820 PRINT "STOP FLOW": SF; "SEC, NEW?"
3825 GETR4: IF R4 = "N" THEN 3845
3830 IF R4 <> "Y" THEN 3825
3840 INPUT "SF": SF
3845 PRINT "PEAKING:" PS; "PTS, NEW?"
3850 GETR4: IF R4 = "N" THEN 3890
3855 IF R4 <> "Y" THEN 3855
217

3860 INPUT "PNTS/SEC"; PS
3865 TS = 256 / PS
3866 PRINT "SCANTIME = "; INT(TS); " SEC";gosub 1880
3870 REM ***** CALCULATE VALUES FOR CLOCK *****
3875 X = 1E6 / PS; I = INT(X / SE + 0.5)
3877 IF I < 1 THEN I = 1
3878 Y = X / I - 2; Q = FNH(Y); R = Y - 256 + Q + 0.5
3880 POKE 240, I; POKE 241, R; POKE 242, 0
3886 gosub 3960
3890 PRINT "DELAY = "; CDN " SEC. NEW?"
3895 GetR$; if R$ = "N" then 3910
3896 IF R$ = "Y" THEN 3915
3900 IF R$ = "Y" THEN 3915
3910 PRINT "WT = "; WT; " NEW WASH TIME?"
3915 GetR$; if R$ = "N" THEN 3930
3920 IF R$ = "Y" THEN 3915
3925 PRINT "WASH TIME (SEC) = "; WT
3930 PRINT "SR LOAD TIME (SEC) = "; LT; " NEW?"
3935 GetR$; if R$ = "N" THEN 3950
3940 IF R$ = "Y" THEN 3935
3945 PRINT "SR LOADTIME (SEC) = "; LT
3950 RETURN
3960 REM ***** ML SETUP AND ZERO SUB
3962 IF R$ = "N" THEN 3970
3965 POKE 4, FNL(SL); POKE 5, FNH(SL); Q = USR(0)
3970 POKE 4, FNL(ZL); POKE 5, FNH(ZL); Q = USR(0)
3975 IF W3 = 0 THEN RETURN
3978 IF R$ = "N" THEN 3985
3980 POKE 4, FNL(SH); POKE 5, FNH(SH); Q = USR(0)
3985 POKE 4, FNL(ZH); POKE 5, FNH(ZH); Q = USR(0)
3988 RT = 0
3990 RETURN
4000 REM ***** MANUAL CONTROL SUBROUTINE ********************
4010 PRINT "LO OR UP I/O OR X"
4015 GetR$; if R$ = "U" then 4170
4110 IF R$ = "L" THEN 4115
4112 IF R$ = "X" THEN 4105
4113 RETURN
4115 PRINT "PB0,1,2,3,4,5,6,7,X"
4120 GetR$; if R$ = "0" then 4200
4125 IF R$ = "1" then 4220
4130 IF R$ = "2" then 4230
4135 IF R$ = "3" then 4240
4140 IF R$ = "4" then 4260
4145 IF R$ = "5" then 4280
4150 IF R$ = "6" then 4300
4155 IF R$ = "7" then 4320
4160 IF R$ = "X" then 4115
4165 goto 4100
4170 PRINT "PB0,1,2,3,4,5,X"
4175 GetR$; if R$ = "0" then 4340
218
IFR$="1"THEN4050:RETURN
4182 IFR$="2"THEN4050:RETURN
4184 IFR$="3"THEN4050:RETURN
4186 IFR$="4"THEN4050:RETURN
4188 IFR$="5"THEN4050:RETURN
4190 IFR$="X"THEN4170
4195 GOTO4100
4200 GOSUB4400:GOSUB4505:RETURN:REM *PB0*
4220 GOSUB4400:GOSUB4525:RETURN:REM *PB1*
4240 GOSUB4400:GOSUB4545:RETURN:REM *PB2*
4260 GOSUB4400:GOSUB4565:RETURN:REM *PB3*
4280 GOSUB4400:GOSUB4585:RETURN:REM *PB4*
4300 GOSUB4400:GOSUB4605:RETURN:REM *PB5*
4320 GOSUB4400:GOSUB4625:RETURN:REM *PB6*
4340 GOSUB4400:GOSUB4645:RETURN:REM *PB7*
4360 GOSUB4400:GOSUB4705:RETURN:REM *PB0*
4380 GOSUB4400:GOSUB4725:RETURN:REM *PB1*
4400 GOSUB4400:GOSUB4745:RETURN:REM *PB2*
4420 GOSUB4400:GOSUB4765:RETURN:REM *PB3*
4440 GOSUB4400:GOSUB4785:RETURN:REM *PB4*
4460 REM HIGH-LOW SUBROUTINE
4470 PRINT"HIGH OR LOW"
4480 GOSUB4445
4482 GOSUB4445
4484 X=0:RETURN
4485 X=1:RETURN
4500 REM ***** I/O LINE(LOWER PORT)HIGH-LOW SUBROUTINE*****************
4510 ONX=160TO4515,4520
4515 POKECL,PEEK(CL)AND254:RETURN
4520 POKECL,PEEK(CL)OR1:RETURN
4525 REM *** PB1***
4530 ONX=160TO4535,4540
4535 POKECL,PEEK(CL)AND253:RETURN
4540 POKECL,PEEK(CL)OR2:RETURN
4545 REM *** PB2***
4550 ONX=160TO4555,4560
4555 POKECL,PEEK(CL)AND251:RETURN
4560 POKECL,PEEK(CL)OR4:RETURN
4565 REM *** PB3***
4570 ONX=160TO4575,4580
4575 POKECL,PEEK(CL)AND247:RETURN
4580 POKECL,PEEK(CL)OR8:RETURN
4585 REM *** PB4***
4590 ONX=160TO4595,4600
4595 POKECL,PEEK(CL)AND239:RETURN
4600 POKECL,PEEK(CL)OR16:RETURN
4605 REM *** PB5***
4610 ONX=160TO4615,4620
POKE CL, PEEK (CL) AND 223: RETURN
POKE CL, PEEK (CL) OR 32: RETURN
REM *** PB6 ***
ONX+10 TO 4635, 4640
POKE CL, PEEK (CL) AND 191: RETURN
POKE CL, PEEK (CL) OR 64: RETURN
REM *** PB7 ***
ONX+10 TO 4655, 4660
POKE CL, PEEK (CL) AND 127: RETURN
POKE CL, PEEK (CL) OR 128: RETURN
REM ***** I/O LINE UPPER PORT ************
REM *** PB0 ***
ONX+16 TO 4715, 4720
POKE CH, PEEK (CH) AND 254: RETURN
POKE CH, PEEK (CH) OR 1: RETURN
REM 444 PB1 ***
ONX+16 TO 4735, 4740
POKE CH, PEEK (CH) AND 253: RETURN
POKE CH, PEEK (CH) OR 2: RETURN
REM *** PB2 ***
ONX+10 TO 4755, 4760
POKE CH, PEEK (CH) AND 251: RETURN
POKE CH, PEEK (CH) OR 4: RETURN
REM *** PB3 ***
ONX+10 TO 4775, 4780
POKE CH, PEEK (CH) AND 247: RETURN
POKE CH, PEEK (CH) OR 8: RETURN
REM 444 PB4 ***
ONX+16 TO 4795, 4800
POKE CH, PEEK (CH) AND 239: RETURN
POKE CH, PEEK (CH) OR 16: RETURN
REM 444 PB5 444
ONX+18 TO 4815, 4820
POKE CH, PEEK (CH) AND 223: RETURN
POKE CH, PEEK (CH) OR 32: RETURN
REM ***** VIEW PEAK SUBROUTINE ************
PRINT "PEAK ONE PEAK TWO X"
GETR=.; IFR$="0" THEN GOSUB 5500
IF R$="T" THEN GOSUB 5600
IF IFR$="X" THEN 5010
RETURN
REM ***** PEAK VIEWING SUBROUTINE ***********
PRINT "CURSOR: RL AB X"
POKE 4, FNH (VU): POKE 5, FNL (VU): O = USR (O)
I = PEEK (DH) OR PEEK (KY)
IF I = 759 THEN H = 1: GOTO 5170
IF I = 139 THEN H = 3: GOTO 5170
IF I = 177 THEN A = 1: PRINT "A SET": GOSUB 1800: GOTO 5010
IF I = 127 THEN B = 1: PRINT "B SET": GOSUB 1800: GOTO 5010
IF I = 0 THEN 5010
IF A1 = A2 = 0 THEN PRINT "SET LIMITS FIRST": GOSUB 1800: GOTO 5110
220
2165 GOTO5235
2170 POKE4,FNL(CU):POKE5,FNM(CU):POKE246,M:Q=USR(0)
2175 I=PEEK(255):PRINT"CURS=";I;"HT=";INT(FND(I)+0.5)
2180 GOTO5115
2185 IF1 THEN5110
2190 GOSUB1000;GOTO5170
2195 IF2 THEN5110
2200 PRINT"W=";W;"NEW LIMITS?"
2205 GETR4:IFR4="Y"THEN GOSUB1000:GOTO5170
2210 IFR4="N"THEN5205
2215 W=A+W-(1)+M:IFB>255 GOTO5225
2220 POKE255,B:GOTO5170
2222 N=N-1
2225 PRINT"OFFSCALE BY";W-255:GOSUB1000:B=255
2226 IFKP=2 THEN PRINT!"RUN NO.";N2%
2230 A2=1:GOTO5245
2235 IF I<B THEN5245
2240 PRINT"LIMITS REVERS";GOSUB1000:GOTO5110
2245 Z=0;BA=0;PA=0
2246 POKE255,A
2247 A1=0;A2=0
2250 PRINT"CALCULATING"
2255 FOR I=A-1 TO A4
2260 IF I<0 THEN Z+1: NEXTI
2265 BA=BA+FND(I): NEXTI
2270 BA=BA/(11-Z)
2275 BA=FNV(BA)
2280 W=A-PH=BA
2285 FORI=AT08
2290 PA=PA+(FNV(FND(I))-BA)
2295 IF FND(I)<PH THEN5310
2300 PH=FND(I):PK=I
2310 NEXTI
2315 PH=FNV(PH)-BA
2320 RT=(CD*10+INT(10*PK/PS+0.5))/10
2325 IFN2%=1 THEN N2%=1
2326 IFKP<1 THEN PRINT!"RUN NO.";N1%
2328 IF KP=1 THEN PRINT!"PEAK 1"
2330 IF KP=2 THEN PRINT!"PEAK 2"
2335 IF KP=3 THEN PRINT!"PK HT=";INT(100*ABS(PH)+0.5)/100*S
2339 PRINT!"PK AREA=";INT(PA/0.5)
2339 PRINT!"AT";RT;"SEC"
2340 S=FNV(BA):PRINT!"BASELINE=";INT(100*ABS(BA)+0.5)/100*S
2345 PRINT!"W=";W;"(";INT(W/PS+0.5);"SEC")"
2349 PRINT!"PNTS/SEC"
2352 X=3:GOSUB1900
2355 IFN1%>10 THEN5010
2360 IFN2%>10 THEN5010
2365 IFVP,-1 THEN5400
5380 IF KP = 2 THEN 5450
5400 A1(N1%) = PA: P1(N1%) = PH: R1(N1%) = RT
5410 N1% = N1% + 1
5415 IF N1% = 10 THEN  PRINT! "START NEW FILE"
5420 KP = 0: RETURN
5450 A2(N2%) = PA: P2(N2%) = PH: R2(N2%) = RT
5455 N2% = N2% + 1
5460 IF N2% = 10 THEN  PRINT! "START NEW FILE"
5465 KP = 0: RETURN
5500 REM ***** PEAK ONE *****
5510 PRINT "VIEW PEAK ONE": GOSUB 1800
5520 VU = VL: CU = LC: BF = 81: KP = 1
5525 PS = P1: CD = D1
5530 GOSUB 1900: RETURN
5600 REM ***** PEAK TWO *****
5610 PRINT "VIEW PEAK TWO": GOSUB 1800
5620 VU = VH: CU = HC: BF = 82: KP = 2
5625 PS = P2: CD = D2
5630 GOSUB 1900: RETURN
DATA ACQUISITION AND DISPLAY ROUTINE FOR PEAK ONE

```
4000 48 PHA
4001 A0 LDA A004
4004 C6 DEC FA
4006 68 PLA
4007 40 RTI
4008 A9 LDA #C0
400A 05 STA F3
400C A9 LDA #50
400E A2 LDS #51
4010 05 STA F5
4012 86 STX F9
4014 80 LDA #80
4016 85 STA F4
4018 86 STX F9
401A 20 JSR 4051
401E 58 CLI
4022 4C JMP 4027
4023 A9 LDA #11
4025 05 STA FA
4027 20 JSR 4048
4028 20 JSR 4046
402A 00 LDV #00
402C A9 LDA #50
4031 85 STA FA
4033 A9 LDA #31
4035 85 STA FC
4037 20 JSR 409C
4039 20 JSR 4048
403B 20 JSR 4040
4040 BC LDA #FFF
4043 00 TYA
4044 E8 BEE 4020
4046 76 SEI
4047 60 RTS
4049 20 JSR 4066
404B CE DEC 4074
404D 00 BNE 4048
4050 60 RTS
4051 A5 LDA F3
4053 80 STA A00E
4056 A9 LDA #40
4058 80 STA A008
405A A5 LDA F1
405D 8D STA A004
4060 A5 LDA F2
4062 80 STA A005
4065 60 RTS
4066 A5 LDA FA
4068 FB BEQ 406E
406A 68 RTS
406B A5 LDA F0
406D 85 STA FA
406F A2 LDS #01
4071 BE STX A001
4074 CA DEY
4075 BC STX A002
4079 A9 LDA #EB
407A 8D STA A00C
407D A0 LDA A008
4088 B1 STA (F4,X)
408B A9 LDA #09
408D 8D STA A001
408F B0 ORA A000
408F A1 STA (FB,X)
4090 BC EB INX
4093 BE STX A001
4095 E6 INC F4
4097 E6 INC F6
4094 D8 BNE 4096
4095 60 PLA
4097 60 PLA
4099 60 PLA
409A 76 SEI
409B 68 RTS
409C 20 JSR 4081
409F CB INV
40A0 8B BNE 409C
40A2 BC STV A001
40A5 68 RTS
40A6 A9 LDA #FF
40A8 8D STA A002
40AA A9 LDA #CA
40A8 8D STA A00C
40B0 60 RTS
40B1 B1 LDA (FD),Y
40B3 8D STA A008
40B5 B1 LDA (FB),Y
40B8 8D STA A001
40B9 A9 LDA #CC
40BC 8D STA A00C
40C0 A9 LDA #CE
40C2 8D STA A00C
40C5 A9 LDA #CC
40C7 8D STA A00C
40CA 68 RTS
40CA A5 LDA #F0
40CC 85 STA FE
40CF A2 LDS #01
40D1 A9 LDA #01
40D3 A0 LDS #00
40D5 91 STA (FD),Y
40D7 CB INV
40D8 8B BNE 40D5
40DA 8E INC FE
40DC 8A DEC
40DD 8D BNE 40D5
40DF 6B RTS
40E0 A5 LDA #FF
40E2 8D STA #03
40E5 A9 LDA #08
40E7 8D STA #400
40F0 6B RTS
40F5 A9 LDA #7D
40F7 85 STA FF
40F9 68 RTS
40FA 84 LDA FF
40F0 CA R5 LDA #0D
40F2 39 AND 5100,Y
40F3 99 STA 5108,Y
40F4 99 STA 5108,Y
40F5 8E0 410F
40F6 DEY
40F7 CPY 1FF
40F8 8E0 4114
40F9 39 AND 5100,Y
40FA 84 STY FF
40FB 60 RTS
40FC 80 STV A001
40FF 84 STY FF
4100 60 RTS
```

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DATA ACQUISITION AND DISPLAY ROUTINE FOR PEAK TWO

4200 A8 PRA
4201 A0 LDA A004
4204 C6 DEC FA
4206 68 PLA
4207 40 RTI
4208 A9 LDA #C0
420A 85 STA F3
420C A9 LDA #52
420E A2 LDX #52
4210 85 STA F5
4212 86 STX F9
4214 A9 LDA #06
4216 85 STA F4
4218 85 STA F0
421A 85 STA FA
421C 20 JSR 4251
421F 50 CLI
4220 4C JMP 4227
4222 A9 LDA #11
4224 85 STA F8
4226 20 JSR 4248
4228 20 JSR 4246
422A 4F LDA #0B
422C 4C STY $FF
422D 90 STY #FF
422F A9 LDA #FF
4231 85 STA FE
4233 A9 LDA #53
4235 85 STA FC
4237 20 JSR 429C
4239 20 JSR 4248
423D 20 JSR ED00
423F 9C LDX #FF
4241 99 TYA
4243 F8 BEQ 422D
4246 78 SEI
4248 68 RTS
424A 2C LSR #00
424C 4E DEC #FF
424E 9E BNE 4248
4250 68 RTS
4251 A5 LDA F3
4253 8D STA #08
4256 A9 LDA #48
4258 6D STA #00
425A A5 LDA F1
425C 8D STA #04
4260 A5 LDA F2
4262 8D STA #00
4265 60 RTS
4266 A5 LDA FA
4268 6B BEQ 426B
426A 68 RTS
426B A5 LDA FA
426D 65 STA FA
426F 42 LDX #01
4271 8E STX A001
4274 8E DEX
4275 8E STX A002
4278 A9 LDA #ED
427A 8D STA A08C
427D AD LDA A000
427D 81 STA (F4,X)
4280 B1 STA (<F4,X>)
4282 A9 LDA #09
4284 8D STA A081
4287 8D DRA A080
428A B1 STA (<F8,X>)
428C EB INX
428D 8E STX A081
428F 8E INC F4
4292 8E INC F0
4294 8D BNE 42A6
4296 68 PLA
4297 68 PLA
4298 68 PLA
4299 68 PLA
429A 78 SEI
429C 28 JSR 42B1
429E 78 SEI
429F 68 RTS
42A0 8D BNE 429C
42A2 8C STY A001
42A5 60 RTS
42A6 8D BNE 429C
42A8 A9 LDA #08
42A9 8D STA A082
42AD 8D STA A00C
42B0 60 RTS
42B1 B1 LDA (<FD>,Y)
42B3 8D STA A000
42B5 8D BNE 42B1
42B7 8D STA A001
42B9 A9 LDA #CC
42BB 8D STA A00C
42BC 8D STA A08C
42BE 8D STA A00B
42C0 A9 LDA #CE
42C2 8D STA A08C
42C4 A9 LDA #CC
42C6 8D STA A00C
42C8 60 RTS
42CB A5 LDA #52
42CD 85 STA FE
42CF A2 LDS #02
42D1 A6 LDA #01
42D3 A6 LDY #00
42D5 91 STA (<FD>,Y)
42D7 CB INY
42DB 8D BNE 42D5
42DA 8D INE FE
42DC CA DEX
42DD DB BNE 42D5
42DF 60 RTS
42E0 A9 LDA #FF
42E2 8D STA A083
42E3 A9 LDA #00
42E5 8D STA A080
42EB A9 LDA #48
42EC BD STA A481
42EF A9 LDA #06
42F1 85 STA FE
42F3 85 STA FD
42F5 A9 LDA #7D
42F7 85 STA FF
42F9 68 RTS
42FA 8D STA #FF
42FC A9 LDA #FD
42FE 39 AND 5388,Y
4301 99 TYA
4304 A5 LDA F6
4306 E8 BEO 430F
430B CB CPY #00
430E 98 BEQ 4314
4311 FD BEO 4314
4313 CB INY
4314 A9 LDA #02
4316 1C PLA 5388,Y
4319 99 LDA 5388,Y
431C 84 STY #FF
431E 60 RTS
COUNTDOWN CLOCK ROUTINE

4120 A9 LDA #E0
4122 8D STA 620B
4125 A9 LDA #8A
4127 8D STA 6208
412A A9 LDA #00
412C 8D STA 6209
412F A9 LDA #C2
4131 8D STA 6204
4134 A9 LDA #B0
4136 8D STA 6205
4139 A9 LDA #20
413B 2C BIT 620D
413E F0 BNE 413B
4140 A9 LDA #0A
4142 8D STA 6208
4145 A9 LDA #00
4147 8D STA 6209
414A 60 RTS
APPENDIX III

SUPPLEMENTARY DATA
The supplementary data from studies with the automated on-line ion exchange trace enrichment system described in Chapter 3 are shown in Figure III.1 to Figure III.9. The supplementary data from studies with the automated two-column ion exchange system described in Chapter 4 are shown in Figure III.10 to Figure III.14. The experimental conditions used to obtain these data are identical to those discussed in Chapter 3 and 4 unless specified otherwise.
Figure III.1. Effect of Solution pH on the Retention of Cu$^{2+}$ Ion by the Chelex-100 Column.

Sample: 600 µg/L Cu$^{2+}$. 
Figure III.2. Multiple Injection Operation Using a 1-mL Sample Loop.

Sample: 50 μg/L Cu$^{2+}$.
Figure III.3. Multiple Injection Operation Using a 10-mL Sample Loop.
Sample: 5.0 μg/L Cu$^{2+}$.
Figure III.4. Calibration Curves for Cu with the Automated On-line Ion Exchange Trace Enrichment System using a 1-mL Sample Loop and the Flame AA Spectrophotometer.
Figure III.5. Calibration Curve for Cu with the Automated On-line Ion Exchange Trace Enrichment System using a 10-mL Sample Loop.
Figure III.6. Calibration Curves for Cd with the Automated On-line Ion Exchange Trace Enrichment System using a 1-mL Sample Loop and the Flame AA Spectrophotometer.
Figure III.7. Calibration Curve for Cd with the Automated On-line Ion Exchange Trace Enrichment System using a 10-mL Sample Loop.
Figure III.8. Calibration Curves for Mn with the Automated On-line Ion Exchange Trace Enrichment System using a 1-mL Sample Loop and the Flame AA Spectrophotometer.
Figure III.9. Calibration Curve for Mn with the Automated On-line Ion Exchange Trace Enrichment System using a 10-mL Sample Loop.
Figure III.10. Effect of the EDTA Concentration on the Speciation of Cu(II)-EDTA Solutions.

Sample: 3.2 μM Cu(II) at pH 7.
Figure III.11. Effect of the Cu(II) Concentration on the Speciation of Cu(II)-EDTA Solutions.

Sample: 3.0 μM EDTA at pH 7.
Figure III.12. Calibration Curve for Cu(II) with the Automated Two-column Ion Exchange System using a 10-mL Sample Loop.
Figure III.13. Calibration Curve for Cd(II) with the Automated Two-column Ion Exchange System using a 10-mL Sample Loop.
Figure III.14. Calibration Curve for Zn(II) with the Automated Two-column Ion Exchange System using a 10-mL Sample Loop.
Table III.1  Calculated Concentrations of CuL at the First Three Titration Points in the Determination of Cu(II) Complexing Capacity and Conditional Stability Constants of Ligands in Water Samples$^a$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$c_{Cu}^b$</th>
<th>$[\text{CuL}]_1^c$</th>
<th>$[\text{CuL}]_2^d$</th>
<th>$[\text{CuL}]_3^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 μM NTA</td>
<td>1.26</td>
<td>1.26</td>
<td>1.10</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>2.52</td>
<td>2.51</td>
<td>2.06</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>5.04</td>
<td>3.69</td>
<td>3.11</td>
<td>3.07</td>
</tr>
<tr>
<td>4.0 mg/L HA</td>
<td>1.26</td>
<td>1.24</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>2.52</td>
<td>2.45</td>
<td>0.86</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>5.04</td>
<td>3.61</td>
<td>1.46</td>
<td>1.25</td>
</tr>
<tr>
<td>River Water</td>
<td>1.26</td>
<td>1.18</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2.52</td>
<td>2.26</td>
<td>0.29</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>5.04</td>
<td>3.37</td>
<td>0.54</td>
<td>0.60</td>
</tr>
</tbody>
</table>

$^a$ see Chapter 5 for more information. The concentrations are in μM.

$^b$ $c_{Cu}$ is the total Cu(II) concentration.

$^c$ $[\text{CuL}]_1$ is the concentration of CuL in the original sample solution calculated using the experimentally determined values of the Cu(II) complexing capacity and conditional stability constant of ligands. The Cu(II) complexing capacity used in the calculations was 3.7 μM. The log conditional stability constants used were 8.64 for the NTA solution, 7.46 for the HA solution, and 6.78 for the Willamette River water sample.

$^d$ $[\text{CuL}]_2$ is the concentration of CuL in the solution that passes through the Chelex-100 column calculated using the experimentally determined values of the Cu(II) complexing capacity and conditional stability constant of ligands and the retention efficiency of the Chelex-100 column. The retention efficiency of the Chelex-100 column used was 160.

$^e$ $[\text{CuL}]_3$ is the concentration of CuL determined experimentally.