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APPLICATION OF NAFION MEMBRANES FOR METAL PRECONCENTRATION AND SPECIATION

BY

Charles L. Bourque

Submitted in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in CHEMISTRY at Dalhousie University, Halifax, Nova Scotia, Canada, July, 1988.

This thesis is dedicated to my wife and best friend, Claudette, whose help, encouragement and love during this four year period proved to be of invaluable assistance.

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Abstract

Photometric and electrochemical modules were characterized as possible metal ion detectors after ion-exchange separations. The strong-acid, high performance resin (Aminex A-9) was suitable for separation of Cu, Zn, Ni, Pb, Co and Cd in 18 minutes with a tartrate eluent. The same resin and a citric acid eluent could determine Cd, Mn, Ca and Mg in 15 minutes. A simple tungsten lamp - 520 nm interference filter-phototransistor detector could determine these metals using 4-(2-pyridylazo)resorcinol reagent at the 0.05 to 0.10 nmole level using a 1 mL injection. A Donnan dialysis preconcentration using 0.20 M tartaric acid as receiver electrolyte and a Nafion cation-exchange membrane gave a 68-fold preconcentration in 30 minutes using a 200 mL sample to 2 mL receiver volume ratio. High ionic strengths and dissolved organic matter were found to lower preconcentration factors. The Nafion membrane was found to be useful for metal speciation when sample and receiver electrolytes had the same ionic strength (0.30M $NaNO_3$). The membrane is permeable to free cations, excludes anions, and can size exclude large neutral and cationic species.

List of Abbreviations and Symbols

1- ASV - anodic stripping voltammetry

2- BAS-GCE - Bioanalytical Services glassy carbon electrode

3- BPC - bonded - phase chromatography

4- BTB - bromothymol blue

5- CA - citric acid

6- DDTC - diethyldithiocarbamate

7- DME - dropping mercury electrode

8- DOM - dissolved organic matter

9- DPP - differential pulse polarography

10- DTPA - diethylenetriaminepentaacetic acid

11- Dz - dithizone

12- E_h - redox potential

13- EC - electrochemical

14- EDTA - ethylenediaminetetraacetic acid

15- EF - enrichment factor

16- EGTA - ethyleneglycol bis(2-aminoethylether) tetracetic acid

17- FA - fulvic acid

18- FIA - flow injection analysis

19- GCE - glassy carbon electrode

20- GFC - gel filtration chromatography

21- HA - humic acid

22- HMDE - hanging mercury drop electrode

23- HPLC - high performance liquid chromatography

24- HPLC/PD - ion-exchange separation/system 2 photometric detection

25- ICP - inductively coupled plasma

26- IEC - ion-exchange chromatography

27- IPC - ion-pair chromatography

28- ISE - ion selective electrode

29- LC - liquid chromatography

30- LED - light-emitting diode

31- LLC - liquid-liquid chromatography

32- LSC - liquid-solid chromatography

33- NTA - nitrilotriacetic acid

34- PAR - 4-(2-pyridylazo) resorcinol

35- PP - pulse polarography

36- PS-DVB - polystyrene - divinylbenzene

37- SiP - silicon phototransistor

38- UF - ultrafiltration

39- %D - percent dialysis

40- α - alpha coefficient (Ringbom, 1979, p 38)

41- β - stability product

42- δ - interaction intensity parameter

43- ε - molar absorptivity

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The charm of knowledge would be small if so much shame did not have to be overcome on the road to it.

Nietzsche

CHAPTER 1

1.0 Introduction

Government regulations on water quality are usually expressed in terms of total metal concentrations. A recommended total metal level in drinking water is 5 ppb for mercury and 1000 ppb for copper (Burrell, 1974). The total metal concentration can be determined by atomic absorption, atomic emission using inductively coupled plasmas, neutron activation analysis, and (after appropriate sample treatment) anodic stripping voltammetry. The metal ions in a natural water sample, however, will be present in a number of physicochemical forms.

This distribution of metal species is illustrated in Figure 1-1. Metal ions in natural waters can be present as truly dissolved complexes (for example, aquo and hydroxy complexes, chloro complexes, and complexes with dissolved organic matter, DOM), adsorbed onto colloidal humic matter and hydrous metal oxides, and adsorbed onto suspended material. Analytical speciation, as defined by Florence (1982), is the determination of the individual physicochemical forms of the element present in the sample. The distribution of a metal among the possible species depends on the total composition and history of the water sample

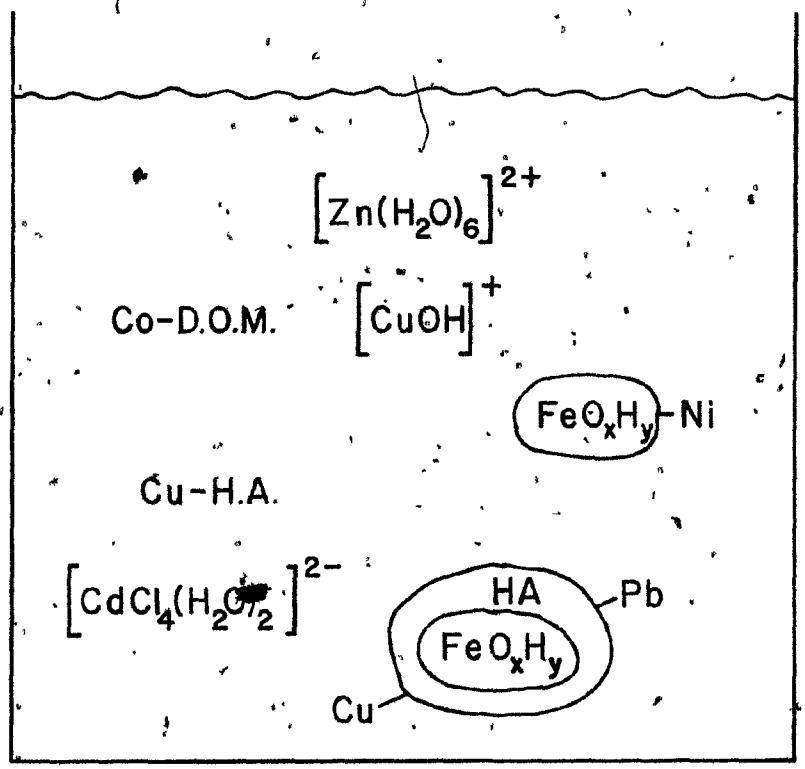


Fig. I-1: Typical metal species present in an unfiltered natural water sample.

and may be a better water quality parameter than total metal concentrations. The importance of analytical speciation results relates to the role of species distribution in environmental toxicology and metal ion transport in aquatic systems.

1.1 Toxicology

All substances, including water and sodium chloride, are toxic if present in large enough amounts. Certain elements are considered essential for life: Fe, Ca, Na, K, Mn, Zn, Cu, Cr, F, Ni, Mg, Mo, Co, V, Se and Sr (Schroeder and Darrow, 1973). Lead, cadmium and mercury are considered toxic. The essential elements are also toxic when present above certain levels. For example, selenium is considered essential but the recommended concentration limit in water is 0.01 ppm (Brooks, 1977). Copper, although not listed as toxic to humans, is known to be particularly toxic to aquatic species. Steemann Nielsen and Wium-Andersen (1970), for example, have suggested that the low level of copper in the marine environment controls the growth of marine algae. The concentration range over which the metal satisfies the requirements for essentiality and nontoxicity can be quite narrow. This narrow concentration range and the possible role of chemical speciation

in metal ion distribution complicates the definition of acceptable water quality parameters.

The toxicity of trace metals is very dependent on their chemical form. It is generally accepted that the free hydrated metal ion is the species most toxic to aquatic life (Florence and Batley, 1980). The role of copper speciation in aquatic toxicity has been studied extensively. Wagemann and Barica (1979) have suggested that in addition to Cu^{2+}, the species $CuOH^{+}$ and $Cu(OH)_2^{o}$ may also be toxic to algae. Guy and Kean (1980) have presented evidence that suggests that complexes with ethylenediamine and citric acid ($Cuen^{2+}$ and $CuCitOH^{2-}$) may also be toxic to algae. Both reports are based on indirect evidence using computer calculated speciation and not direct analytical determination of species. It is possible, for example, that the apparent species toxicity may be a result of poor equilibrium constant data.

Strong metal complexes with aminopolycarboxylate ligands and metal ions adsorbed onto particulate or colloidal matter are usually not toxic. Poldoski (1979) reported that humic and aminopolycarboxylic acids decreased cadmium uptake by Daphnia magna but diethyldithiocarbamate increased uptake of cadmium. Albert (1971) has found that 8-hydroxyquinoline augments the toxic effects of metals

towards bacteria through chelation. Ligands may enhance the toxicity of metal ions by forming species that are more soluble in the biological membranes and hence increase transport of the toxic metal ion into the cell.

Organisms can also determine to some extent the forms of heavy metals to which they are exposed. Bacteria are known to detoxify their environment either by transformation of species to less toxic forms or by immobilization after uptake of the metal (Sterritt and Lester, 1980). Johnson (1978) has reported that phytoplankton blooms can affect the As(III)/As(V) distribution. It is evident, therefore, that biological systems can alter the chemical speciation of an element. It is important to develop an analytical speciation method suitable for monitoring chemical speciation changes induced by biological activity before one can unequivocally relate speciation and toxicity.

1.2 Metal Transport

The metal ion species illustrated in Figure 1-1 aid in the transport of metals from the terrestial and sediment compartments to the marine environment. River, stream and run-off waters can carry soil and sediment derived particulates into lakes and estuaries. One can envision four

water quality changes that may affect metal ion transport through species redistribution: changes in pH, salinity, dissolved organic matter and E_h. Adsorbed metal ions on clay particulates, humic colloids and metal hydroxy colloids interact via a combination of ion-exchange and complexation reactions. The humic colloids are capable of binding metal ions using salicylic and phthalic acid groups and the free/bound distribution is pH dependent (Guy and Chakrabarti, 1976a). The charges of hydroxy colloids of iron and manganese are controlled by the pH of the solution. Ferric colloids, for example, have a zero point charge at pH between 5 and 6. Cation adsorption onto the iron colloids is enhanced considerably above pH 6 because of the negative charge of the colloid. The clay and hydroxy metal colloids interact with metal cations via ion-exchange. Hence an increase in salinity favors desorption of the metal ions. A second effect of changes in salinity is to compress the double layer and enhance coagulation of the colloids. Humic acid and iron or manganese hydroxy colloids are known to precipitate in the fresh water - seawater mixing zones and the metal ions are deposited in the sediment (Lee, 1974).

The addition of dissolved organic matter to the aquatic environment can increase metal transport both by

chelation and by change in E_h. The ion-exchange equilibrium:

$$\text{clay-M} + n\text{Na}^+ \rightleftharpoons \text{clay-Na}_n + \text{M}^{n+} \qquad (1\text{-}1)$$

will shift to the right if a complexing ligand is present to bind the free M^{n+}. Guy and Chakrabarti (1976a) have found that tannic acid could solubilize copper from bentonite. Hydrous manganese dioxide and ferric hydroxy colloids can be solubilized by reduction and chelation with dissolved organic matter. Baker (1973, 1978) has illustrated the effect of dissolved organic matter on the solubilization of minerals using humic acid.

The species distribution of a metal ion in natural waters will be dependent on the sample pH, ionic strength and complexing agents present. A characterization of the water sample should include potential distribution changes as a function of pH and ionic strength. This means that the analytical speciation should not be a simple free versus bound measurement but a map of free metal ion as a function of pH, ionic strength and E_h. The complexity of the species distribution illustrated in Figure 1-1 and the dependence of the distribution on pH and ionic strength will limit the operations available for use in analytical speciation techniques.

1.3 Speciation Methods

Analytical chemical speciation problems can be divided into three groups:

Group 1: differentiation between covalently bonded organometallic compounds, e.g., CH_3HgCl vs $(CH_3)_2Hg$ or ϕ_3Sn^+ vs ϕ_2Sn^{2+}, etc.

Group 2: differentiation between oxidation states of metal ions, e.g., Fe(II) vs Fe(III) or Cr(III) vs Cr(VI).

Group 3: differentiation between free and bound forms of simple cations, e.g., Cu^{2+} vs $CuCO_3$ vs $CuOH^+$ vs Cu-humic acid, etc.

Group 1 and 2 speciation type problems can be solved using chromatographic and electrochemical methods because of the well-defined nature of the species. The Group 3 speciation problem, however, is considerably more difficult. The humic acid and metal hydroxy colloids are poorly defined and tend to be aggregates of smaller species. The free metal ion is in equilibrium with simple complexes (e.g., $CuCO_3$ and Cu-citrate) but it is possible that the adsorption reactions with the colloidal species are not in equilibrium. The speciation methods developed to date for Group 3 systems are based on operational procedures - the analytical data obtained can only be

useful if the assumptions behind them are completely understood.

1.3.a. Computer modelling: Computer calculated speciation studies have been reported at two levels of difficulty. The first level assumes the concentration of metal ions, inorganic and organic ligands, pH and E_h values, and calculates the equilibrium distribution using known equilibrium constants corrected for temperature and ionic strength. The E_h value is used to calculate the oxidation states of important species (e.g., SO_4^{2-} vs S^{2-}, Fe(II) vs Fe(III)), and the pH and equilibrium constants are used to determine the number of solid species (metal sulfides, metal carbonates, etc.) and the concentration of each soluble species. The speciation obtained depends on the choice of suitable equilibrium constant data. The results in Table 1-1 illustrate this dependence on choice of equilibrium constant data.

A second level of computer calculated speciation is to couple the equilibrium calculations for soluble species with empirical adsorption isotherms onto clays and hydrous metal oxides. Benjamin and Leckie (1981, 1982) have used such a model to study the adsorption of cadmium onto oxide surfaces. A comparison of model calculations and

Table 1-1*

Effect of equilibrium constants on calculated speciation

	% in seawater		
Zn species	Ref 1	Ref 2	Ref 3
Zn^{2+}	14.1	17.2	26.6
Zn-chloro	79.5	10.6	47.0
$ZnSO_4^o$	1.7	3.5	4.3
$ZnOH^+$	0.9	0.2	4.4
$Zn(OH)_2^o$	0.9	62.8	-
$ZnHCO_3^+$	3.8	0.7	1.0
$ZnCO_3^o$	3.8	5.0	16.7

* - Florence and Batley (1980).

experimental results suggested that adsorption of metal complexes was possible. The chloro and sulphate complexes adsorbed less strongly than the free metal cation. The net effect was to decrease the fractional amount of cadmium adsorbed. Thiosulfate, however, adsorbed onto γ-Al_2O_3 and Fe_2O_3 and enhanced the adsorption of cadmium. The authors used the comparison of calculated speciation and experiment to suggest that $CdCl_x$ and $Cd(SO_4)_x$ adsorbed with the metal closer to the surface and $Cd(S_2O_3)_x$ with the ligand closer to the oxide surface.

Lerman and Childs (1973) incorporated a Freundlich isotherm into the computer calculated speciation. The computer model was applied to a consideration of a well-mixed reservoir with a constant rate of input of metal pollutant. The model suggested that the immediate effect of equilibrium ion exchange or adsorption was to lower the concentration of metal in solution but does not affect the steady-state concentration of pollutant (i.e., eventually the adsorbent is effectively saturated).

Computer modelling of speciation has several potential applications. The first is the characterization of the complex chemical interactions in multication and multi-ligand systems. Morel et al (1973) have used a level 1 type of program to illustrate the "interaction intensity

parameters" (defined as $\delta_{x,y} = \partial p[x]/\partial p[y]_T$) when one varies one component in a multicomponent system. The computer study suggested that organic complexing material plays a very important role in speciation. In a purely inorganic system, cadmium was buffered by precipitation of $CdCO_3$ ($\delta_{Cd,Cd} = 10^{-3}$) but in the presence of NTA $\delta_{Cd,Cd} = 1.1$. Also, in the complexing media, transition metal interactions were increased: for example $\delta_{Cd,Cu} = 0.13$ vs $\delta_{Cd,Cu} = 5.0 \times 10^{-4}$ in the inorganic system. This computer model study indicates that multication speciation techniques are necessary for a complete understanding of metal interactions in a multicomponent system.

MacCarthy and Smith (1979) have used computer calculations (again level 1) for simple model systems to illustrate species distribution during a complexing capacity experiment in a multiligand system. The principal implication of the study was that for multiligand systems it was impossible to determine a unique binding constant for the system. The binding constant that one would determine analytically would be a weighted average of the binding constants for each individual species. This is important when one realizes that the humic substances in natural waters can act as a multiligand system.

A second application of computer modelling is the development and characterization of simple chemical systems for the study of the relationship between speciation and toxicity. The computer calculated equilibrium distribution for a system containing the metal of interest (e.g., Cu^{2+}), nutrient (NO_3^-, PO_4^{3-}, Ca^{2+}, Mg^{2+}, CO_3^{2-}), and complexing ligands (Cl^-, OH^-, EDTA, NTA, etc.) is used to correlate the species present with the behavior of the biological system (Wagemann and Barica, 1979; Guy and Kean, 1980). As indicated earlier in this chapter, the comparative results suggest that it is the free metal ion that is toxic to aquatic species.

1.3.b. Ion selective electrodes (ISE): It is well known that ion selective electrodes respond to the activity of the free hydrated metal ions (Ross, 1969). Metals bound to ligands or adsorbed onto colloids and particulates in principle are not measured. These electrodes, therefore, offer the unique ability of measuring free metal activities without altering the equilibria in any way. An example of such use is the work of Gardiner (1974). He studied cadmium complexation in the presence of various ligands (OH^-,Cl^-,CO_3^{2-}, SO_4^{2-}, HA) at environmental concentrations. The results indicated that a substantial fraction of the

total cadmium was in the free form (e.g., 35-92%) and that humic acid complexation accounted for most of the bound fraction (e.g., 12-39%). These electrodes, however, are not reliable below 10^{-6} M and are limited to use on contaminated waters or on model systems. For example, Gardiner reported departure from linearity at 4.5×10^{-6} M cadmium and at 10^{-7} M cadmium the electrode ceased to respond. Florence (1982) has noted that the copper ISE may be responding to species other than copper ion, e.g., hydroxo and bicarbonato complexes of copper, natural and synthetic complexing agents.

1.3.c. Anodic stripping voltammetry (ASV): The most widely used of all speciation techniques is anodic stripping voltammetry. When used in conjunction with the pulse mode (i.e., differential pulse polarography, DPP), it offers the advantage of having sufficient sensitivity (10^{-9} - 10^{-10} M) for the direct analysis of Cu, Cd, Pb and Zn in natural waters (Batley and Florence, 1974; Nurnberg, 1979; Florence, 1980a). The ASV experiment consists of two steps: a deposition step in which the metal ions in solution are reduced to form an amalgam in the mercury drop (HMDE) or film and a stripping step in which the metals are

oxidized out of the amalgam. The stripping current is proportional to the amount of metal ion reduced.

Davison (1978) has considered the relationship between the measured reduction current and the species in solution. The system of interest was an electroactive species M_a in equilibrium with an electroinactive species M_i:

$$L + M_a \underset{k_b}{\overset{k_f}{\rightleftharpoons}} M_i \qquad K = \frac{k_f}{k_b} \qquad (1\text{-}2)$$

$$M_a \xrightarrow{ne^-} M(Hg)$$

In an ASV experiment, the solution is stirred and the associated current, $(i_d)_s$, is convective diffusion limited:

$$(i_d)_s = \frac{nFADc}{\delta} \qquad (1\text{-}3)$$

where n is the number of electrons involved in the electrochemical reaction, F is Faraday's number, A is the surface area of the mercury drop or film, D is the diffusion coefficent of the electroactive species, c is its concentration and δ is the Nernst diffusion layer. A second contribution to the current is caused by the dissociation of M_i and this contribution is given by $(i_k)_s$ where:

$$(i_k)_s = \frac{nFADc}{\delta + \frac{D^{\frac{1}{2}}[L]K^{3/2}}{k_f^{\frac{1}{2}}(1+K[L])^{\frac{1}{2}}}} \qquad (1\text{-}4)$$

Davison assumes that the species M_i will contribute significantly if:

$$\frac{(i_k)_s}{(i_d)_s} \geq 0.1 \qquad (1\text{-}5)$$

When (1-3) and (1-4) are substituted into (1-5), one obtains:

$$\frac{K^{3/2}[L]}{k_f^{\frac{1}{2}}(1+K[L])^{\frac{1}{2}}} = \frac{9\delta}{D^{\frac{1}{2}}} \qquad (1\text{-}6)$$

Using Cu^{2+} as an example: $k_f^{max} = 10^9$ $mol^{-1}Ls^{-1}$, $D = 10^{-5}cm^2s^{-1}$, $\delta = 10^{-3}$ cm and a typical ligand concentration of 1×10^{-6} M, then a limiting value of 9×10^7 is obtained for K. Above this value, the species would be "nonlabile". This treatment agreed with the experimental results of Chau et al (1974).

Davison (1978) also considered the case of transient measuring techniques such as pulse polarography (PP) and differential pulse polarography (DPP). In differential pulse polarography, the controlling factor is the

measurement time given by the duration of the pulse and the sampling interval. This is usually between 1 and 100 msec. In ASV, the controlling factor is the convective diffusion layer δ and not the electrolysis time. An estimate of the effective measuring time in ASV can be obtained by equating the current in the steady state case (for convective diffusion) to the time necessary for the transient current to attain this value:

$$\begin{aligned} (i_d)_s &= (i_d) \\ \frac{nFADc}{\delta} &= \frac{nFADc}{(\pi Dt)^{\frac{1}{2}}} \\ \delta &= (\pi Dt)^{\frac{1}{2}} \end{aligned} \qquad (1\text{-}7)$$

where t is the time of measurement. For the case cited above for Cu^{2+}, the value of t would be 32 msec.

It is evident from Davison's treatment of anodic stripping voltammetry that the species measured depends on a number of operational parameters. The applied deposition potential defines the electroactive species and the stirring rate controls δ. Equation (1-6) defines the species measured in terms of the free ligand concentration and the complex stability constant. The experimental results of Chau et al (1974) have shown that at pH 7.0 and typical operating conditions for copper with

$[L] = 1.5 \times 10^{-6}$ M, tartrate, glycine and citrate species were labile whereas NTA and EDTA species were nonlabile.

There are, however, some important limitations when using ASV in natural waters. Organic compounds are reported to influence results either by adsorbing onto the mercury surface and altering peak currents and peak potentials or by causing tensammetric waves, i.e., currents due to adsorption-desorption processes and not redox reactions (Florence, 1982). Ernst et al (1975) applied DPASV to the determination of copper and lead stability constants with various ligands. They reported that the method could not be applied to humic acid due to its adsorption onto the mercury drop. Brezonik et al (1976) reported that a large number of organic compounds found in natural waters (e.g., proteins, fats, oils, detergents, polysaccharides and organophosphates) may sorb onto the HMDE surface and thus lead to erroneous interpretations of speciation data. Buffle et al (1976) reviewed the various voltammetric techniques used in speciation and suggested that disagreement in the interpretation of results between various authors may be due to under-estimation of the role of adsorption phenomena. Possible interferences from organics must therefore be carefully evaluated when applying DPASV

and other high-frequency voltammetric methods to the analysis of natural waters.

1.3.d. Ion-exchange techniques: Two ion-exchange techniques have been used for trace metal speciation in natural waters. Cantwell et al (1982) have used a cation-exchange column to determine free nickel in sewage. The sample is passed through a short column until total nickel in the column effluent is equal to total nickel in the sample. If the concentration of Na^+ is the same in all samples, if the capacity of the resin is much greater than the metal adsorbed, and if the only species adsorbed is the free cation, then:

$$[M^{n+}] = \frac{[R_nM]}{\lambda_o} \qquad (1\text{-}8)$$

where $[M^{n+}]$ is the free metal concentration, $[R_nM]$ is the amount of metal adsorbed onto the resin and λ_o is the distribution coefficient for the free cation. The metal on the resin is eluted and determined by atomic absorption.

A second exchange method uses a complexing resin (i.e., Chelex-100) to determine a labile fraction. Figura and McDuffie (1979) used a calcium saturated resin (1.3 g resin, 2-3 mL flow rate) with a sample contact time of 6-9 seconds. The procedure should determine as "labile":

(i) free metal ion that reacts with the iminodiacetate functional groups.

(ii) the fraction of metal species that dissociates during the contact time.

A comparison by Figura and McDuffie (1979) of DPASV and the Chelex-100 method suggests that the Chelex-100 method will measure a larger fraction of the metal. DPASV, for example, gave 50% and 0% labile copper in 5×10^{-5} M EDTA and 3.5 ppm humic acid, respectively. Chelex-100 gave recoveries of 61% and 54% for the same samples. The measurement time for the DPASV method was estimated to be 2×10^{-3} seconds (cf section 1.3.c).

1.3.e. Size separations: Chemical speciation can also be described in terms of the size distribution of the various physicochemical forms present in a water system. An example of a size classification as applied to metals is shown in Table 1-2. This speciation approach often aims at separating a small and therefore potentially bioavailable fraction from larger forms which are generally considered not to interact with biota. Size separations have been reported using gel filtration chromatography (GFC), ultrafiltration (UF) and dialysis.

In gel filtration or size exclusion chromatography, species having small enough diameters to penetrate the resin pores will be retained whereas larger forms will be

Table 1-2*

Metal speciation according to size

Species	Example	Approx. diameter (nm)
1 - Simple hydrated metal ions	$Cd(H_2O)_6^{2+}$	0.8
2 - Simple inorganic complexes	$Pb(H_2O)_4Cl_2$	1
3 - Simple organic complexes	Cu-glycinate	1-2
4 - Stable inorganic complexes	PbS, $ZnCO_3$	1-2
5 - Stable organic complexes	Cu-fulvate	2-4
6 - Adsorbed on inorganic colloids	$Cu\text{-}FeO_xH_y$	10-500
7 - Adsorbed on organic colloids	Cu-humic acid	10-500
8 - Particulate	Retained by 0.45 µm filter	>450

* - Florence (1982)

excluded and will therefore not be retained. The smaller species able to penetrate the gel particles do so to varying degrees depending on their size and shape. Species are therefore eluted in the order of decreasing molecular size. Effective size separations are dependent on resin swelling and are described in terms of fractionation ranges. Commercial packings available offer a wide choice of fractionation ranges, e.g., 0-700 increasing to 1,000-200,000.

Gel filtration chromatography as applied to metal speciation suffers from a few significant limitations. For example, both Fe(III) and humic acid are known to interact with Sephadex gels (Guy and Chakrabarti, 1976b). Chromatographic dilution may also be substantial, a major concern when analyzing natural water samples containing very low levels of trace metals (Florence, 1982).

Ultrafiltration separates species according to their ability to pass through a membrane of particular pore diameter. The method offers the possibility of separating molecular from colloidal species and a further size fractionation of colloidal substances. Ultrafiltration membranes are available with nominal pore diameters ranging from 1 to 15 nm. These membranes, however, suffer from adsorption and contamination problems. Guy and Chakrabarti (1976b) reported 40% losses when ultrafiltering Cu-EDTA

through Amicon UM2 filters. No such losses were observed when using Amicon PM10 filters. Ultrafiltration is also suspected of altering chemical equilibria as a result of concentration gradients at the membrane surface (Guy and Chakrabarti, 1975; Florence, 1980b).

Dialysis is similar to ultrafiltration in that separation is based on the ability of a species to pass through a membrane. Analyte will tend to diffuse from the sample to a receiver solution until equilibrium is attained, i.e., diffusible analyte concentrations are the same on both sides of the membrane. Since the membrane offers a certain selectivity, separation (and speciation) will occur resulting from size and/or charge parameters. The commonly used cellulose dialysis membranes separate using pore diameter restrictions. Spectra/Por membranes are available with molecular weight cut-off (MWCO) values of 1,000 to 50,000. Once dialysis equilibrium is attained, it is possible to analyze the receiver solution using any appropriate technique.

There are some important advantages in using dialysis as a speciation tool. First, the method has a more general applicability than most other speciation methods (Truitt and Weber, 1981). It is not restricted to only a few metals as with ASV or limited by lack of sensitivity as with ion selective electrodes. Most divalent metals

dialyze at approximately the same rate and therefore speciation studies can include such important metals as calcium, magnesium, manganese, iron and the common transition metals. Second, the effect of dialysis on chemical equilibria is probably less than that of other speciation methods such as ASV and Chelex-100. When used under proper experimental conditions, the effect on equilibria is equivalent to a minor dilution, typically on the order of 5%. Third, the presence of organic surfactants and colloids does not appear to seriously affect results as is the case with ASV.

Dialysis has been applied using a variety of experimental approaches. Benes (1967) described a dialysis cell consisting of two compartments of equal volume separated by a cellophane membrane (pore size 2-8 nm). Both sample and receiver solutions were stirred with 15 hours required to reach equilibrium. The method was applied to the speciation of manganese and gold (Benes, 1967) and of mercury (Benes, 1969) and to the study of yttrium adsorption onto colloidal iron (Benes and Kucera, 1971a, b).

A more common approach and one which has less effect on chemical equilibria is to use large sample volumes with small receiver volumes, typically in the ratio of ~20:1. This corresponds to a dilution of less than 5%. This

method was applied by Guy and Chakrabarti (1976a) to the study of interactions between Cu, Cd, Pb and Zn with natural ligands such as humic and tannic acids. A similar dialysis arrangement was used to determine the complexing capacities of soil fulvic acid for Cu and Cd (Truitt and Weber, 1981) and for Cu, Cd, Mn, Ni and Zn (Rainville and Weber, 1982). Benes and Steinnes (1974) reported the development of an "in situ" dialysis technique whereby a dialysis bag was placed in a natural water system and left to equilibrate for 1-4 weeks. The method reportedly had the advantage of saturating adsorption sites on the membrane and thereafter attaining an equilibrium truly indicative of the undisturbed speciation. The method was applied to the study of the effects of sample storage on chemical speciation (Benes and Steinnes, 1975) and to trace metal-humic acid interactions in fresh waters (Benes et al, 1976).

A novel approach has been to couple dialysis to preconcentration methods. This has been reported by Hart and Davies (1977) and by Benes (1980). The method described by Hart and Davies consisted of a dialysis membrane placed in between two circulating solutions (sample and receiver). The receiver was pumped through a Chelex-100 column, resulting in a "dialyzable and ion-exchangeable" fraction as well as a "dialyzable but not

ion-exchangeable" fraction. The method was applied in conjunction with total metal analysis and batch ion-exchange to describe a total speciation scheme (Hart and Davies, 1981).

Benes' method was simply to add an adsorbent (e.g., FeO_xH_y) to the receiver solution inside a dialysis bag to maintain the receiver free metal ion concentration at less than 1%. The concentration gradient was therefore maintained and diffusion continued at a constant rate. After an appropriate time, the receiver solution was analyzed by activation analysis. One problem with these approaches which effects both a speciation and a preconcentration is that they can shift all of the chemical equilibria present. For example, Hart and Davies (1977) reported complete dialysis of Fe(III) even though calculations indicated that the iron should be present as colloidal $Fe(OH)_3$.

There are two principal limitations to dialysis as a speciation technique. First, it is a relatively slow process, often requiring days to attain chemical equilibrium. For example, Truitt and Weber's dialysis titration required a 48 hour equilibration per metal aliquot added and 30 days to complete (i.e., 15 aliquots). Second, the dialyzable fraction does not necessarily represent that which is bioavailable. It may consist

primarily of species not capable of interacting with biota, e.g., $PbCO_3^o$. What would be of greater value would be a dialysis method permitting the determination of the toxic fraction, i.e., the free aquated metal ion.

1.4 Binding Capacity

Natural waters have the ability to detoxify metals by converting the free metal to nonavailable species. One use of the speciation methods described in section 1.3 is the determination of the binding capacity of the sample. A typical binding capacity curve is shown in Figure 1-2. As copper is believed to be one of the more toxic of the common metal ions to aquatic biota, binding capacity measurements are most often determined for this element. The water sample being characterized is titrated with copper and, after a suitable equilibration period, the free metal is determined by an appropriate speciation technique. Typical values for sea and river waters are 1-5 x 10^{-8} M and 1-50 x 10^{-8} M, respectively. As in the case for speciation determinations, binding capacity determinations are also method-dependent. Among the methods reported are ASV (Hart, 1981), ion selective electrodes (McCrady and Chapman, 1979), copper solubilization (Campbell et al, 1977), dialysis titration (Rainville and Weber, 1982) and fluorescence quenching (Ryan and Weber, 1982).

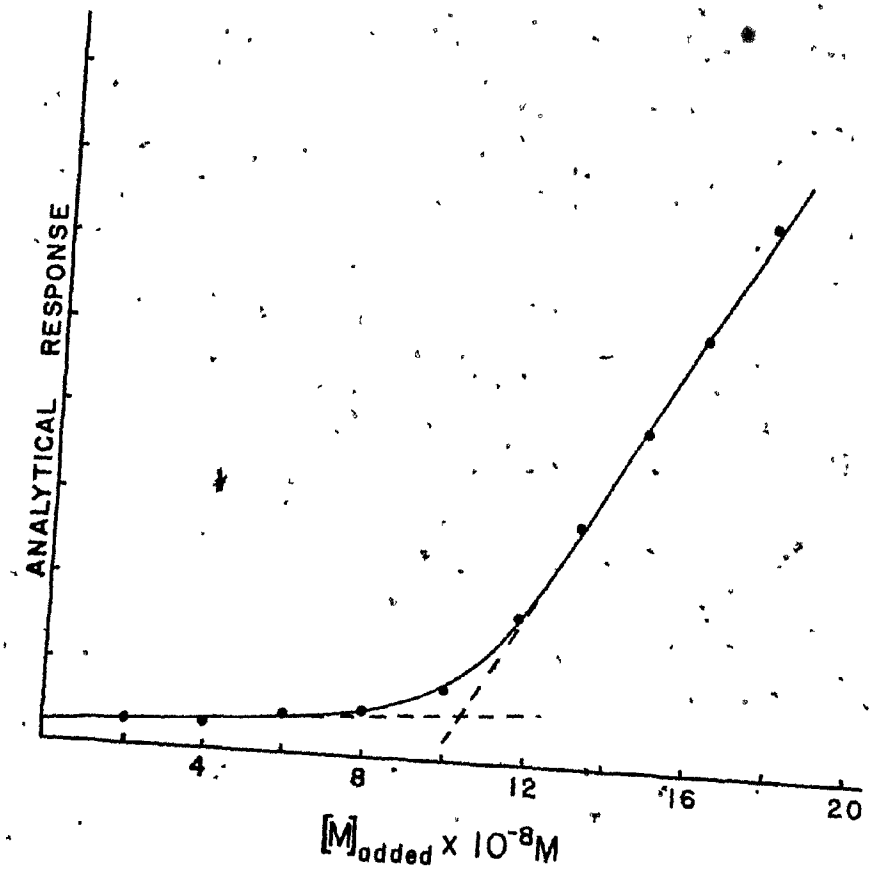


Fig. I-2: Typical binding capacity titration results for a strong metal-ligand complex.

1.5 Multication Speciation

The computer model studies of Morel et al (1973) reported earlier suggested that organic ligands in natural waters may increase transition metal interactions. This enhancement of trace metal interactions may play an important role in water quality with respect to toxicity to aquatic biota. An example of this was the work of Petersen (1982) who studied the effects of copper and zinc on the growth of freshwater algae. He reported that algal growth rates were decreased to 50% when the concentrations of free copper or free zinc were $10^{-8.8}$ M and $10^{-5.1}$ M, respectively. However, when both metals were present in the sample, competition between Cu and Zn for a metal-buffering ligand present in the nutrient (i.e., EDTA) resulted in greater interdependent behavior. For example, in experiments where total copper was maintained constant and total zinc was increased, zinc became toxic at concentration levels lower than $10^{-5.1}$ M. This was interpreted to be a result of zinc displacing the copper bound to EDTA. Similarly, when total zinc was maintained constaht and copper was added at very low concentrations (i.e., below Cu toxic levels), algal growth rates increased because copper was displacing bound zinc and thereby making available this essential element.

Other examples of a multielement approach to toxicity have been reported by Baldry et al (1977) and by O'Shea and Mancy (1978). Baldry et al studied the effects of heavy metals on bacteria and reported that low concentrations of Cd or Zn increased the toxicity of Cu. Similarly, mixtures of Cu and Cr(VI) gave an additive response whereas mixtures of Cd and Cr(VI) were antagonistic. O'Shea and Mancy reported that calcium, because of its presence at relatively high concentrations, was able to compete with cadmium for labile sites present in humic colloids. Calcium was much less competitive with the other metals studied. In a complexing medium, toxic and essential metals may therefore interact in a synergistic fashion (i.e., the displacement of the bound copper by zinc) or in an antagonistic fashion (i.e., the copper displacing bound zinc essential for growth).

1.6 Research Objectives

A summary of the present state of Group 3 speciation is as follows:

(i) Bioassays suggest that the metal species of interest is the free aquated cation.

(ii) Computer simulation and bioassay experiments suggest that a multication speciation is necessary when complexing ligands are present.

(iii) Bioassays suggest that the toxic species (free metal is usually present at 10^{-9} M to 10^{-8} M for toxic effects to be observed. This requires either a very sensitive detector or the application of a preconcentration technique.

(iv) The usual speciation methods (potentiometry, ASV, size separation or ion-exchange) either lack sensitivity or provide analytical data that is difficult to interpret in terms of the free metal cation.

The objectives of this project were to develop a multication speciation procedure that was suitable for determination of the free cation. The method of choice was a dialysis separation using a tubular cation-exchange membrane Nafion 811X- a product of DuPont used as a separator in electrochemical cells. The cation-exchange membrane is permselective to cations and excludes anionic species. Difference in dialysis rates allows one to distinguish between free cations and neutral species. A multication analysis procedure was devised using high performance ion-exchange separations coupled to spectrophotometric or

amperometric detectors. Preconcentration procedures to provide sensitivity were based upon the use of Donnan dialysis with Nafion 811X membrane or ion-exchange pre-columns.

The results are described in four chapters. Chapter 2 compares the performance of electrochemical and spectro-photometric detectors, by using flow injection analysis. Chapter 3 presents the results for the ion-exchange separation of Cu, Zn, Ni, Pb, Co, Cd, Mn, Mg and Ca and gives the detection limits for the ion-exchange/detector systems. Chapter 4 describes the two preconcentration methods suitable for use prior to the ion-exchange separation. Chapter 5 characterizes the Nafion 811X speciation using simple model systems.

CHAPTER 2

Metal Ion Detectors

2.0 Introduction

One of the primary objectives of this thesis was the development and characterization of an analytical system capable of multication analysis. The system would permit the study of multication speciation in model fresh waters. This application imposes a number of constraints on the system. It must be sensitive enough to detect micromolar levels of transition metal ions and at the same time sufficiently selective to permit the determination of trace metal mixtures in millimolar levels of calcium and magnesium. Other considerations were the possibility of small sample volumes, the desirability of short analysis times (<20 minutes per sample) and cost. A possible method for achieving selectivity is to do a preliminary ion-exchange separation of the cations using columns packed with small diameter resins. The problem of sensitivity is solved by appropriate selection of detectors which is the subject of this chapter.

Liquid chromatographic detectors are usually batch methods applied to flowing streams. Among the methods of metal ion analysis that provide multication capability are the photometric detection of metal complexes and electro-

chemical methods. Commercially available liquid chromatographic detectors are variable wavelength UV-visible photometers, amperometric and coulometric detectors, and conductivity detectors.

2.0.a. UV-visible photometers and spectrophotometers: The most widely used detectors in LC are photometers based on the absorption of UV and visible radiation. These detectors are usually capable of providing a readout in absorbance which is proportional to the concentration of analyte in the flow cell ($A = \varepsilon bc$). When properly designed, these detectors are relatively insensitive to flow variations, are capable of high sensitivity (0.002 AUFS with 1% noise), have a good linear dynamic range ($\sim 10^5$), are reliable and easy to use.

Most common amongst light absorbing detectors is the 254/280 nm UV detector. With a low-pressure Hg lamp as its source, it may be used directly (254 nm) or with a suitable phosphor (280 nm). The result is a detector of high stability, high sensitivity and sufficient flexibility to satisfy most demands when analyzing organic compounds.

Metal complexes used in chemical analysis, however, absorb strongly in the visible region of the spectrum and the use of a variable wavelength UV-visible detector is often necessary. A deuterium lamp (UV) and a tungsten lamp

(visible) coupled with a monochromator presents the operator with the choice of any region between 190-650 nm, an option which may greatly enhance either sensitivity or selectivity. It does, however, suffer from greater background noise and more complicated optics.

In applying a photometric detector to the determination of metal ions, a derivatization agent is required since most metal ions are not by themselves suitable for direct analysis. One can form the complex either pre-column and separate the complexes or one can do the derivatization after the separation.

Post-column derivatizations after ion-exchange separations using 4-(2-pyridylazo) resorcinol (PAR) have been reported by Fritz and Story (1974), Elchuk and Cassidy (1979) and Cassidy and Elchuk (1980, 1981 a, b). This reagent is versatile, giving rapid color-forming reactions with many of the common transition metals and with most of the lanthanides. The complexes have high molar absorptivities; for example, the manganese and zinc complexes have ε values of 7.8×10^4 and 8.1×10^4, respectively (Ahrland and Herman, 1975). Fritz and Story (1974) compared PAR with Arsenazo I and Arsenazo III and reported that "PAR was by far the most versatile and convenient of the photometric reagents to use. It was also the most sensitive".

Typical examples of pre-column derivatization are those reported by Uden and Walters (1975), Uden et al (1975), and Gaetani et al (1976). They used the pre-column formation of strong metal organic complexes, e.g., copper complexed with tetradentate β-ketoamines. After liquid-liquid partition chromatography, the complexes were monitored using absorption photometry at 254 nm.

A combination of the two procedures has recently been reported by Beckett and Nelson (1981). They separated metals complexed with 4-aminophenylethylenediaminetetraacetic acid using anion-exchange chromatography followed by post-column derivatization of the metal complexes with fluorescamine. The derivatives were monitored using fluorescence detection.

The detection limits for the three methods described above are typically 1 ng for PAR, 0.5 ng for β-ketoamines and 60 pg for the fluorescence method. The detection limits for the latter two methods appear more favorable but are somewhat compensated for by the decreased resolution in the chromatographic separations.

Molecular absorption bands of metal complexes are broad and consequently the use of a variable wavelength detector may be superfluous. A simple detector for flow injection analysis (FIA) reported by Betteridge et al (1978) incorporated a light-emitting diode (LED)/silicon

phototransistor (SiP) assembly as an inexpensive, dedicated but efficient continuous flow detector. This system was reportedly capable of measuring metals at the parts per 10^9 level using PAR as the photometric reagent. It was decided to investigate the possible application of this LED-SiP detector to the liquid chromatographic separation of metal ions.

2.0.b. Electrochemical detectors: Second only in importance to the photometric detectors are those based on some electrochemical process. These EC detectors are rather varied in design, electrode material and mode of operation. There are some inherent and significant advantages to these detectors, most important being greater sensitivity and selectivity than other conventional LC detectors. In comparing the commonly used LC detectors, Snyder and Kirkland (1979) report the EC detector as the most sensitive, with a "sensitivity to favorable sample" an order of magnitude greater than that of the photometric detector.

Sensitivity to variations in flow rates and restrictions placed on the carrier represent two of the more significant limitations of EC detectors. Whereas most other LC detectors are not very flow sensitive, flow variations do represent a major stumbling block when using

EC detectors, especially when a post-column addition of reagent or electrolyte is required. The method commonly used to alleviate this problem is the incorporation of pulse dampeners. As well, electrochemical detection requires a carrier which is conductive. Its use has therefore been restricted mostly to reverse phase and ion-exchange chromatography.

Three types of electrochemical detectors are those based on measurement of either conductivity, current or potential. The first type, the conductivity detector, responds to differences in conductivity between sample and eluent. For maximum sensitivity, it is advantageous to have a low conductivity eluent and highly conductive samples, i.e., an ion as sample dissolved in water. Ion chromatographic analysis of cations, for example, use suppressor columns to remove eluent ions. An example of this is the analysis of alkali metals using dilute HCl as the eluent (Small et al, 1975; Sawicki et al, 1978). The suppressor column is a strong base anion-exchanger

$$HCl + Resin - OH \rightleftharpoons Resin - Cl + H_2O \qquad (2\text{-}1)$$

which neutralizes the acid and binds the chloride. The result is an eluent of pure deionized water. Sevenich and Fritz (1983) have recently used a single column separation without suppressor and a low concentration eluent to determine some transition metal ions and alkaline earths using a

conductivity detector. They were able to bypass the use of a suppressor column by measuring decreases in conductivity, the sample cation having a lower equivalent conductance than that of the eluent cation. Detection limits for Ca and Mg were 8.0 and 4.8 ng, respectively.

The second type of EC detector is based on a measurement of current resulting from an electrochemical reaction occurring at the electrode surface. This reaction can be described by Faraday's law:

$$Q = nFN \qquad (2-2)$$

where Q is the number of coulombs, n the number of electrons involved in the electrochemical process, F the Faraday constant and N the number of moles converted to product in the flow cell. The detector actually measures "instantaneous current i_t" given by:

$$i_t = \frac{dQ}{dt} = nF \frac{dN}{dt} = 9.65 \times 10^4 \times \left[\text{equivalents converted}/\text{s} \right] \qquad (2-3)$$

A chromatogram is, therefore, a measure of i_t as a function of time.

Coulometric detectors are defined as those having conversion efficiencies of ~100%. Thus, in principle, the use of a calibration curve is not required. However, to achieve ~100% efficiency requires electrodes of such dimensions as to decrease relative sensitivity when

compared to the less efficient amperometric detectors (Kissinger, 1977). This can be explained as follows. As more electrode surface is added to a coulometric detector to increase efficiency, each increment of surface area contributes proportionally less to the amount of material converted but approximately equally to background current, resulting in a lower signal to noise ratio.

Examples of coulometric detectors applied to the LC determination of metal ions are the studies reported by Takata et al (1973, 1975, 1977) and by Girard (1979). They studied the determination of transition metals using the post-column exchange reaction:

$$M_2\text{-}L + M_1 \rightleftharpoons M_2 + M_1\text{-}L \qquad (2\text{-}4)$$

where M_1 is the metal eluting from the column and M_2-L an appropriate complex. The released metal M_2 is detected coulometrically using carbon cloth electrodes. Takata used Hg-DTPA (1973, 1975) and Cu-DTPA (1977) whereas Girard used only Cu-DTPA (DTPA = diethylenetriaminepentaacetic acid).

Amperometric detectors also measure current but with much lower conversion efficiencies than the coulometric detectors, typically 1-10%. In spite of this, they have the greatest relative sensitivity (S/N) of any of the electrochemical detectors. A great variety of detector

flow cell designs have been reported. Of interest are those published by Kissinger (1977) and by Fleet and Little (1974). Kissinger's model uses a Teflon gasket sandwiched between blocks of Kel-F into which have been imbedded appropriate electrodes to achieve a low-volume (~5μL) flow cell with fluid flow parallel to the surface of the electrodes. Another option is to have the flow impinge directly onto the surface of the working electrode, as in the "wall-jet electrode detector" reported by Fleet and Little. This design has the advantage of increasing mass transfer of electroactive material to the electrode surface thus increasing sensitivity.

Amperometric detectors also offer the flexibility of mode of operation -constant potential, pulse and differential pulse amperometry. The pulse modes have two important advantages as applied to LC detectors. Fleet and Little (1974), Swartzfager (1976) and Mayer and Greenberg (1979) have reported enhanced selectivity using pulse modes of amperometry. By a judicious choice of potential and pulse height, selectivity not attainable by either UV-visible photometry or constant potential amperometry is routinely accomplished. A second advantage of pulse modes reported by Myers et al (1974) and Swartzfager (1976) is an

important decrease in flow rate dependence. They showed that convection has little effect on the current provided that the Nernst diffusion layer is small in comparison to the convective shear layer. This condition is more readily attained in the pulse modes and flow variations should present less of a problem.

The vast majority of applications of amperometric detectors has been in the determination of organic compounds in the oxidative mode. The reductive mode presents the problem of dissolved oxygen and metal contaminants. Efficient degassing of solvents and the use of high-grade stainless steels help in decreasing the problem. A few examples of amperometric detectors applied to the HPLC determination of metals have been reported. MacCrehan and Durst (1978) determined cationic organomercury species in biological samples by the reverse phase LC separation of their neutral 2-mercaptoethanol complexes. A differential pulse amperometric detector was used in the reductive mode. Bond and Wallace (1982) separated mixtures of four transition metals using reverse phase LC of their neutral dithiocarbamate complexes. The determination was done by oxidative amperometry using a glassy carbon electrode. Detection limits were 0.1 to

0.5 ng. Lyle and Saleh (1981) used a dropping mercury electrode to determine mixtures of copper, cadmium and zinc separated by ion-exchange chromatography. The detector was used in the amperometric reductive mode. However, detection limits were poor; e.g., 300 ng for copper.

The third type of EC detector is one based on measurement of potential. This is accomplished by use of either an inert electrode (e.g., platinum foil) or with ion selective electrodes (ISE). There are two serious limitations associated with potentiometric detectors. First, they have higher detection limits than other commonly used EC detectors. Second, their response is non-linear, following a logarithmic relationship dictated by the Nernst equation, i.e., 29.6 mV per 10-fold change in concentration of divalent cation. As a result, these detectors have not been applied to liquid chromatographic systems. They have, however, been used in flow injection analysis to monitor nitrate, potassium, sodium, copper, glucose and ascorbic acid (Betteridge, 1978).

The separation of transition metal ions on a high-capacity cation-exchange resin followed by the amperometric determination of a displaced metal according to equation 2-4 should be feasible. It was decided to explore this

possibility using three different electrodes - the dropping mercury electrode (DME), the hanging mercury drop electrode (HMDE) and a glassy carbon electrode (GCE).

2.1 Experimental

The experimental arrangement for the flow injection analysis comparison of detectors is shown in Figure 2-1. The pump used was a Constametric III (Laboratory Data Control, Riviera Beach, Florida) constant flow pump and the sampling valve was a Rheodyne 7125 high pressure valve fitted with a 250 µL stainless steel sample loop. Carrier flow rates were 1.2 mL/min. All tubing between the sample valve and detector was 1/16" O.D. x 1/32" I.D. Teflon tubing. All connections were made with flanged tubing, plastic fittings and couplings (1/4" x 28). Preliminary studies on Dowex 50 suggested that a 0.20 M tartaric acid eluent adjusted to pH 3.74 with NaOH would be suitable for ion-exchange separations. The flow injection analysis studies, therefore, used this solution as the principal flowing stream. The pressurized delivery system and detectors will now be described in more detail.

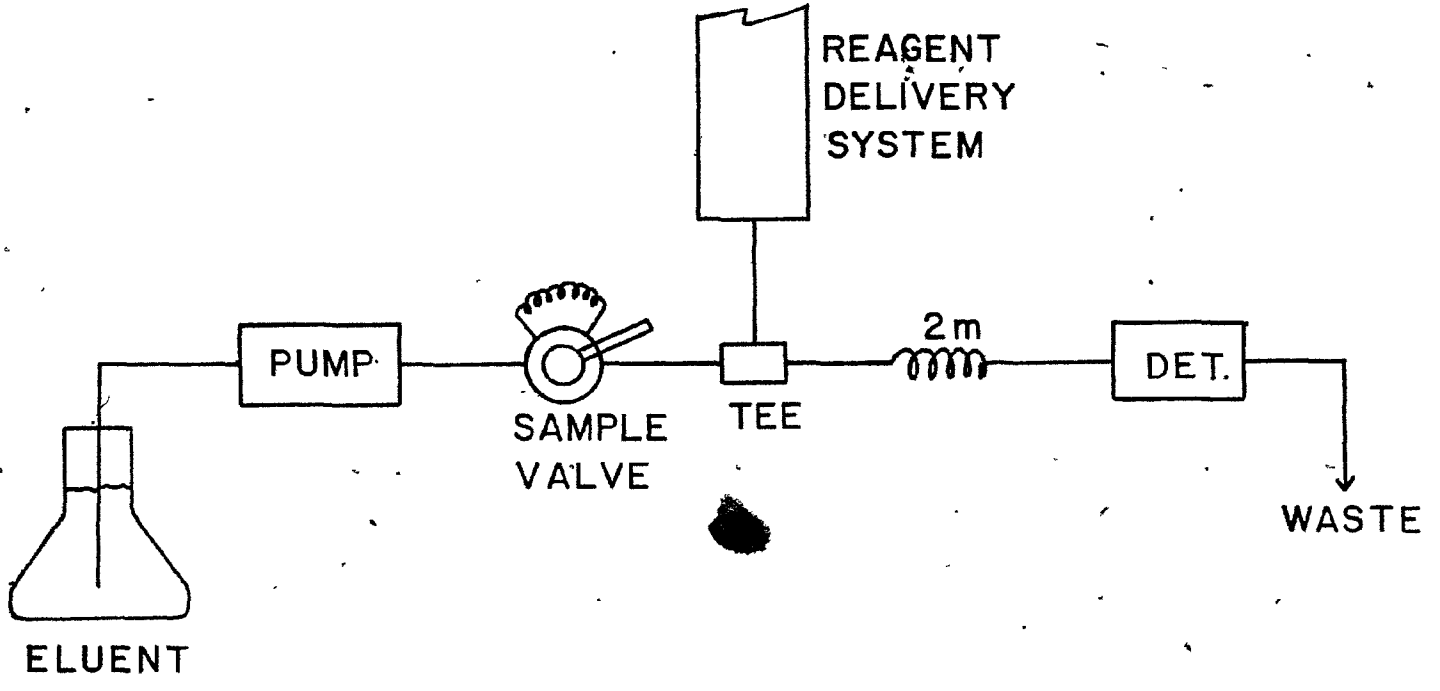


Fig.2-1: Flow injection system for comparison of detectors.

2.1.a. Detection reagent delivery systems: Two delivery systems were studied. The first system was a Sage Model 341A Syringe Pump (Sage Instruments, Cambridge, Mass.) and a 50 mL polypropylene syringe (Becton-Dickinson 5663, Fisher Scientific). The connection between syringe and Teflon tubing was made with an Uptight female luer connector. The second system was a N_2-pressurized system described in Figure 2-2. The reservoir was machined from nylon and had a volume of approximately 300 mL. The base of the unit was machined from plexiglass and the flowmeter was a Gilmont Valve Assembly, Size No.1, 0-4 mL/min with micrometer control. The tube connecting the flowmeter and plastic tee was 30 cm of 0.01" I.D. Teflon tubing. This small diameter tubing was used to increase the backpressure in the system to 1-2 psig and, thereby, achieve greater control and constancy of delivery. The syringe pump and pressurized delivery systems were used to deliver derivatization reagents at the rate of 0.50 mL/min.

2.1.b. Spectrophotometric detectors: Two spectrophotometric systems were studied. Systems 1 and 2 used a common flow cell described in Figure 2-3. All parts (A-E) were machined out of plexiglass. Parts A and C were made to

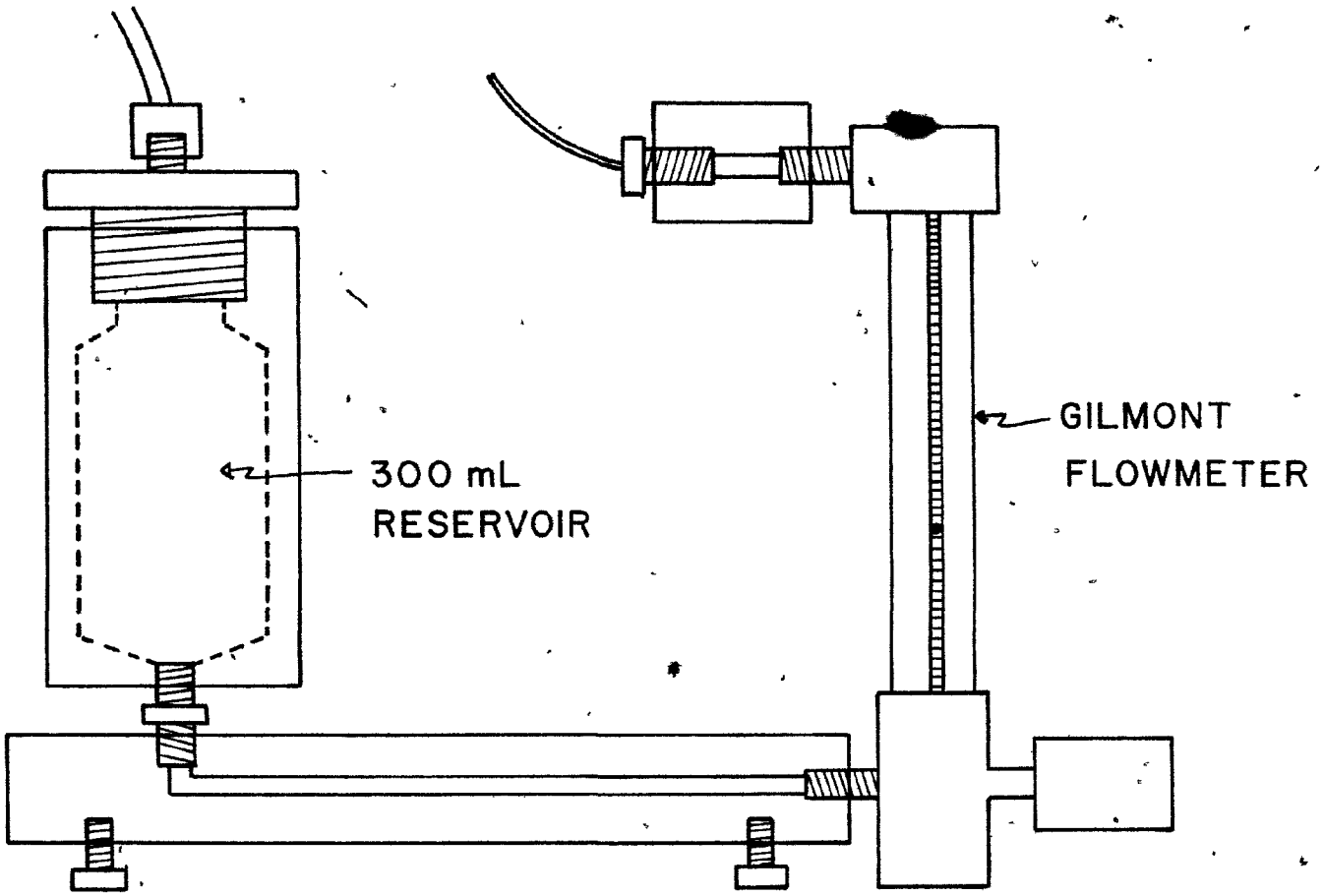


Fig. 2-2: Pressurized delivery system.

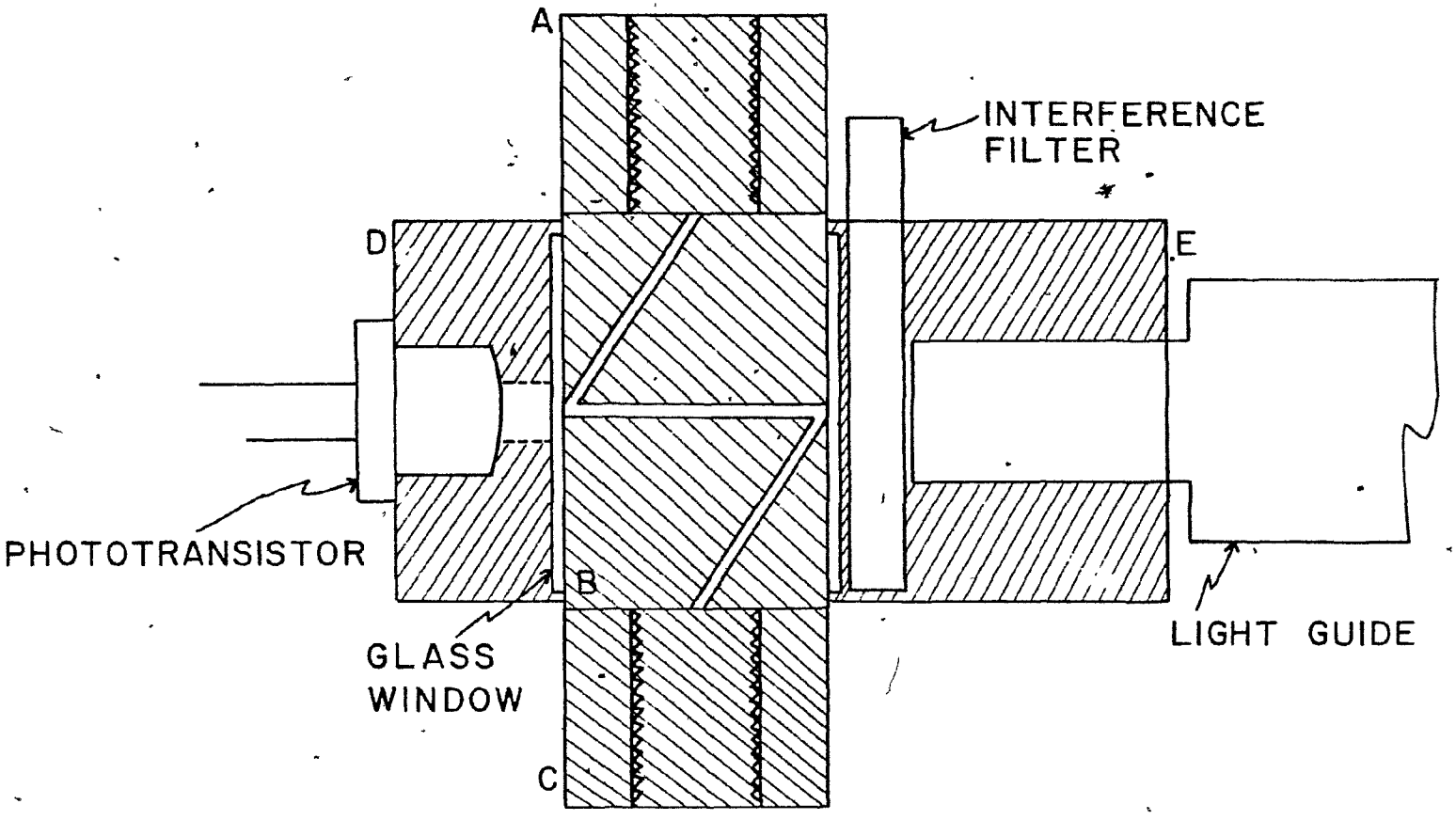
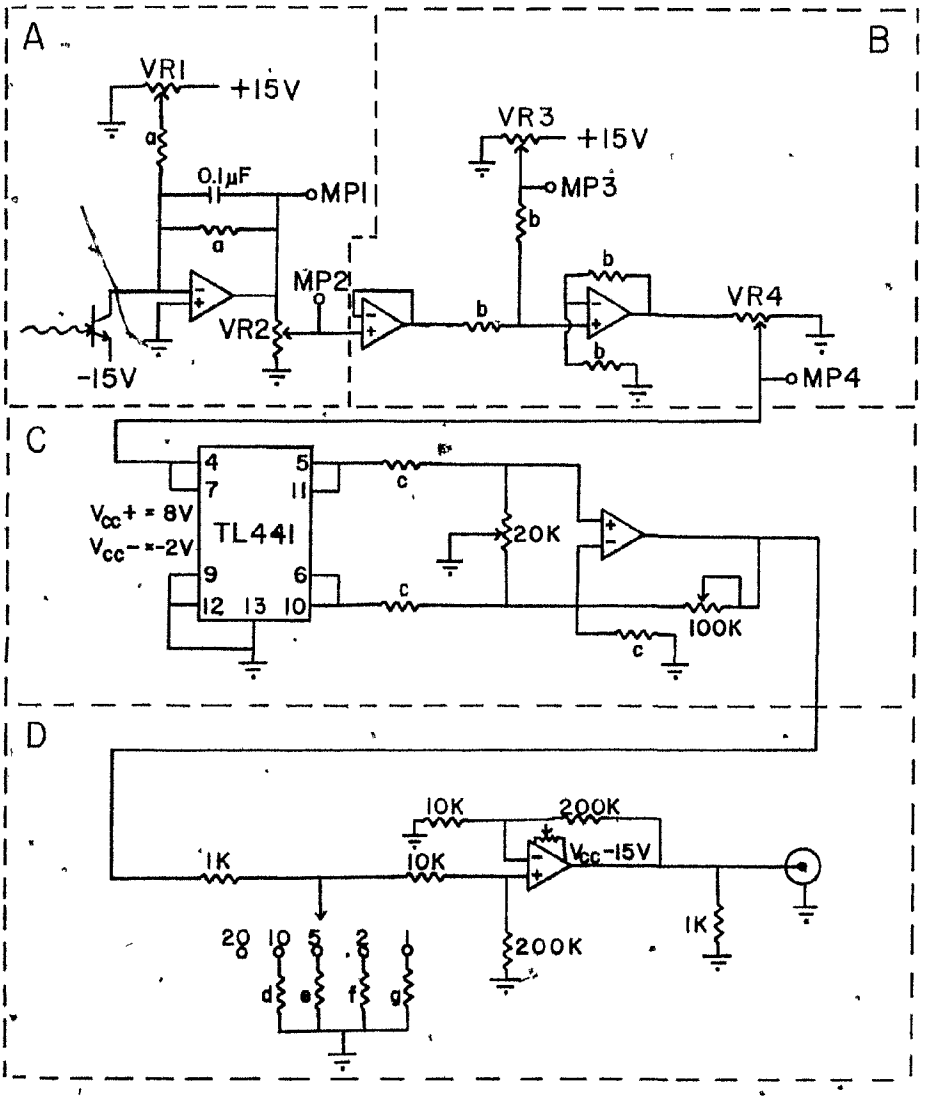


Fig. 2-3: Top view of flow cell .

accept standard 1/4" x 28 plastic connectors and were glued to central unit B using acrylic cement. Part D was machined so as to accept by push-fit a silicon phototransistor TIL-78 (Texas Instruments). Part E held the source - a gallium phosphide light-emitting diode (LED)(Texas Instruments) in system 1, or a fibre optic light guide and removable dielectric interference filter (Edmund Sci.Co., New Jersey) in system 2. In most cases a 520 nm filter was used. Parts D and E were held to the central unit using a nut and bolt assembly. The cell windows were 18 mm diameter microscopic slide cover glasses and were glued using silicone sealant. The entire cell was lacquered black and placed in a light-tight box in order to exclude ambient radiation. Flow channels were 1 mm diameter, giving a cell volume of 10.2 µL. The light guide was coupled to a high intensity tungsten halogen lamp (Dolan-Jenner Ind.Inc., Model 170 D).

The phototransistor was operated using the transducer circuit shown in Figure 2-4. System 1 used only the circuit enclosed within the dashed lines of section A and was essentially the same as that reported by Betteridge et al (1978). The phototransistor, biased at a constant -15 volts, acted as a current source whose output was



VR1=1K; VR2=100K; VR3=5K; VR4=10K

a=3.3M; b=12K; c=1.8K; d=1K; e=330; f=110 ; g=55

Fig. 2-4: Transducer circuits for system 1 and 2.

dependent on the incident illumination. System 1 was operated as follows. The 100% light intensity was adjusted with both detector solution and eluent flowing through the light path. Potentiometer VR1 was used to set the potential at metering point MP1 to 0.00 volts. The 0% light intensity was adjusted by disconnecting the LED from the circuit and potentiometer VR2 was used to set an arbitrary gain (V_T). The output voltages (V_O) were recorded on a Fisher Recordall chart recorder.

System 2 modified system 1 by adding a linearization circuit. The 100% adjustment was the same as in system 1. The 0% light intensity was adjusted by turning off the tungsten lamp and setting VR2 such that MP2 read -1.00 volts. Potentiometer VR3 was used to adjust the voltage at MP3 to +1.00 volts. If one assumes that the voltage output V_O is proportional to the light intensity, then the second part of the circuit (section B) gives us the transmittance. Potentiometer VR4 is used to adjust the value at MP4 to read between 0 and 400 mV. This is the approximate linear range of the logarithmic amplifier (TL441, Texas Instruments). The final section of the circuit (section D) provided multiple gain and offset to match the input of the recorder. The choice of the gain was as follows: 1.04, 1.98, 4.96, 10.0 and 20.0.

2.1.c. Electrochemical detectors: Three flow cells were utilized in these experiments. The HMDE cell is described in Figure 2-5a. The Princeton Applied Research Model 303 Static Mercury Drop Electrode (SMDE) was equipped with a glass sleeve which fit snuggly around the capillary of the Hg electrode. The distance between the mercury drop and the flow outlet could be adjusted by raising or lowering the sleeve. Optimum results were obtained when this distance was 6 mm. A reservoir served to make electrical contact with the reference and counter electrodes.

The second flow cell is described in Figure 2-5b. The main body was machined from plexiglass and accepted a 1/4" x 28 plastic male connector. This connector was for 1/8" O.D. tubing and accepted a glass sleeve held in place with silicone sealant. This sleeve acted as a support for the 1/32" I.D. x 1/16" O.D. Teflon tubing outlet. The cell was attached to the DME of a PAR Model 1746 dropping mercury assembly.

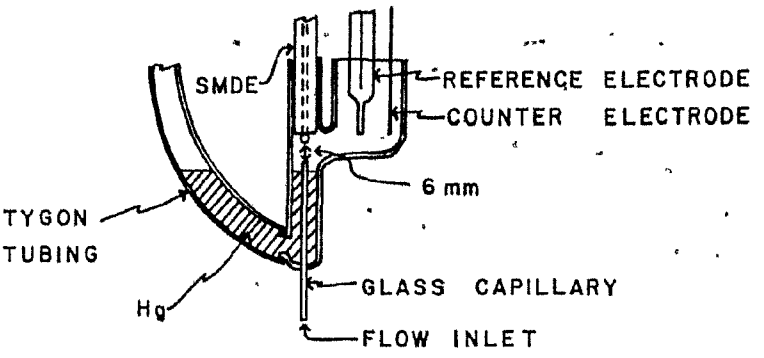


Fig. 2-5a HMDE detector

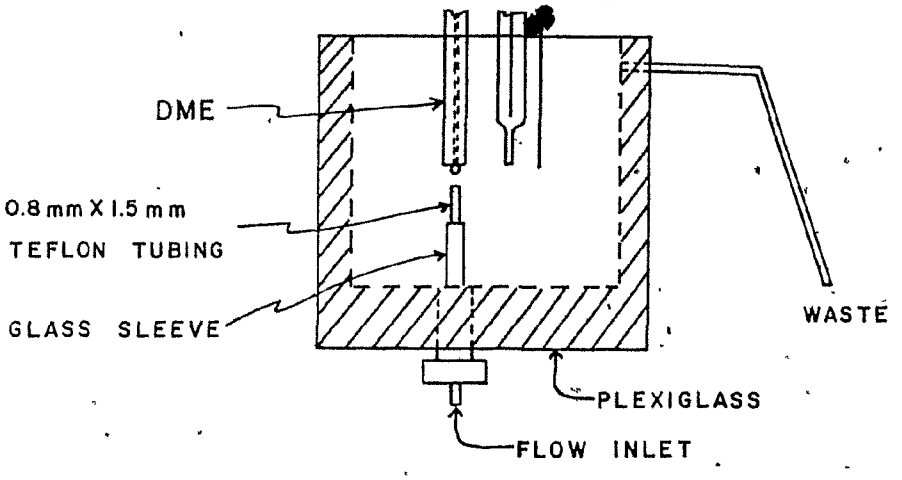


Fig. 2-5b: DME detector

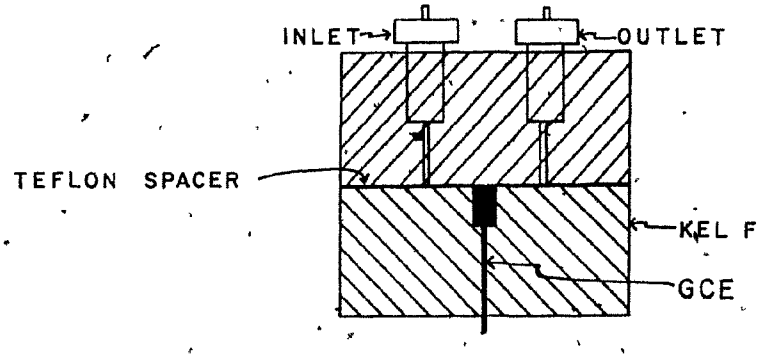


Fig. 2-5c: BAS-GCE detector

The third electrochemical detector was the Bioanalytical Services (BAS) Model TL-3 flow cell illustrated in Figure 2-5c. This cell was used with a BAS Model RE-1 Ag/AgCl reference electrode and a Model RC-2A auxiliary electrode assembly. The three flow cells described above used the PAR Model 174A Polarographic Analyzer as the potentiostat.

2.1.d. Chemicals: The monitoring of metal ions either by spectrophotometric or electrochemical detectors was done either by direct reaction of the cation with reagent or by a metal-displacement reaction. All metal-displacement reagents (for example 2.5 mM Zn-EDTA; 1.0 mM Cu-DTPA; 0.50 mM Cd-EDTA and 0.50 mM Cd-DTPA) were prepared by titrating the ligand in 2.0 M NH_3/1.0 M NH_4OAc buffer with the appropriate metal using either PAR (i.e., Zn) or ion selective electrodes (i.e., Cu, Cd) as the indicator. The 4-(2-pyridylazo) resorcinol (PAR) detection reagent was prepared by dissolving 21.5 mg of indicator grade compound (G.F. Smith Co.) in 500 mL of 2 M ammonia/1 M NH_4OAc buffer.

All metal stock solutions (0.100 M) were prepared by dissolving either metal (Cu, Zn, Cd) or reagent grade salts (Mg, Ca, Ni, Pb, Mn and Co) in nitric acid. These were standardized using EDTA titrations.

2.1.e. FIA procedure: All flow injection analysis studies were done using the apparatus described in Figure 2-1. The carrier stream flow rate (tartrate carrier) was 1.2 mL/min and the detection reagent was 0.5 mL/min. All samples were made up in tartrate carrier to minimize effects of changes in refractive index (for photometric detectors) or electrolyte (electrochemical detectors).

2.2 Results and Discussion

The method selected for comparison of the detectors was flow injection analysis (FIA). The advantage of this method was that all samples have the same bandbroadening controlled by injection volumes, coil lengths, flow rates and detector volumes. One thus avoids the bandbroadening introduced by retention of a species on a column. The disadvantage of this method was that the pulse dampening resulting from column operation was absent, hence a high background noise was observed. The sensitivities reported in this chapter are for the comparison of detectors and detection reagents whereas those reported in the next chapter are for the comparison of the various separation/detection systems.

2.2.a. Detector reagent delivery systems: The two reagent delivery systems were compared using a PAR-Zn-EDTA reagent,

the tartrate eluent and photometric system 2 detector. Baseline noise tracings are illustrated in Figure 2-6.

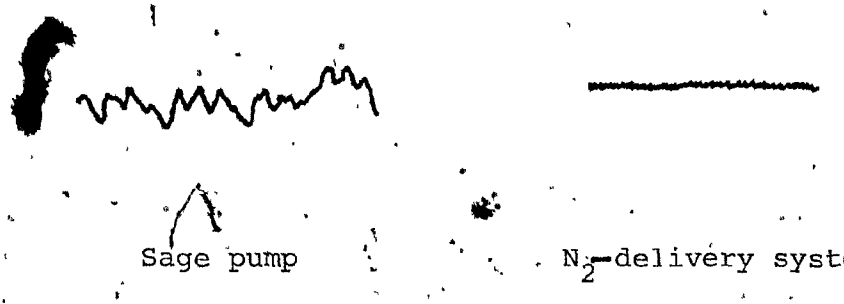


Fig. 2-6: Baseline tracings of reagent delivery systems.

The Sage pump noise was thought to result from sticking of the plunger against the syringe walls. The N_2-delivery system has a noise level that results from the Constametric III pump. The N_2-delivery system was used in all subsequent studies.

2.2.b. Photometric detectors: The first detector studied was the LED-SiP system 1 detector described by Betteridge et al (1978). Figure 2-7a presents tracings of the system response for individual injections of several metal ions into the flowing stream using PAR as detection reagent. Cobalt and copper were found to be 10 to 25 times more sensitive than the other metals studied. Figure 2-8 illustrates the limitations of the simple LED-SiP system.

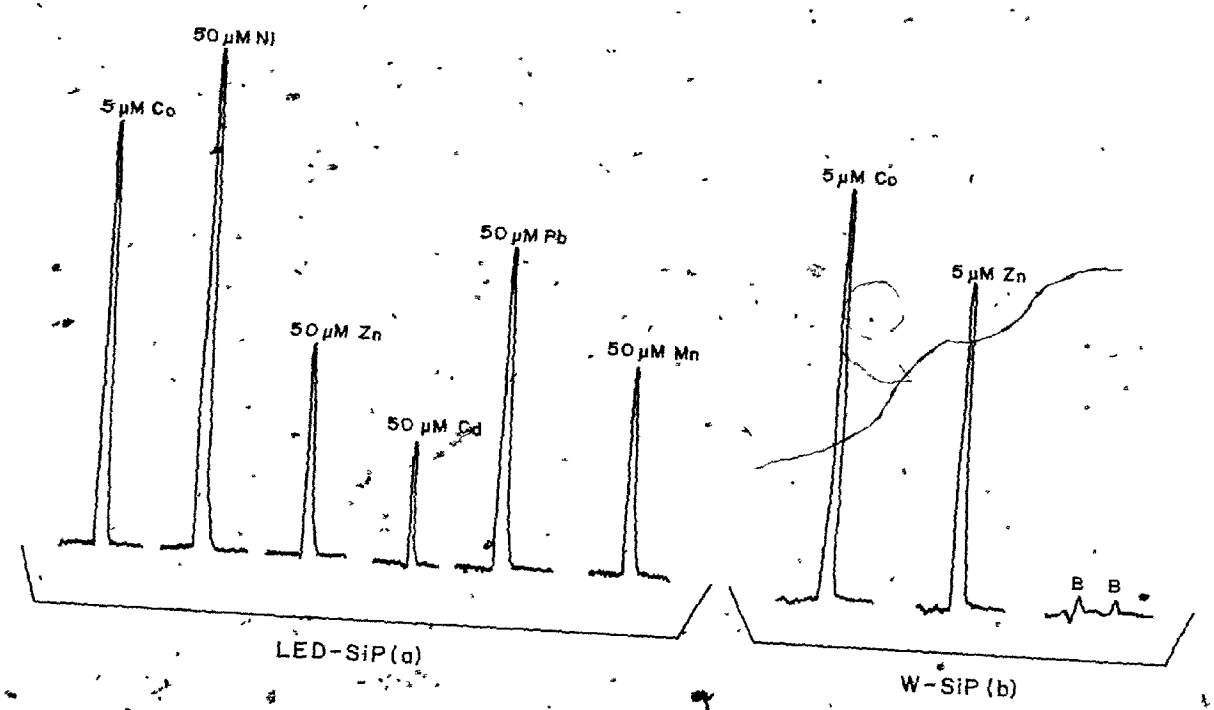


Fig. 2-7 Relative responses for system I detector. -100 μL injections, 1.0 mL/min carrier and 0.14 mL/min PAR

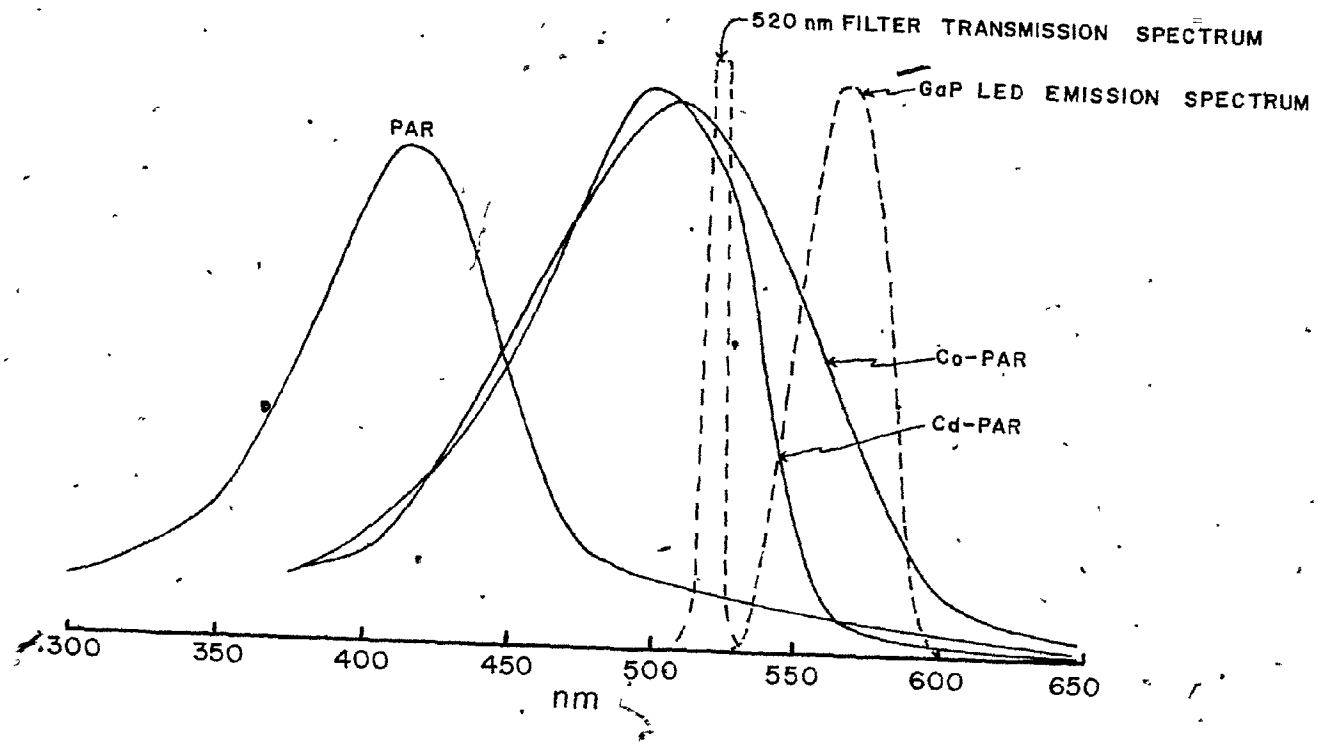


Fig.2-8: Source emission and M-PAR absorption spectra.

The LED emission maximum at 565 nm overlaps better with the cobalt and copper complex absorption spectra than with those of other metal complexes. To obtain better overlap of source emission and metal complex absorption, one needs a lower source wavelength. For example, 520 nm would be a good compromise between metal complex absorption maxima and free indicator absorption minima. To effect this change, the LED in system 1 was replaced by a quartz iodide tungsten lamp and a 520 nm interference filter but with no change in the electronic circuitry. The improved response for Zn with respect to Co using the 520 nm source is shown in Figure 2-7b.

To verify the linear response of the system 1 unit, a calibration curve for bromothymol blue (BTB) was done using 0.01 M borax carrier and a 620 nm interference filter. The response for system 1 (V_O) is given in curve A of Figure 2-9. The calibration is definitely non-linear. This can be readily explained using Figure 2-10.

As indicated in Figure 2-10, assuming that the response of the phototransistor is linear, the voltage output for system 1 (V_O) should be proportional to the amount of light absorbed. For a linear calibration curve, one needs a plot of absorbance (or - log T) versus concentration and not a plot of absorption versus concentration. Since T = 1 -(fraction of light absorbed),

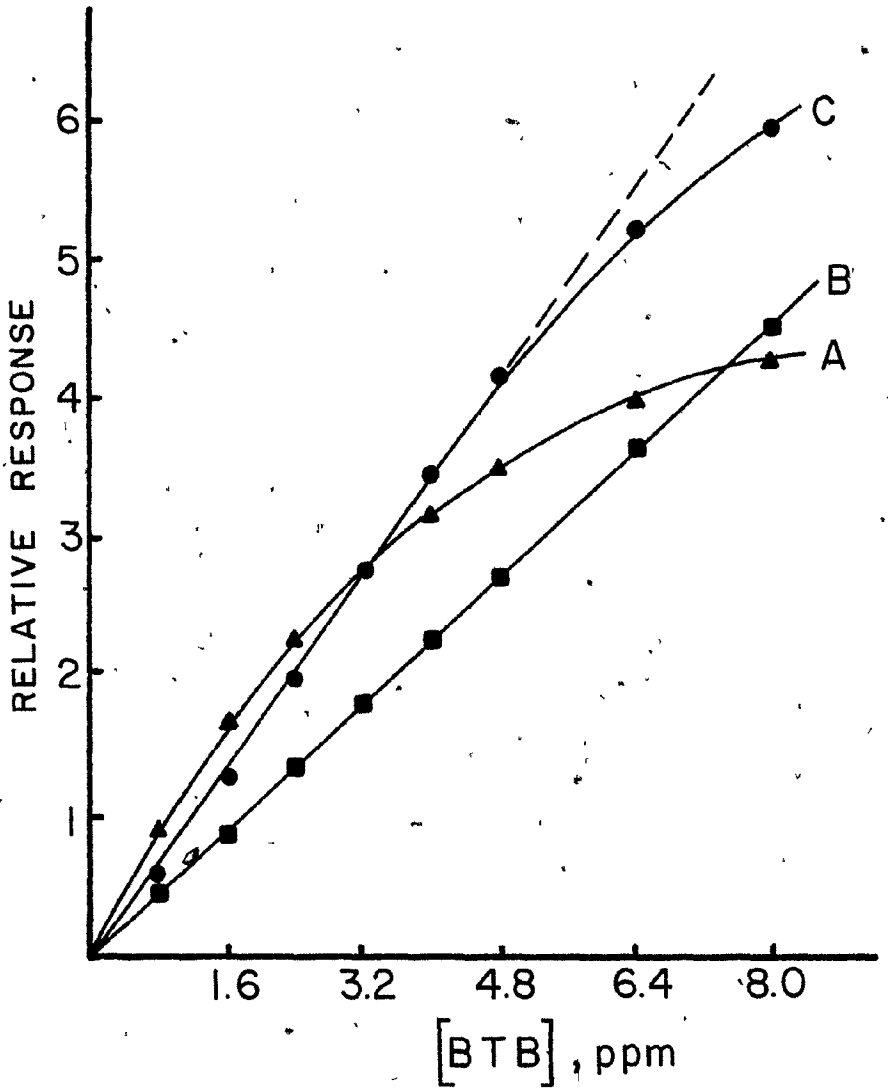


Fig. 2-9: BTB calibration curve.

1 unit = 200 mV(A); 0.2AU(B); 40 mm (C)

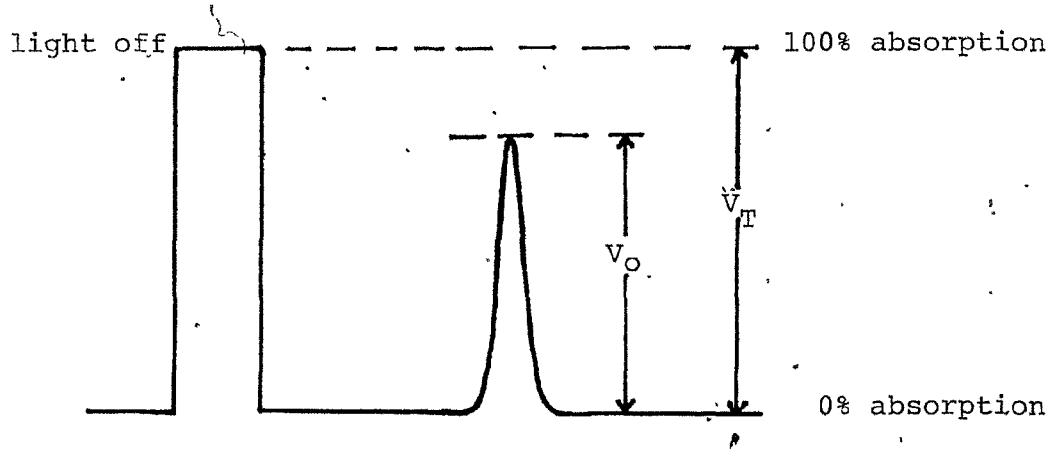


Fig. 2-10: Schematic of system 1 detector

a plot of -log $(1 - V_O/V_T)$ versus concentration should be linear. The values of V_O and V_T from curve A have been used in this expression to obtain curve B in Figure 2-9. In this case a linear calibration curve was obtained.

The electronic circuitry in sections B and C of Figure 2-4 was used to calculate V_O/V_T, $1 - V_O/V_T$ and log $(1 - V_O/V_T)$ automatically. The BTB calibration curve using system 2 is shown in curve C of Figure 2-9. An extended linear range was observed followed by curvature at high BTB concentrations. This curvature is not due to the phototransistor which has a linear response from 100% T (0.00 A) to at least 12% T (0.90 A) as shown by curve B in Figure 2-9. It appears that the observed departure from linearity is due to the logarithmic amplifier. Figure 2-11

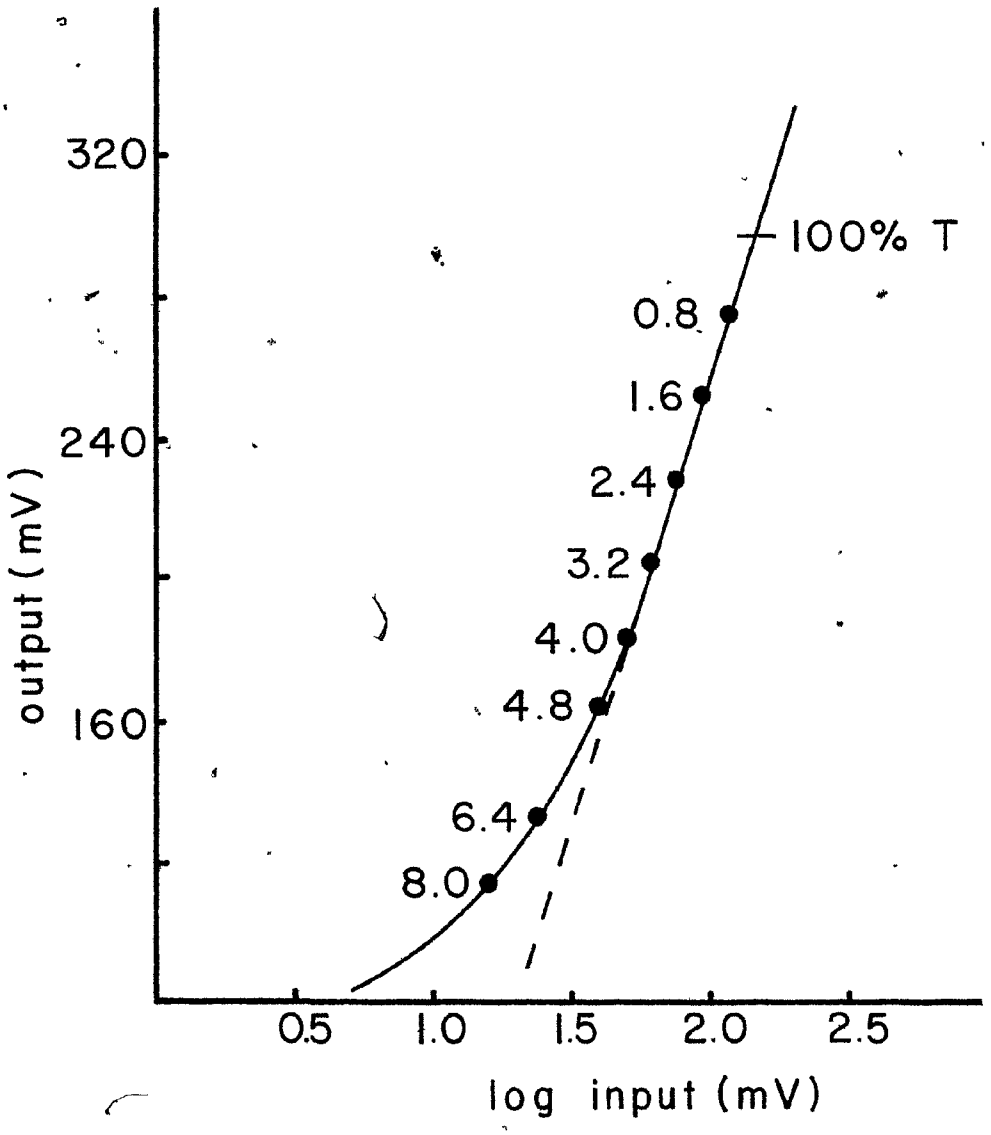


Fig. 2-11: Log amp response.

- smooth curve is a result of 15 equally spaced measurements (not shown)
- points shown correspond to BTB calibration curve, Fig. 2-9

shows the response of the log amp as a function of input voltage. The response was not linear over the 0-100% absorption interval with curvature starting at the input potential corresponding to 72% absorption. This onset of curvature in the log amp response (Figure 2-11) corresponded to that in the BTB calibration curve (Figure 2-9c).

A calibration curve for Zn was done using the system 2 detector with PAR as the photometric reagent. The results are shown in Figure 2-12. As in the case of BTB, a linear range was observed followed by negative curvature above 0.6 absorbance. The calculated $-\log (1 - V_O/V_T)$ curve was again linear over the entire range studied, i.e., 0-0.74 absorbance. Despite the curvature noted, the system 2 detector represents a definite improvement over the Betteridge model in two respects. First, with the exception of Co and Cu, sensitivities are much improved through the use of a more appropriate wavelength (520 nm vs 565 nm). Second, linear calibration curves are obtained over the 0-0.6 absorbance range with a useful working range up to 0.8 absorbance.

The PAR photometric reagent, while giving good response for metals such as Co, Ni, Cu, and Zn, gave relatively poor response for such metals as Cd and Pb. An example of this is shown in Figure 2-13. The slope of the

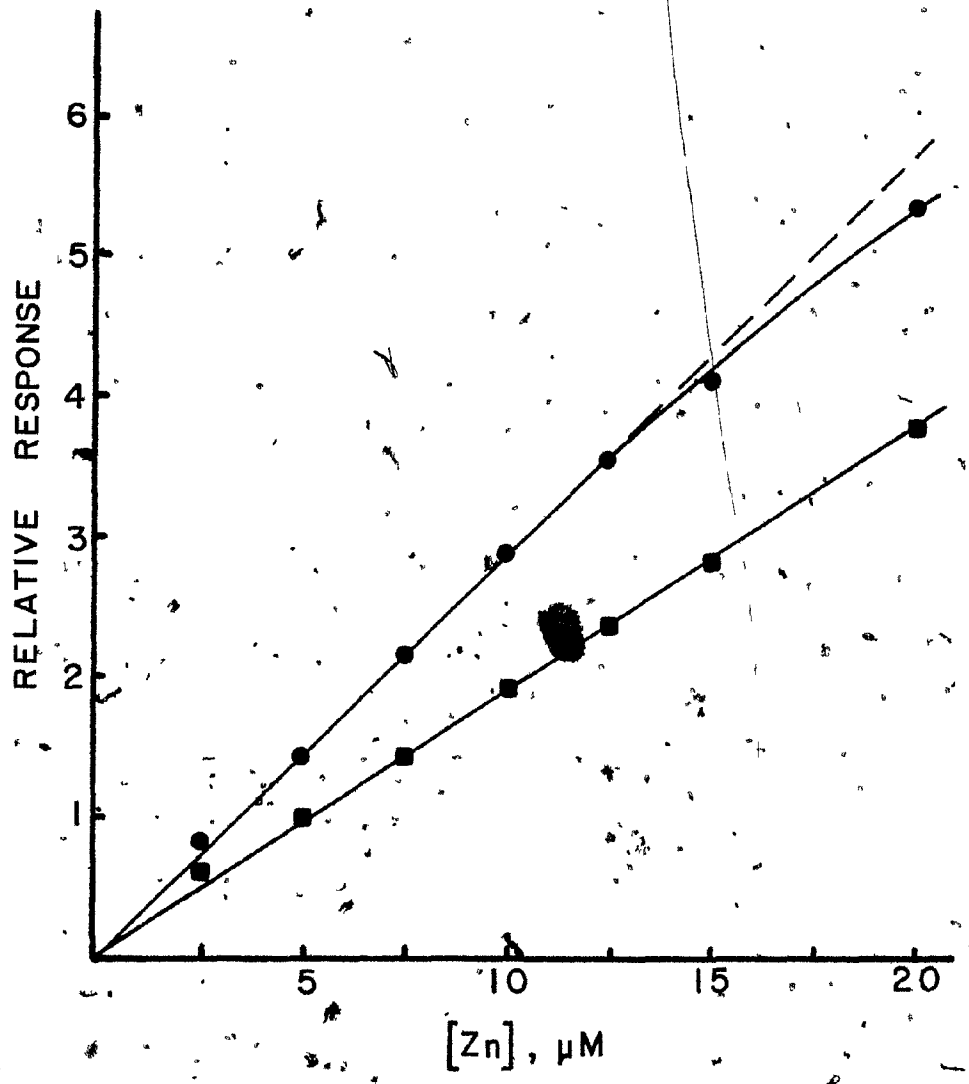


Fig. 2-12: Zn calibration curve.

1 unit = 40mm (peak height, ●); 0.2AU (calc., ■)

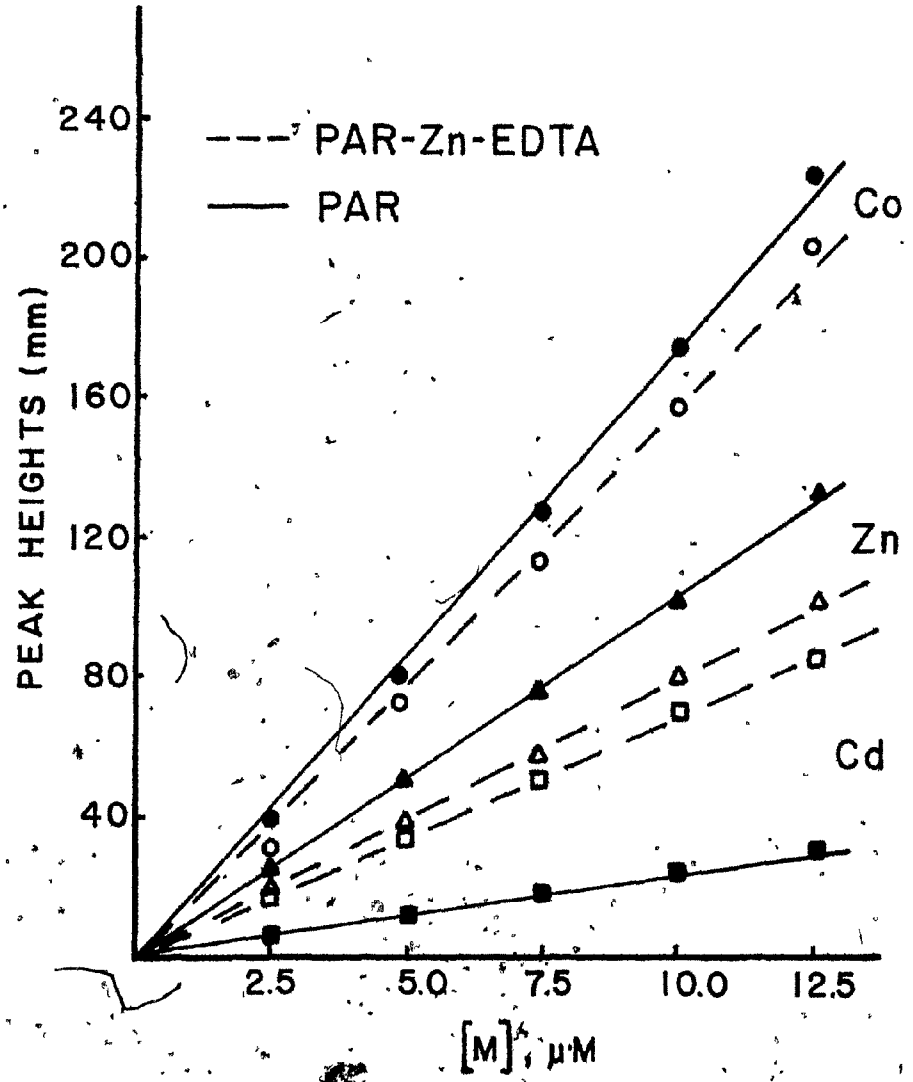


Fig. 2-13: Calibration curves with PAR and PAR-Zn-EDTA.

cadmium calibration was only 14% and 23% the cobalt and zinc slopes, respectively. This is directly related to the molar absorptivities of the metal complexes.

An improvement in sensitivity might be expected if one used a displacement reaction similar to reaction 2-4. Preliminary calculations of conditional stability constants for metal-EDTA complexes suggested that at pH 10 Zn-EDTA would be a suitable candidate for study. The results for a PAR-Zn-EDTA reagent are also shown in Figure 2-13. A comparison of the PAR and PAR-Zn-EDTA results indicates a 280% increase in sensitivity for cadmium but decreases of 7.5% and 22% for cobalt and zinc, respectively.

If displacement is occurring, a decrease in sensitivity for cobalt would be expected as $\varepsilon^{520}_{Co\text{-}PAR} > \varepsilon^{520}_{Zn\text{-}PAR}$. The observed decrease for Zn, however, was more surprising. It was noted that the PAR-Zn-EDTA photometric reagent had a higher absorbance at 520 nm than the PAR reagent. This increased absorbance corresponded to a free zinc concentration of approximately 7.5 micromolar. Since the initial Zn-EDTA concentration was 100 micromolar (assuming no dissociation), one can readily calculate a conditional stability constant for this solution:

$$K'_{Zn'\text{-}EDTA'} = \frac{92.5 \times 10^{-6}}{(7.5 \times 10^{-6})^2} = 1.64 \times 10^{6} \qquad (2\text{-}5)$$

This conditional stability constant was used to calculate a calibration curve for the addition of zinc ion into the equimolar Zn-EDTA solution. The results are given in Figure 2-14. A second curve is shown assuming 2.5 micromolar free zinc and a calculated conditional stability constant of 1.56×10^7 (i.e., using values from Ringbom, 1979). The two curves show slight curvature near the origin but the linear regression slopes were 0.71 and 0.86 for the experimental and calculated conditional constants, respectively. This suggests that the presence of PAR and dissociation of the Zn-EDTA complex is one of the main causes for the observed decreased sensitivity. The addition of a post-column displacement reaction may enhance sensitivity by forming a PAR complex with greater molar absorptivity (e.g. Cd). The sensitivity may be decreased, however, if the displacement reaction results in species of lower molar absorptivity or if the metal ion interacts with the free EDTA present in the eluent/detection reagent solution.

Table 2-1 presents the relative sensitivities for nine metal ions reacting with both PAR and PAR-Zn-EDTA photometric reagents. The choice of the more suitable reagent will depend on which metals are to be analyzed. The advantage of the PAR-Zn-EDTA reagent is that significant increases in sensitivity are achieved for Cd,

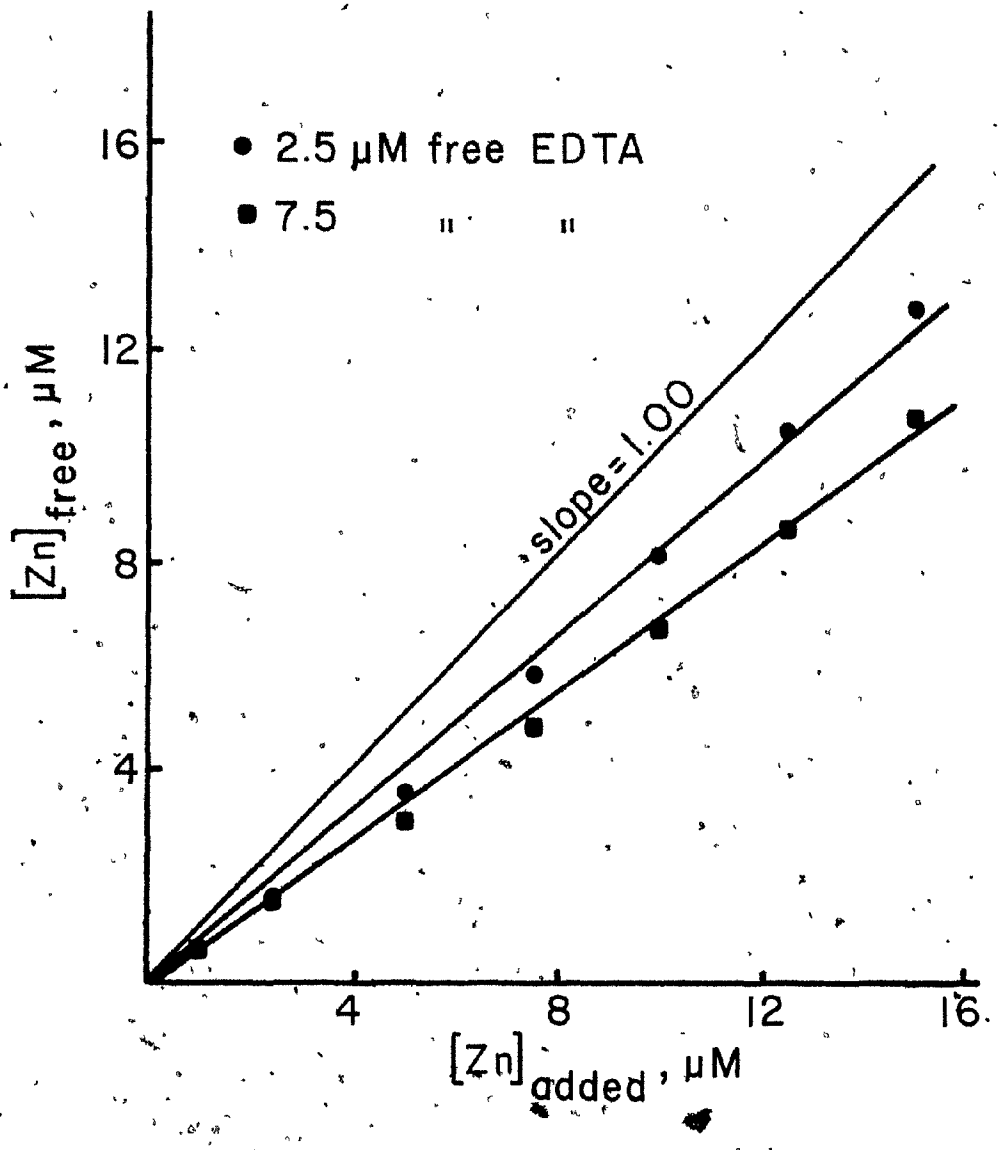


Fig. 2-14: Effect of free EDTA on Zn calibration curves.

Table 2-1

Sensitivity* Data for Photometric Detection

Metal ion	PAR	PAR-Zn-EDTA
Co(II)	69.2	60.0
Ni(II)	62.4	56.8
Cu(II)	45.2	42.0
Zn(II)	41.4	30.3
Mn(II)	44.0	27.2
Pb(II)	9.2	28.0
Cd(II)	9.6	25.3
Ca(II)	0.0	24.0
Mg(II)	0.0	5.2

* - sensitivity = response in mm/nmole injected
  - gain = 10X
  - recorder = 1VFS

Pb,Ca and Mg while suffering only minor losses for Co, Ni, Cu, Zn and Mn. The greater experimental flexibility offered through this choice of reagents has in some cases considerably facilitated the design of experiments to be reported later in this thesis.

2.2.c. Electrochemical detectors: The first of three electrochemical detectors investigated was the dropping mercury electrode (DME). Preliminary batch studies using various metal-polycarboxylic acid complexes had shown that a mixture of Cd-EDTA, 5 x 10^{-4} M and Cd-DTPA, 5 x 10^{-4}M in tartrate carrier was a promising detection reagent. Relative sensitivities as determined by FIA using this reagent are shown in Table 2-2. Only 5 metals - Cd, Pb, Zn, Co and Cu -gave significant responses. Little exchange was noted for Ni, Mn, Ca, and Mg. The poor exchange for Ca, Mg and Mn was due to the solution pH of 3.74 (hence poor ligand exchange constants) whereas nickel was probably the result of slow kinetics.

The calibration curves for the five metals that gave good response were linear from detection limits to 60 nmoles. No curvature was noted at the 60 nmole level and higher amounts could probably be injected. The main problem observed with the DME detector was noise. High background current combined with the noise gave

Table 2-2

Sensitivity* Data for Electrochemical Detection

Metal ion	Cd-EDTA / Cd-DTPA/DME	Cd-EDTA / Cd-DTPA/HMDE	Cu-DTPA/GCE
Co(II)	0.078	0.365	0.116
Ni(II)	0.006	0.009	0.095
Cu(II)	0.035	0.290	0.150
Zn(II)	0.081	0.411	0.157
Mn(II)	0.005	0.091	0.154
Pb(II)	0.097	0.480	0.153
Cd(II)	0.103	0.491	0.142
Ca(II)	N D	N D	0.113
Mg(II)	N D	N D	0.024

* - sensitivity = response in μA/nmole injected

- N.D = not detected.

unacceptably high detection limits on the order of 5-10 nmoles. The noise was due to several factors - reduction of dissolved oxygen, lack of mechanical stability of the mercury drop, drop size irreproducibility and noise due to the drop knocker. As a result of the poor sensitivity, no further work was done with the DME detector.

The hanging mercury drop electrode (HMDE) using the same detection reagent gave more satisfying results. Background currents and noise were much reduced when compared to the DME. The HMDE has the advantage of eliminating noise associated with the drop knocker and with reproducibility in drop size. Relative sensitivities using this detector are shown in Table 2-2. As expected, results are similar to those using the DME. Calibration curves for the metals were once again linear over the range studied (detection limits to 10 nmoles). The detection limits were about 0.2 nmoles.

One problem observed with the HMDE detector was passivation. Figure 2-15 shows the detector response for repeated injections of a single solution of cadmium. One can see that passivation does occur, being most prominent in the first 5 minutes and leveling off afterwards. The routine application of this detector in a LC system would therefore be somewhat compromised.

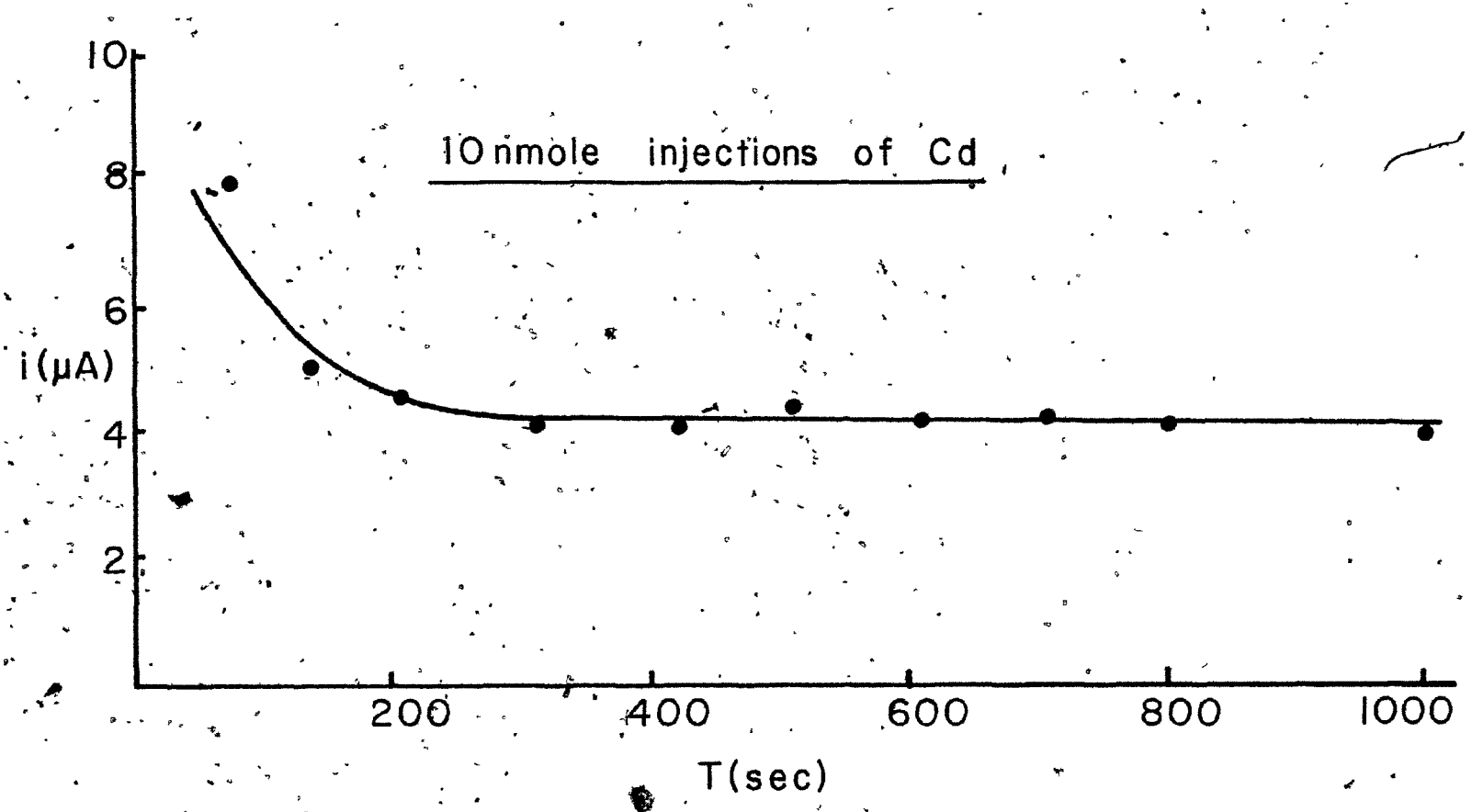


Fig.2-15: Passivation of Cd response with HMDE detector.

The best performance of the three detectors was given by the BAS glassy carbon electrode (GCE). The absence of mercury permitted one to use more anodic potentials than in the HMDE and DME work. The latter electrodes at high pH and anodic potentials gave large mercury oxidation currents. The GCE allowed the use of a Cu-DTPA detector reagent at pH 10 and an applied potential of -0.300 V.

Passivation of this electrode surface did not appear to be a major problem. Slight decreases in sensitivities occurred on start-up but the system response quickly equilibrated (less than 5 minutes). This can be supported by results obtained in a reproducibility study. Six repeat injections of 7.5 nmoles of each of Cu, Zn, Ni, Pb, Co and Cd were run over a 3 hour period with relative standard deviations ranging from 0.3% to 1.6%.

The GCE detector system was also operated in the pulse mode, pulsing from 0 mV to -350 mV (vs Ag/AgCl). Higher background currents and noise resulted and as a consequence a 4-fold decrease in sensitivity. No further studies were done in the pulse mode and all remaining results are for the differential pulse mode, i.e., -300 mV with a 50 mV pulse.

Relative sensitivities as determined by FIA using the GCE detector with the Cu-DTPA reagent are shown in Table 2-2. It can be seen that near quantitative displacement

occurs for Cu, Zn, Pb, Cd and Mn and that important displacements (i.e. > 60%) are observed for Co, Ni and Ca. Only magnesium gave poor results. Examples of calibration curves for Cu and Pb are given in Figure 2-16. Good linearity is observed over the detection limits to 6.25 nmole range studied with detection limits being on the order of 0.5 nmoles for both metals.

2.3 Conclusions

Five detection systems were characterized for the detection of metal ions separated by ion-exchange chromatography. Figure 2-17 presents FIA injections near the detection limit for each of the detection systems. Table 2-3 presents the detection limits for each of the metals for each of the five detection systems. The system 2 photometric detector was the most sensitive detector studied, with FIA detection limits of approximately 0.05-0.1 nmoles for Co, Ni, Cu, Zn and Mn. The detector did exhibit non-linearity at low light levels but its working range of 0-0.8 absorbance units would satisfy most common usage. In addition, the flexibility offered through choice of photometric reagents (PAR or PAR-Zn-EDTA) permitted the determination of trace transition metals in the presence or in the absence of millimolar levels of calcium and

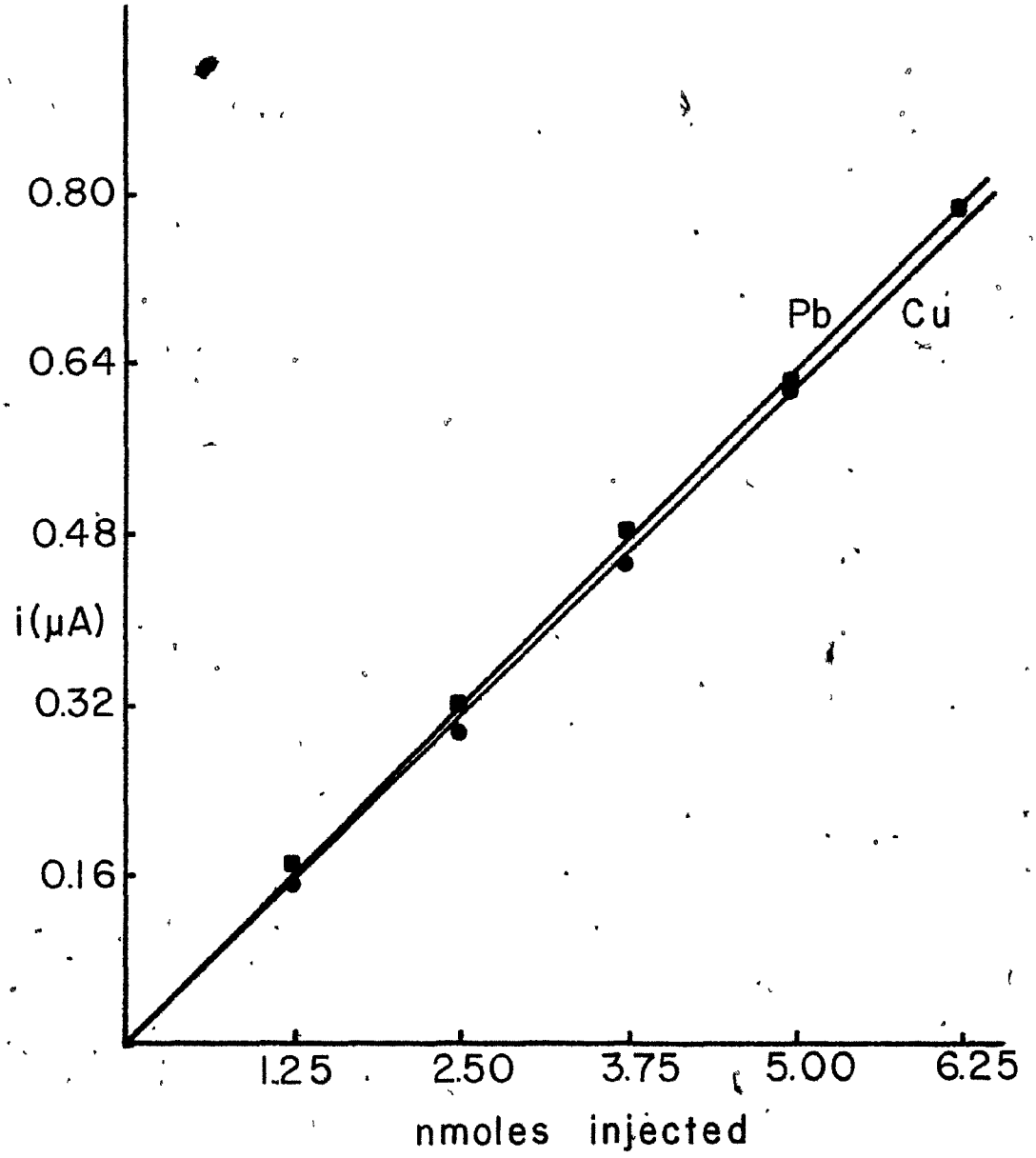


Fig. 2-16: FIA calibration curves using BAS-GCE detector.

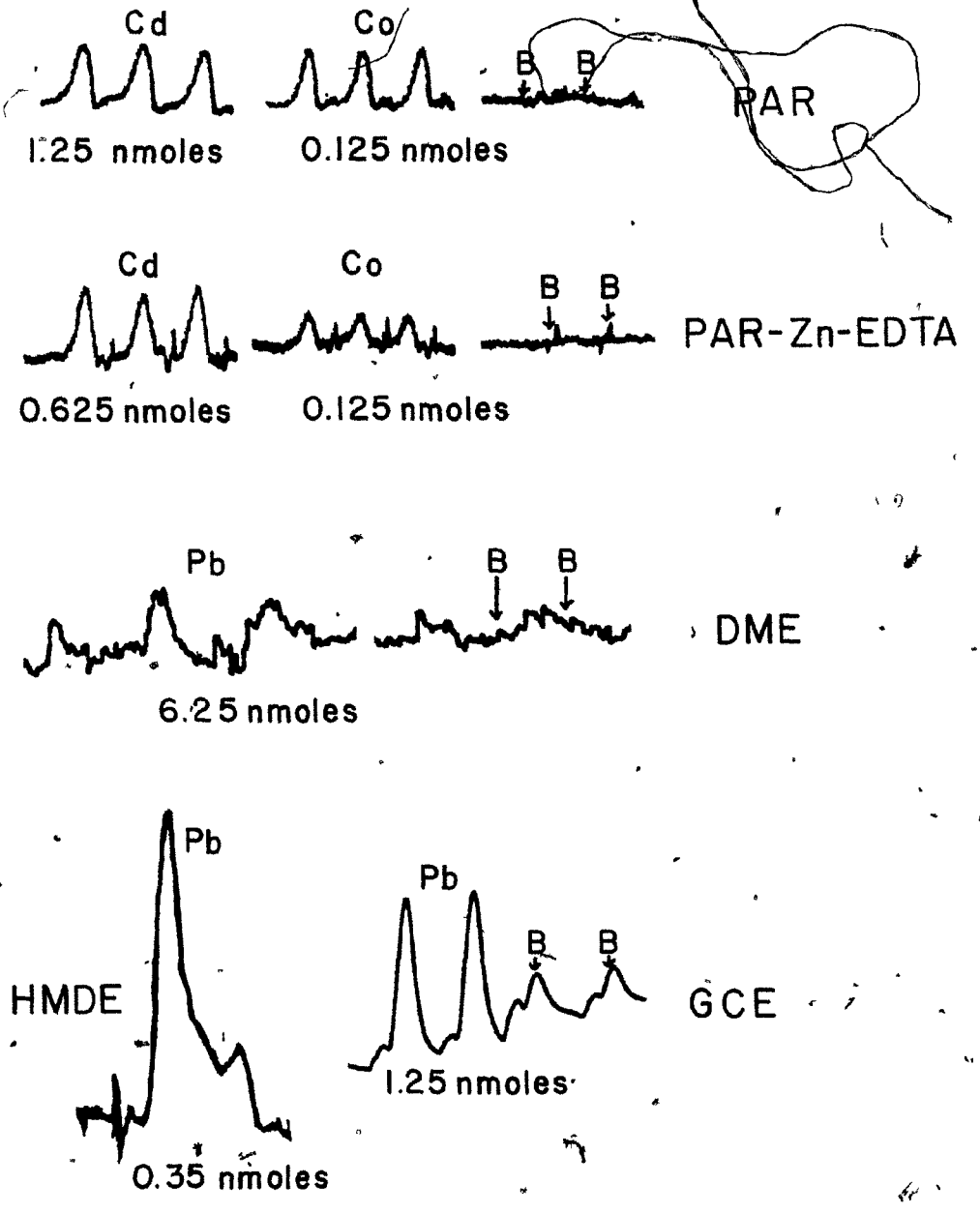


Fig. 2-17: FIA injections near detection limits for 5 detector systems.

Table 2-3

Flow Injection Analysis Detection Limits

M	PAR	PAR-Zn-EDTA	DME	HMDE	GCE
Co(II)	0.05	0.12	8	0.3	0.7
Ni(II)	0.06	0.12	110	9	0.8
Cu(II)	0.08	0.16	18	0.4	0.5
Zn(II)	0.09	0.23	8	0.2	0.5
Mn(II)	0.08	0.25	130	1.0	0.5
Pb(II)	0.45	0.25	6	0.2	0.5
Cd(II)	0.50	0.27	6	0.2	0.5
Ca(II)	-	0.29	-	-	0.7
Mg(II)	-	1.33	-	-	3.2

- detection limits in nmoles (2X blanks)

magnesium. The PAR-Zn-EDTA reagent, however, increased detection limits due to the presence of equilibrium free ligand.

The GCE detector gave the best results of the three EC detectors studied. It demonstrated comparable sensitivities to the photometric system 2 detector with FIA detection limits of approximately 0.5 nmoles for the more sensitive metals. It did have the advantage of linearity over the concentration range of interest.

CHAPTER 3

Liquid Chromatographic Separations

3.0 Introduction

Multication speciation requires an analytical system capable of selectivity, i.e., determining one metal in the presence of varying amounts of several other metals. Selectivity is often achieved chromatographically, isolating the compound or compounds of interest from matrices of varying complexities. Once the selectivity is accomplished, detection and determinations are carried out by the use of an appropriate detector, the subject of Chapter 2. This chapter will describe liquid chromatographic separations of mixtures of metal ions pertinent to multication speciation in model systems.

Liquid chromatography (LC) was first conceived and developed by Tswett at the turn of the century in the form of "open-column" liquid-solid or adsorption chromatography. Research over the following 50 years resulted in the development of various forms of liquid chromatography - partition, paper, thin-layer, ion-exchange and size exclusion or gel permeation. Although gas chromatography succeeded in displacing LC with respect to many separations of volatile organics, liquid chromatography remained the

method of choice when separating non-volatile or thermally unstable compounds and ionic species.

Before the development of modern liquid chromatography in the late sixties, metal ions were often separated by ion-exchange chromatography using large particle synthetic resins such as Dowex-AG50W. These separations were tedious and time-consuming and often required the use of several eluents. An example of this is the work reported by Kraus and Moore (1953). They separated mixtures of six transition metal ions by anion-exchange chromatography using a large particle (200-230 mesh) polystyrene-divinylbenzene (PS-DVB) strong-base resin (Dowex-1). The elution procedure involved six different concentrations of HCl, one for each metal ion. The procedure was further complicated by use of three different detection techniques: spectrography for Mn^{2+}, radiotracer analysis for Fe^{3+} and Zn^{2+} and colorimetry for Co^{2+}, Ni^{2+} and Cu^{2+}. More recently Seymour and Fritz (1973) and Fritz and Story (1974) reported the "forced-flow" chromatographic separations of mixtures of metal ions using either strong-acid or strong-base PS-DVB resins. Eluents were forced through the large particle resin beds by way of pressurized eluent tanks (90 psig). In the former study, 150-200 mesh Amberlyst A-26 anion-exchanger was used, with a different hydrochloric-perchloric acid mixture required for the

elution of each metal. Detection was by photometric determination of the chloro complexes at 225 nm. In the latter paper, the separation of 5 transition metals (Zn, Pb, Cu, Mn, Ni) was accomplished in only 10 minutes using partially sulfonated low capacity resins. However the separation required five different combinations of acetonitrile-hydrochloric acid. Eluted metals were determined photometrically after the post-column formation of PAR complexes.

The development of modern liquid chromatography permitted shorter analysis times through more efficient separations. This increase in efficiency was due to the utilization of smaller particle packings, typically 5-20 μm. The result was an increase in the number of plates per column and, therefore, better separations in less time.

The most common high performance separations of metal ions have been by ion-exchange chromatography. The packing materials consist of either polymeric porous resin particles (e.g., PS-DVB) or silica supports with a chemically or mechanically bonded organic substrate. In both cases, ion-exchange sites are introduced, typically $-SO_3^-$ for cation-exchangers and $-N(CH_3)_3^+$ for anion-exchangers. Transition metal ion mixtures were separated by Takata and Fujita (1975) under isocratic conditions

using various PS-DVB resins. They succeeded in separating Cu, Zn, Ni, Pb, Co and Cd in 7 minutes using 8-11 μm particles and tartrate eluent at 50°C. Cassidy and Elchuk (1980, 1981 a, b) used either tartrate or citrate under both isocratic and gradient conditions to separate mixtures of transition metal ions, e.g., Cu, Co, Zn, Pb, Fe, Mn, Mg, and Ca in 30 minutes. They reported that gradient conditions were preferable when looking at several metals whereas isocratic elution was preferable for maximum baseline stability.

In comparing bonded-phase ion-exchangers to conventional resin ion-exchangers, Elchuk and Cassidy (1979) and Cassidy and Elchuk (1981b) found that although bonded-phase columns gave slightly better peak shapes, their low capacities made them less convenient to use. An example of a bonded-phase anion-exchange separation is the work reported by Beckett and Nelson (1981). They used an anion-exchanger (Partisil 10 SAX) to separate mixtures of Pb, Cd and Zn using the pre-column formation of anionic polycarboxylic acid complexes of the metals. The 3-metal separation, however, required 24 minutes.

Liquid chromatographic separations of alkali metals, alkaline earths and many of the common anions have been reported by ion chromatography originally developed by Small, Stevens and Bauman (1975). This dual-column method

requires an ion-exchange separation of the components followed by elimination of background conductivity by way of a suppressor column. Until recently, the method had not been commonly used to separate transition metal ions because the hydroxide form of the suppressor column would precipitate most transition metal cations. As described in Chapter 2, Sevenich and Fritz (1983) eliminated the suppressor column and separated Zn, Co, Mn, Cd, Cu, Pb, and Sr in 15 minutes using a low capacity cation-exchange resin.

Adsorption or liquid-solid chromatography (LSC) has been used less frequently than ion-exchange chromatography to separate metal ions but has been used to separate neutral metal chelates. Lohmuller et al (1977) separated mixtures of three or four transition metals using the formation of their dithizonates (MDz). Silica was used as the adsorbent, benzene as the eluent, and the metal dithizonates were determined at 525 nm. Separations were not very good because of peak tailing and the separations required 20-40 minutes. Moriyasu and Hashimoto (1978) separated various mixtures of Hg, Cd, Pb, Cr, Bi, and Cu using the formation of their neutral diethyldithiocarbamates (DDTC). The separations used 10 μm silica particles, water saturated hexane as eluent, and spectrophotometric detection at 254 nm. O'Laughlin and

O'Brien (1978) used both DDTC and Dz chelates with 10 µm silica and toluene eluent. However, only 2-metal mixtures could be successfully resolved.

Liquid-liquid or partition chromatography has also been used to separate metal ions by HPLC. Separations are based on distributions of solute molecules between two immiscible liquids present as stationary and mobile phases. The stationary phase is either mechanically held onto an inert support, as in classical LLC, or chemically bonded, as in bonded-phase chromatography (BPC).

An example of LLC applied to the separation of metal ions was the work of Huber et al (1972). They separated neutral metal - β - diketonates using two ternary water -2,2,4-trimethylpentane-ethanol mixtures as stationary and mobile phases. The solid support consisted of 5-10 or 10-20 µm diatomaceous earth. Detection was at 310 nm. A separation of six metal-acetylacetonates (Be, Cu, Al, Cr, Ru, Co) was achieved in less than 25 minutes. Reverse-phase LLC has been used to separate metal ions by Uden et al (1975) and by Bond and Wallace (1982). Uden et al used 10 µm C_{18} columns to separate various Schiff base chelates followed by photometric detection at 254 nm. Only 3-metal separations were given, those of Pd, Cu and Ni. Bond and Wallace also used a C_{18} column to separate DDTC complexes

of Cr, Co, Ni and Cu. Resolution of the 4-component mixture required 16 minutes.

Ion-pair chromatography (also extraction chromatography, soap chromatography and chromatography with liquid ion-exchangers) has recently been developed as an alternative to ion-exchange chromatography. It is based on the formation of "ion-pairs", i.e., sample ions with eluent counter-ions. These "ion-pairs" appear to be resolved by either partition or ion-exchange processes, depending on the size and nature of the counter-ion. As an example, carboxylic acids ($RCOO^-$) can be separated by IPC using as counter-ion tetrabutyl ammonium and C_{18} reverse-phase columns. The eluent is an aqueous buffer solution. Separation is thought to be the result of partition of $[RCOO^- — TBA^+]$ between the C_{18} organic stationary phase and the aqueous buffer mobile phase. However in soap chromatography, where the counter-ions are usually much larger and are thought to be adsorbed onto the organic stationary phase, separation would result from ion-exchange at the liquid-liquid interface.

Ion-pair chromatography has been applied to the separation of metals using both mechanically-held and chemically-bonded organic phases. Horwitz et al (1976, 1977) reported the separation of radionuclides using 5 and 20 μm porous silica microspheres coated with 25-30% (w/w)

of di(2-ethylhexyl) orthophosphoric acid in dodecane. Nitric acid was used as the mobile phase. They separated ^{225}Ac and four daughter nuclides in less than 2 minutes using a short 1 cm column and four different concentrations of HNO_3. Recently, Cassidy and Elchuk (1982) described an IPC separation of transition metals using C_{18} phases bonded to both 5 and 10 μm silica particles in equilibrium with aqueous tartrate solutions (0.045 M) containing either 0.01 M $C_{20}H_{41}SO_4Na$ or $C_6H_{13}SO_3Na$ as counter-ions. The separation of Cu, Pb, Zn, Ni, Co, and Mn required only 10 minutes. Detection was by photometric determination of PAR complexes.

The more promising methods for the liquid chromatographic separation of metal ions are the ion-exchange and the ion-pair methods. The former is more adaptable to both photometric and electrochemical detection whereas the latter gave better resolution and analysis times. Earlier work on the detection systems suggested that a preconcentration method would be necessary before the separation. The Donnan dialysis method to be reported in Chapter 4 requires high salt or complexing ligand concentrations to be effective. This limited the choice of separation to ion-exchange and this chapter summarizes the methods used for the divalent metal ions of interest.

3.1 Experimental

The ion-exchange experiments were done using the arrangement described in Figure 2-1. Two modifications were made to adapt the FIA system to the chromatographic applications. The connection between sample valve and plastic tee was replaced with a column packed with ion-exchange resin. The selection of eluent was facilitated by using a low pressure Altex 6-way Rotary Selection Valve (Rainin Inst. Co., Woburn, Mass.) between the eluent reservoirs and pump inlet. This permitted one to select any one of six possible eluents for step-gradient chromatography.

3.1.a. Reagents: Eluents were prepared from either tartaric acid (Analar, B.D.H.) or anhydrous citric acid (Baker Analyzed Reagent) dissolved in glass distilled water. Inorganic salts to modify the eluents were $NaNO_3$ (ACS Assured, B.D.H.), NaCl (Certified ACS, Fisher) and NaOH (ACS Assured, B.D.H.). All eluents were adjusted to appropriate pH, filtered through 0.2 µm Nuclepore membrane filters and degassed for five minutes using a water aspirator.

Packed ion-exchange columns were prepared using either Aminex A-9 (11.5 ± 0.5 µm) or Aminex A-8 (7.0 ± 1.0 µm) supplied in the sodium form by Bio Rad Laboratories

(Richmond, Calif.). The columns were slurry packed in eluent using an air-driven Haskel pump. The resin suspension in the eluent was ultrasonicated and added to a 13.0 mL reservoir. The resin was transferred from the reservoir to the empty column using an initial pump pressure of 300 psi. The column packing was equilibrated by passing eluent through the column and gradually increasing the pressure to 4500 psi in steps of 500 psi over a period of 30 minutes. Packed columns were stored in a refrigerator (-20°C) between use to inhibit bacterial growth.

After extensive use (three to six months) or to change counter-ions, the resins were regenerated to remove metal impurities. The resins were successively equilibrated with 0.10 M EDTA, 6 M HCl and 2 M base of the appropriate cation (i.e., NaOH for sodium form resin or NH_3/NH_4NO_3 for ammonium form resin). The resins were equilibrated with fresh eluent before packing.

3.2 Results and Discussion

The objective of the study was to develop a rapid exchange separation suitable for future multication speciation studies. The metals of interest-Cu, Zn, Pb, Ni, Co, Mn and Cd at the micromolar level and Ca and Mg at the millimolar level - should be analyzed in the same

separation, if possible. The minimum requirement (for model studies) is to determine two metals in the first group and one in the second group. This would be useful for studying the effect of macro concentrations of alkaline earths on the trace metal binding.

Ion-exchange separations are controlled by the distribution coefficient for the exchange of a metal ion between solution and exchanger. For an exchange reaction

$$M_1^{2+} + 2M_2R \rightleftharpoons M_1R_2 + 2M_2^{+} \qquad (3\text{-}1)$$

in the presence of a complexing buffer, the distribution coefficient D is given by (using the formalism of Ringbom (1979)):

$$D = \left[\frac{[M_2]_R}{[M_2]_a}\right]^2 \frac{K^{M_1}_{2M_2}}{\alpha_{M_1L}} \qquad (3\text{-}2)$$

where $[M_2]_a$ and $[M_2]_R$ represent the concentrations of the metal in solution and resin, respectively; $K^{M_1}_{2M_2}$ represents the selectivity coefficient for the resin, and α_{M_1L} represents the masking reaction between M_1^{2+} and a complexing ligand:

$$\alpha_{M_1L} = \frac{[M_1] \text{ not on resin}}{[M_1^{2+}]} = \frac{[M_1^{2+}] + [M_1L] + [M_1L_2] + \ldots}{[M_1^{2+}]}$$

$$= 1 + \beta_1[L] + \beta_2[L]^2 + \ldots. \qquad (3\text{-}3)$$

The elution volume for a metal ion is given by:

$$V_e = D \cdot g + V_o \qquad (3\text{-}4)$$

where g is the weight of resin in grams and V_o is the void volume of the column in milliliters. It is evident from these expressions that the elution volume depends on the capacity of the resin $[M_2]_R$, the concentration of the counter-ion in solution $[M_2]_a$, and the concentration of free complexing ligand in solution. The concentration of free ligand in solution can be controlled by adjusting the pH of the eluent.

The ability of an ion-exchange system to resolve metal ion mixtures depends on two factors: the separability of the metal ion pairs (Ve_1/Ve_2; Ve_2/Ve_3, etc) and the number of plates on the column. The plate height of a separation system is proportional to the particle diameter - the smaller the resin particle size, the smaller the plate height and the greater the number of plates per column length. The separability of metal ion pairs obviously depends on the distribution coefficients. The principal control for the separation (both elution volumes and resolution) is to alter $\alpha_{M_1 L}$ by either choice of ligand or by varying the pH. The remainder of this chapter summarizes the development of a number of separation systems suitable for speciation studies.

3.2.a. Tartaric acid eluents: The first exchange system studied used the Aminex A-9 resin and ammonium tartrate eluents. Figure 3-1 presents the separation of a Cu, Zn, Pb, Co and Cd mixture using a 0.10 M ammonium tartrate/0.10 M NH_4NO_3 eluent at pH 4.6. The metal ions are well resolved but Ni could not be determined because it eluted after Zn but before Pb. The other problem was the excessively long retention time for cadmium. The elution time for cadmium could be reduced by the addition of chloride ion to the eluent. Elution times for the different ions are presented in Table 3-1 as a function of chloride ion concentration.

To improve the separation between Zn and Pb and allow the inclusion of Ni would require adjusting D for each ion. The stability constants for Zn, Ni and Pb complexes with tartrate are logK = 2.4, 2.1 and 3.8, respectively. Lead forms a more stable complex and its elution volume should be most susceptible to changes in solution pH. A difficulty was noted with the ammonium tartrate system - ammonium hydrogen tartrate is relatively insoluble and was not suitable for further studies as an eluent. Sodium tartrate was used for further studies of pH on the elution volume.

Figure 3-2 presents the results for a separation of Cu, Zn, Ni, Pb, Co, and Cd using 0.20 M sodium tartrate at

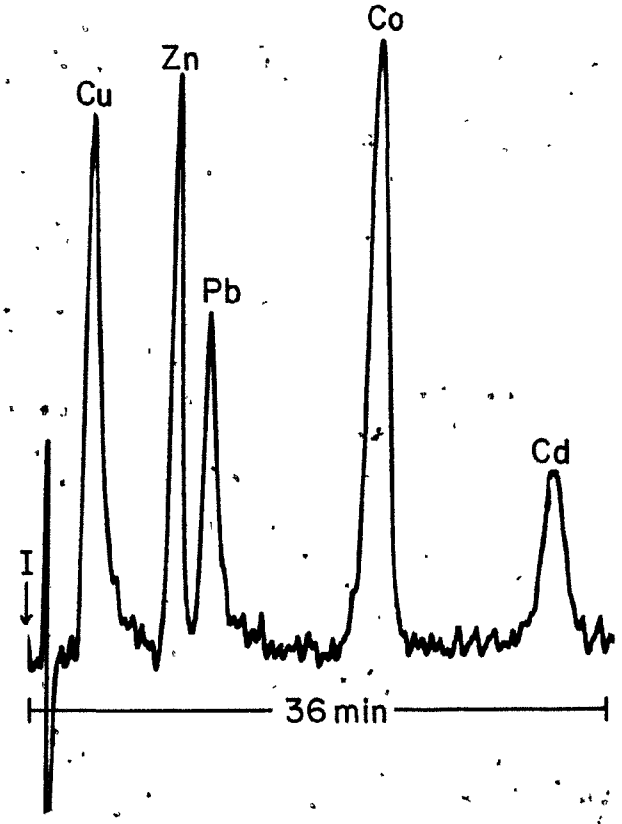


Fig. 3-1: Isocratic A-9/NH_4-tart. separation

eluent - 0.10 M NH_4-tartrate (pH = 4.60)/0.10 M NH_4NO_3
column - 12.5 cm x 4.2 mm Aminex A-9
flow rate - 1.25 mL/min
injection - 1 mL of 5 µM Cu, Zn, Pb, Co; 10 µM Cd
detector - W-SiP system 2 with PAR-Zn-EDTA and Sage delivery system

Table 3-1

Effect of $[Cl^-]$ on t_R

Metal Ion	0.2M NH_4-tart 0.05M NH_4Cl	0.2M NH_4-tart 0.075M NH_4Cl	0.2M NH_4-tart 0.10M NH_4Cl
Cu(II)	3:10	2:50	2:45
Zn(II)	6:45	5:05	4:30
Pb(II)	8:55	6:37	5:40
Co(II)	16:03	11:28	9:40
Cd(II)	19:00	12:32	9:45

- t_R = retention time (min:sec)

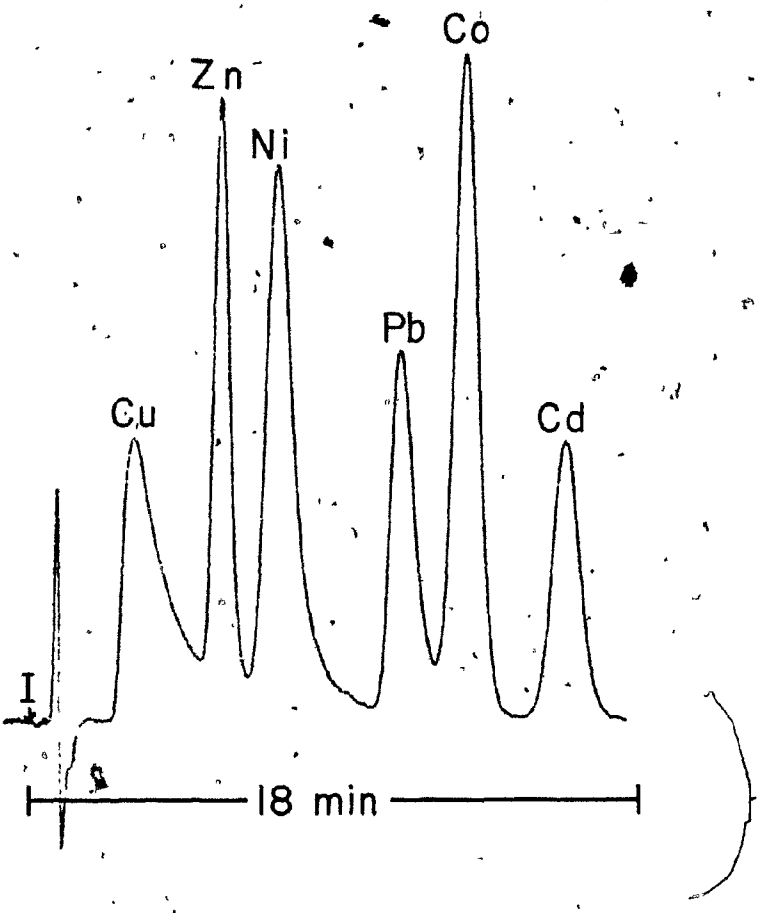


Fig. 3-2: Isocratic A-9/tartrate separation.

eluent - 0.20 M Na-tart (pH = 3.74)/0.05 M NaCl/0.056 M $NaNO_3$
column - 20 cm x 4.2 mm Aminex A-9
flow rate - 1.2 mL/min
injection - 1 mL of 3 µM Cu, Zn, Ni, Co; 6 µM Pb, Cd
detector - W-SiP system 2 with PAR-Zn-EDTA reagent

pH 3.74. Adequate resolution was obtained for the separation but copper gives a poorly shaped peak. A possible explanation for the poor copper peak shape is the presence of strong copper binding sites on the resin. The peak shape rises sharply and exhibits significant tailing. This behavior is typical of a non-linear adsorption isotherm with a mixture of sites. The tracings of copper peaks given in the inset of Figure 3-5 support this hypothesis. At low copper concentrations, the peak shape is more symmetrical suggesting a linear isotherm binding region for the strong sites. The analysis time of 18 minutes for this resin could not be shortened without decreasing the resolution of the separation.

A second resin - Aminex A-8 - with a smaller particle size was used to improve the resolution and allow a shorter analysis time. Figure 3-3 presents the chromatogram for the separation using Aminex A-8 and 0.33 M sodium tartrate at pH 3.65. This system was capable of separating the six ions in 14 minutes with adequate resolution. The difficulty of the Aminex A-8 system was a high backpressure which required a large diameter column (8 mm I.D.) and a large amount of resin. The Aminex A-8 resin increased in backpressure over time, increasing from 2600 psi to 4500 psi in 4-6 weeks. The Aminex A-9 columns could be used for 3-6 months before the build up in backpressure

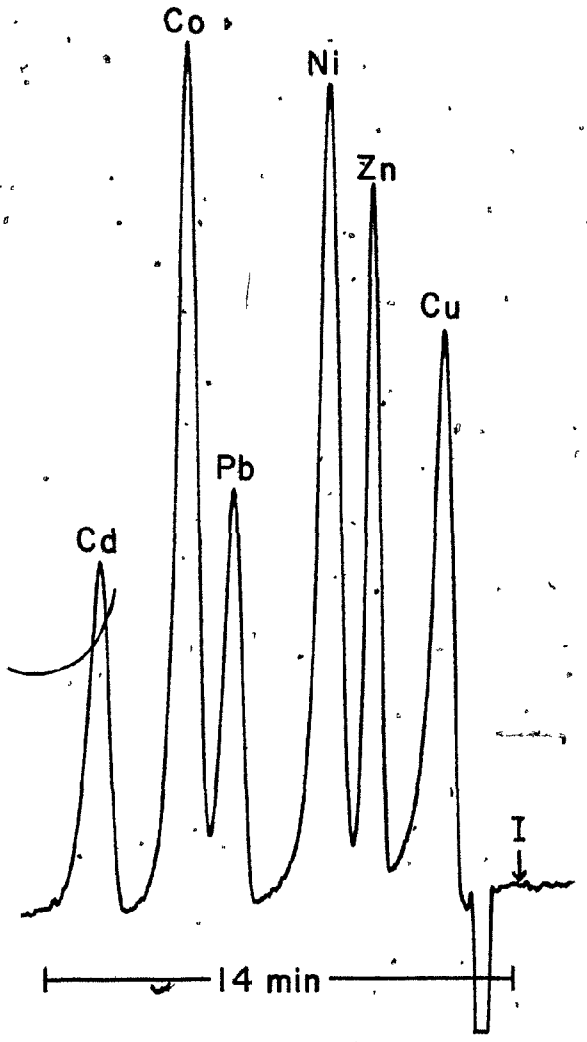


Fig. 3-3: Isocratic A-8/tartrate separation.

eluent - 0.33 M Na-tart (pH = 3.65)/0.042 M NaCl/0.05 M $NaNO_3$
column - 10 cm x 8 mm Aminex A-8
flow rate - 1.2 mL/min
injection - 1 mL of 3 µM Cu, Zn, Ni, Co; 6 µM Pb, Cd
detector - W-SiP system 2 with PAR-Zn-EDTA

required repacking. The difficulty with the Aminex A-8 resin suggested that the Aminex A-9 resin would be the better system for routine studies.

The optimized 6-metal separation presented in Figure 3-2 was used to characterize the detection limits for the separation. Figure 3-4 gives the calibration curves for five of the six metals and the inset gives the chromatogram for the separation near the detection limits. The detection system used for this study was the PAR-Zn-EDTA reagent and system 2 photometric detector. The calibration curves for these five metals were linear but the copper calibration curve was non-linear. Figure 3-5 presents the calibration curves for single injections of copper using both peak height and peak area measurements. The peak area calibration curve was found to be linear which suggests that the problem is a result of poor peak shapes.

Reproducibility studies using 6x5 nmole injections gave relative standard deviations ranging from 0.5% to 1.6%. The detection limits were 0.5 nmole Cu, Cd and Pb, and 0.25 nmole Zn, Ni and Co. These results are twice those obtained by flow injection analysis because of the increased dispersion in the ion-exchange column.

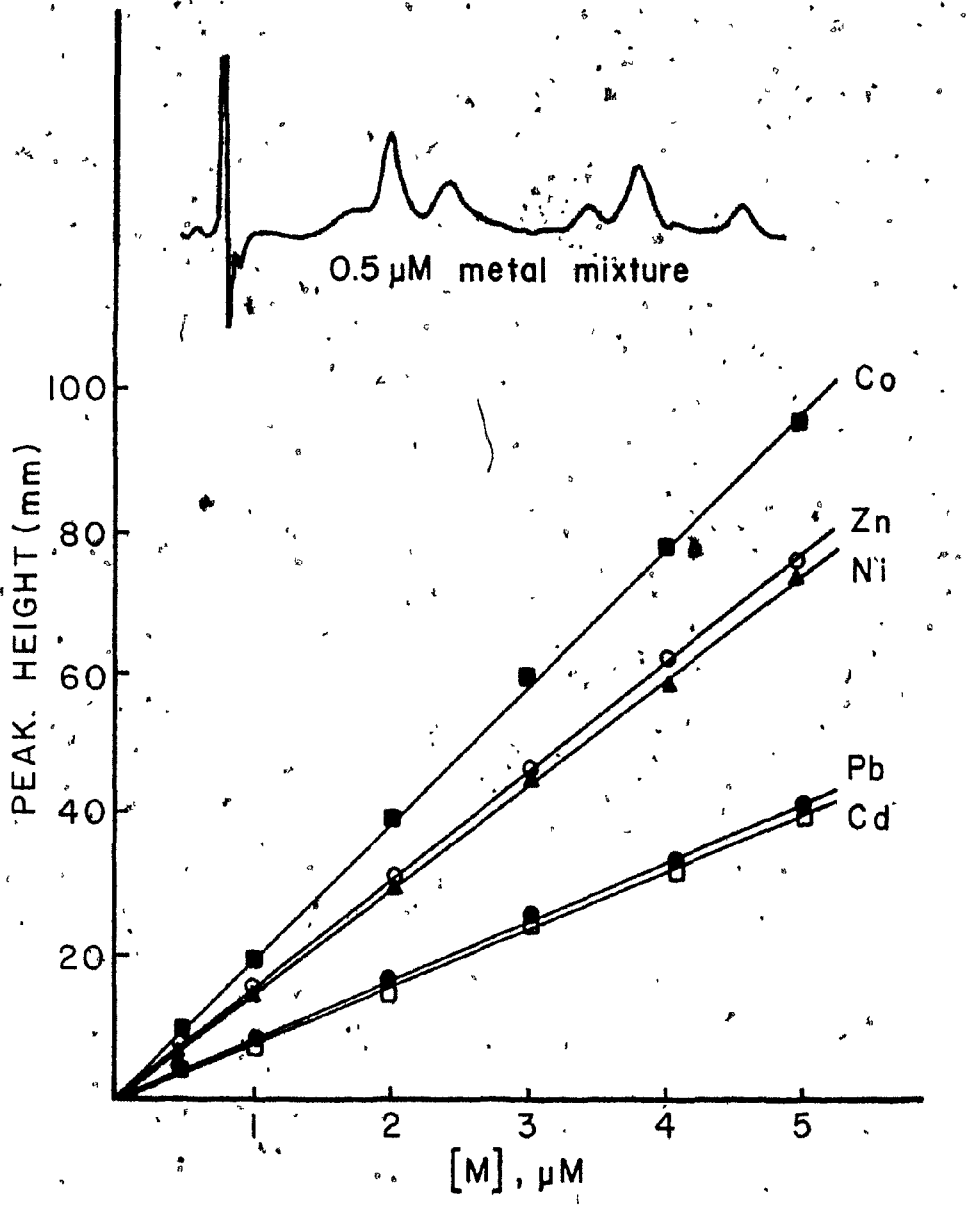


Fig. 3-4: Calibration curves with A-9/tart. - conditions same as in Fig. 3-2.

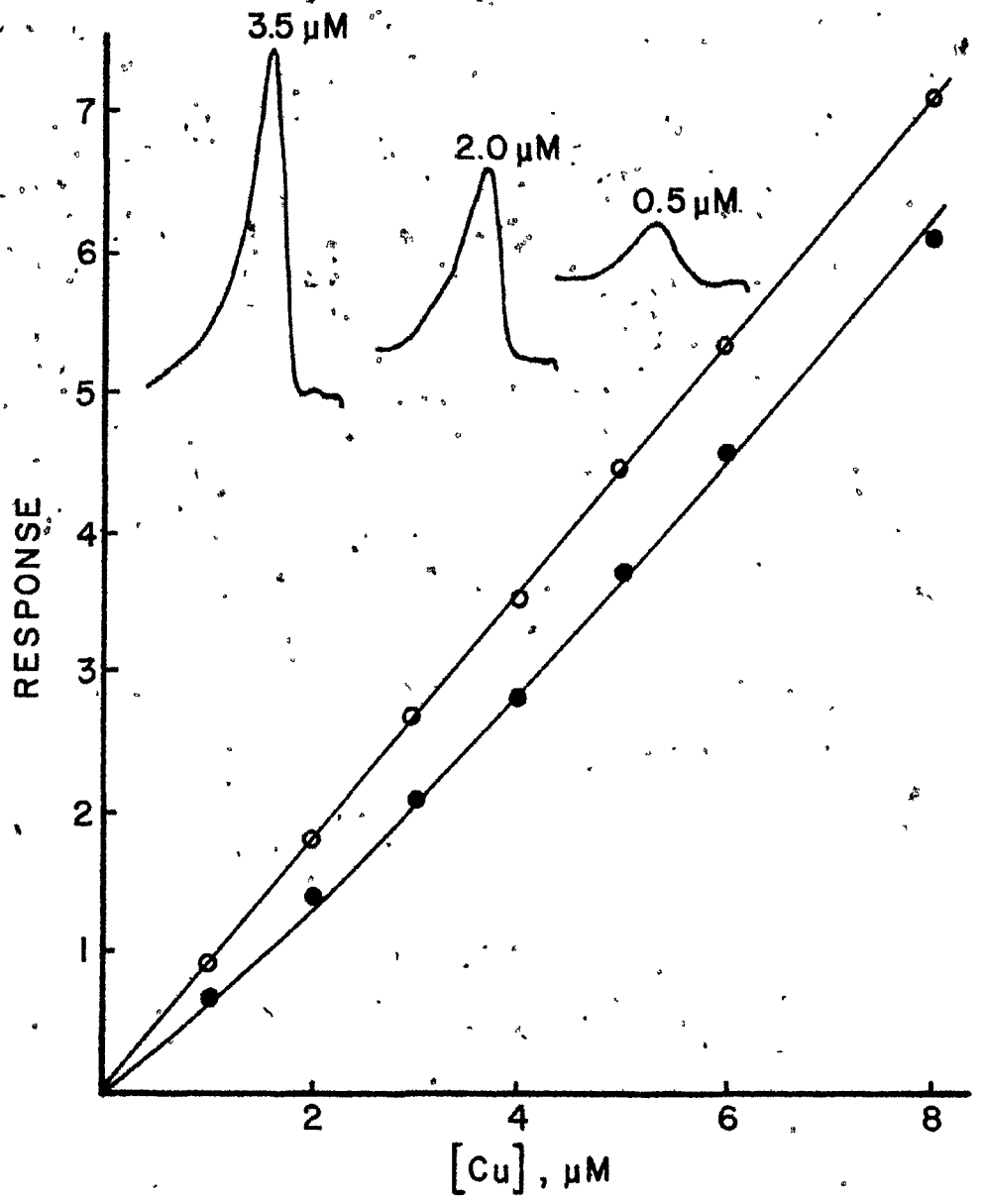


Fig. 3-5: Copper calibration curves.

- conditions same as in Fig. 3-2.
- 1 unit = 20 mm (peak height, ●); 5.0 mg (area, o).

3.2.b. Citric acid eluents: Tartaric acid was not strong enough to elute calcium and magnesium within 40 minutes. To lower the elution times for the alkaline earths required a stronger complexing agent to increase the value of α_{M_1L} in equation 3-2. Citric acid forms much stronger complexes with these metal ions but will not permit the analysis of all of the transition metals of interest. This is illustrated in Figure 3-6 which shows the chromatogram for Cu, Cd, Mn, Mg and Ca using Aminex A-9 and 0.30 M sodium citrate at pH 4.3. The metals Cu, Co, Ni, Zn, and Pb eluted at approximately the same time and could not be resolved. This exchange system would only be suitable for model studies involving only one metal from Cu, Pb, Zn, Ni, and Co with Cd, Mn, Mg, and Ca.

Figures 3-7 and 3-8 present the calibration curves and a chromatogram for the metals near the detection limits using the citrate system. Copper, cadmium and manganese gave linear calibration curves over the range of 0 to 25 μM (or nmoles). Magnesium and calcium, however, were non-linear over the range of interest for model studies (0-250 and 0-1250 μM for Ca and Mg, respectively). This non-linearity is a result of the exchange reaction in the detector where the calcium and magnesium concentrations are of the same order as the Zn-EDTA and PAR in the reagent. Reproducibility studies using 6 injections of 10 nmoles Cu,

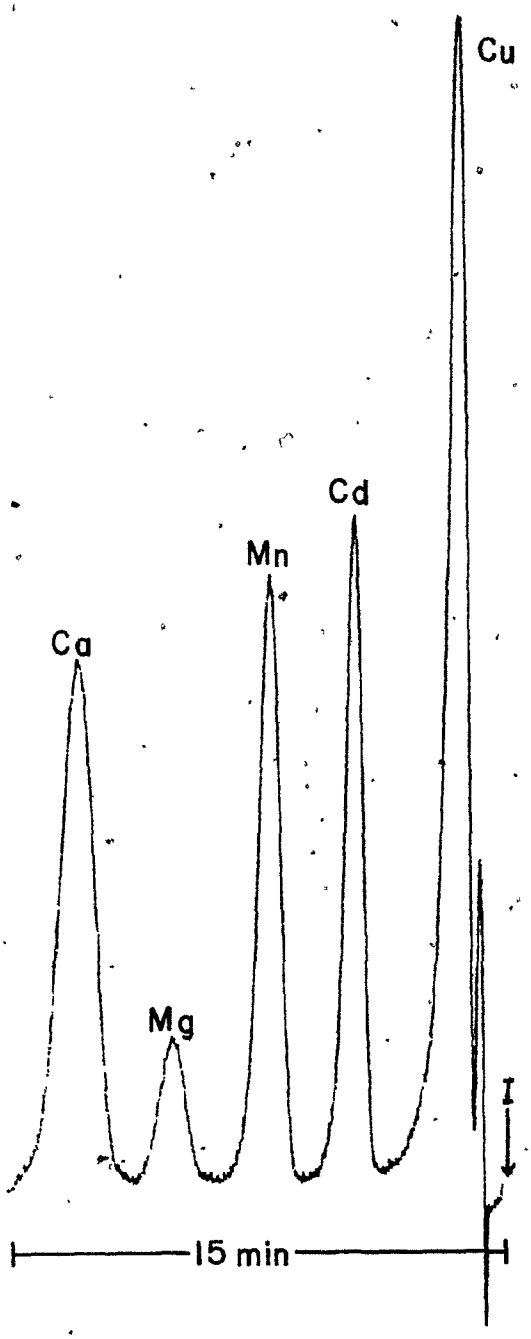


Fig. 3-6: Isocratic A-9/citrate separation.

eluent - 0.30 M Na-citrate (pH = 4.30)
column - 20 cm x 4.2 mm Aminex A-9
flow rate - 1.00 mL/min
injection - 1 mL of 10 µM Cu, Cd, Mn; 500 µM Mg; 100 µM Ca
detector - W-SiP system 2 with PAR-Zn-EDTA reagent

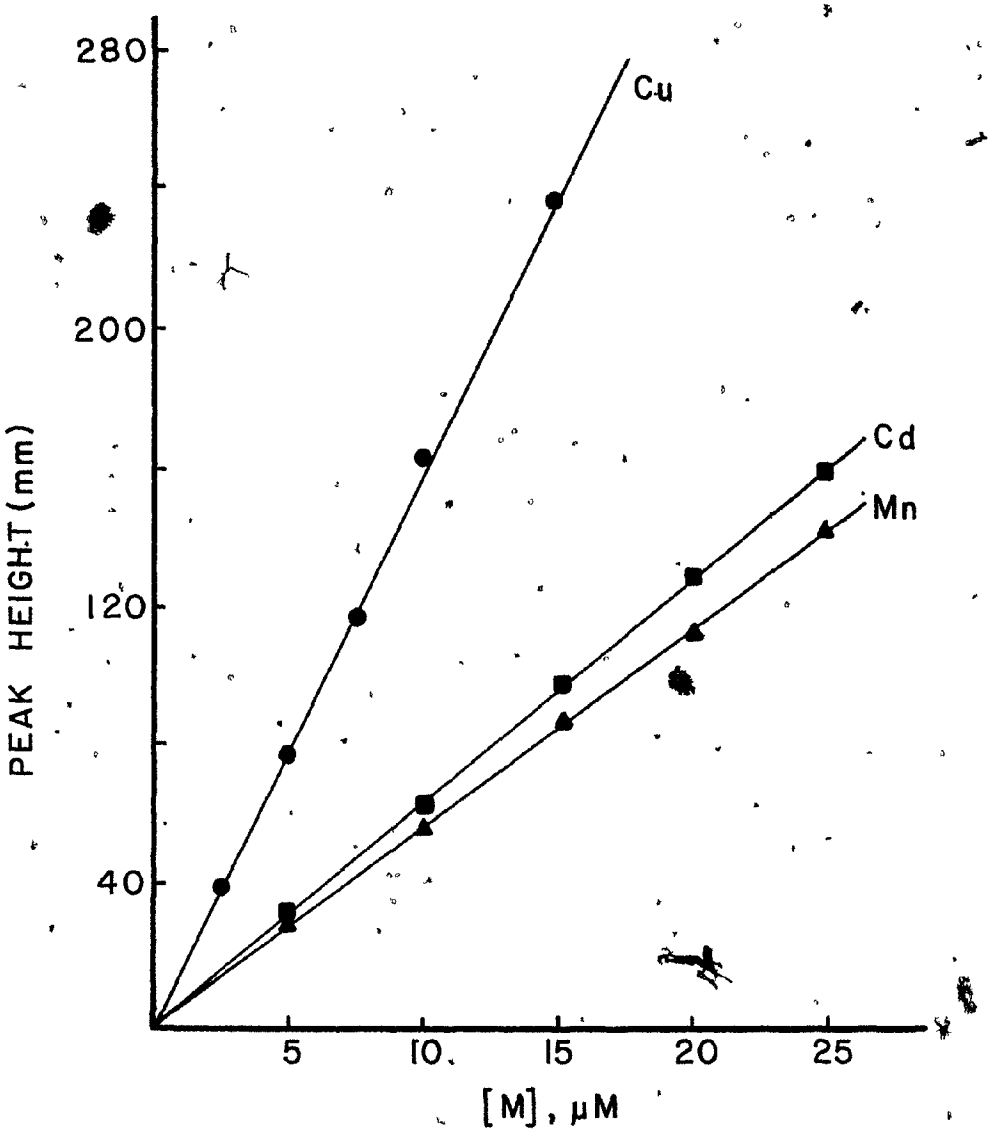


Fig. 3-7: Calibration curves with A-9/citrate.
- conditions same as in Fig. 3-6.

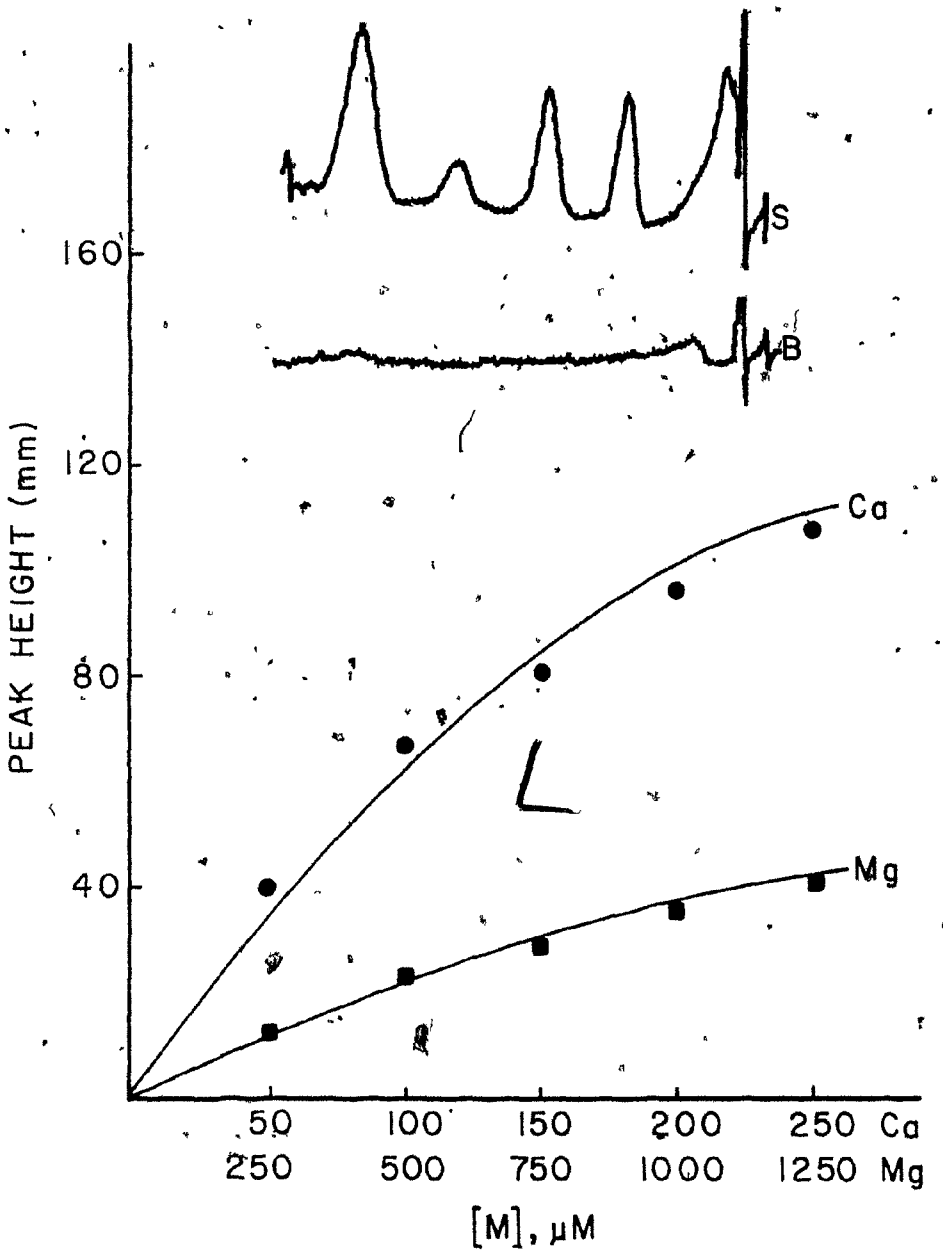


Fig. 3-8: Calibration curves with A-9/citrate.

- conditions same as in Fig. 3-6.
- B = blank ; S = 2 µM Cu, Cd, Mn; 20 µM Ca; 100 µM Mg.

Cd and Mn and 100 nmoles of Ca gave relative standard deviations of 2.5%, 0.5%, 1.2% and 1.3%, respectively. The detection limits were found to be 0.5 nmoles Cu, 0.25 nmoles Cd and Mn, 5 nmoles Ca and 25 nmoles Mg. These values compare favorably with the values determined by flow injection analysis.

3.2.c. Step-gradient systems: The citric acid eluent was too strong to permit the separation of all of the ions of interest in one chromatogram. One way to increase the number of metals separated in a chromatogram is to do a gradient elution with increasing eluent strength. The possibility of using a step-gradient system for the separation was investigated to evaluate the possibility of determining the alkaline earths in the same chromatogram as the transition metal ions. Figure 3-9 illustrates the advantage of the step-gradient system for the ammonium tartrate eluent. The step-gradient allows one to shorten the elution time for cadmium without adding chloride ion but does not allow one to determine Ca, Mg or Ni.

Figure 3-10 presents the results of a 2-step gradient chromatogram for seven metal ions. A low citrate eluent (0.10 M, pH 4.3) permits the elution of cupric ion and a more concentrated eluent (0.30 M, pH 4.3) resolves the

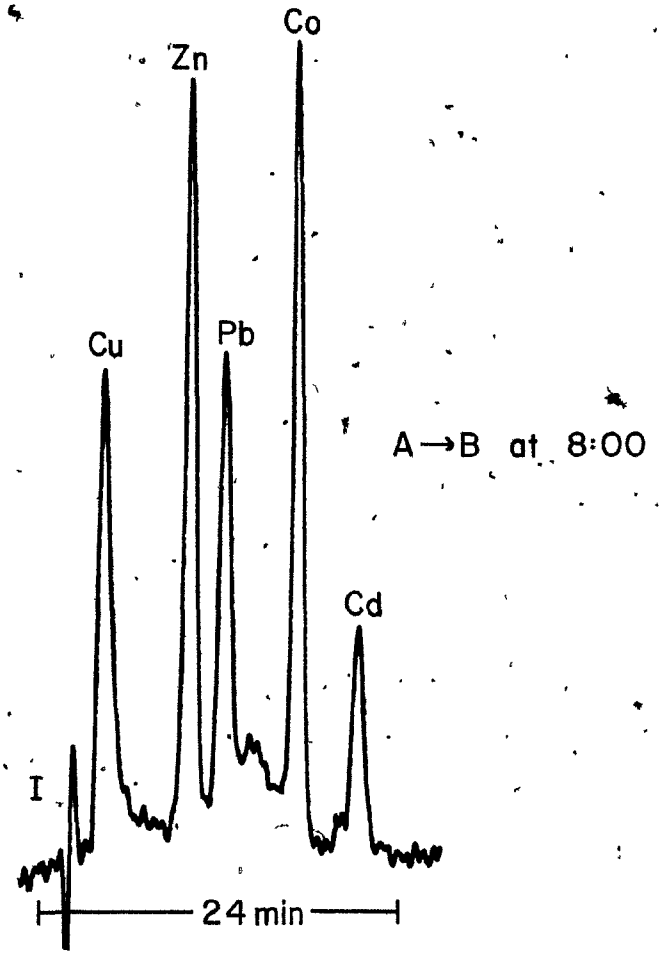


Fig. 3-9: Two-step NH_4-tart gradient separation.

eluents - A = 0.125 M NH_4-tart (pH = 4.6) + 0.075 M NH_4NO_3; B = 0.20 M NH_4-tart (pH = 4.6)

column - 12.5 cm x 4.2 mm Aminex A-9

flow rate - 0.75 mL/min

injection - 1 mL of 5 µM metal

detector - W-SiP system 2 with PAR-Zn-EDTA and Sage delivery system

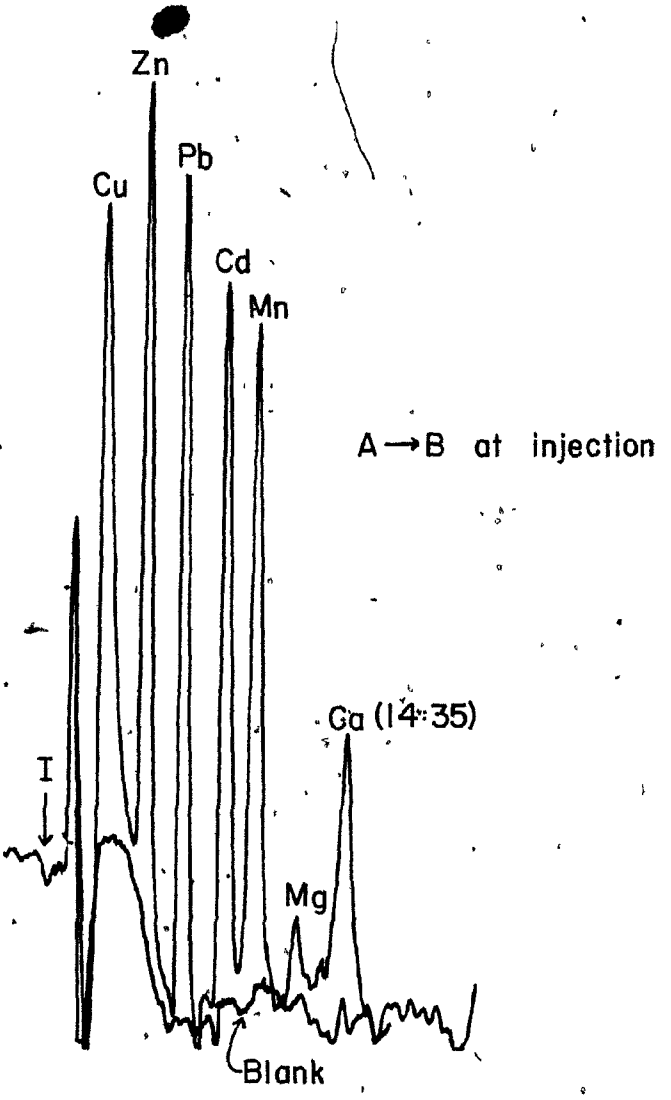


Fig. 3-10: Two-step citrate gradient separation.

eluents - A = 0.10 M Na-Cit (pH = 4.3); B = 0.30 M Na-Cit (pH = 4.3)
column - 20 cm x 4.2 mm Aminex A-9
flow rate - 1.25 mL/min
injection - 1 mL of 10 µM Cu, Zn, Pb, Cd, Mn; 50 µM Ca; 500 µM Mg
detector - W-SiP system 2 with PAR-Zn-EDTA and Sage delivery system

remaining ions. This system would allow the analysis of one of copper or nickel, one of zinc or cobalt, and Pb, Cd, Mn, Mg, and Ca in 22 minutes.

Gradient separations suffer from two problems - a variation in baseline and the possibility of preconcentration of contaminants at low eluent concentrations. Figure 3-11 presents blank and sample chromatograms for 3-step gradient citrate separations that illustrate both of these problems. This procedure applied to a blank run gave a reproducible but varying baseline due to changes in background absorbance associated with changes in citrate concentration. This decrease in baseline stability must ultimately lead to greater irreproducibility. A second disadvantage observed in our 3-step system was the appearance of a calcium contaminant not present in the isocratic or 2-step separations. It is thought that this calcium was present in trace quantities in the citrate eluent. The trace calcium was preconcentrated onto the Aminex A-9 column during the first step and was being eluted during the third step. Step-gradient did permit one to determine the metal ions in a single chromatogram but the error introduced by the contaminant, the demands of precise timing and variable

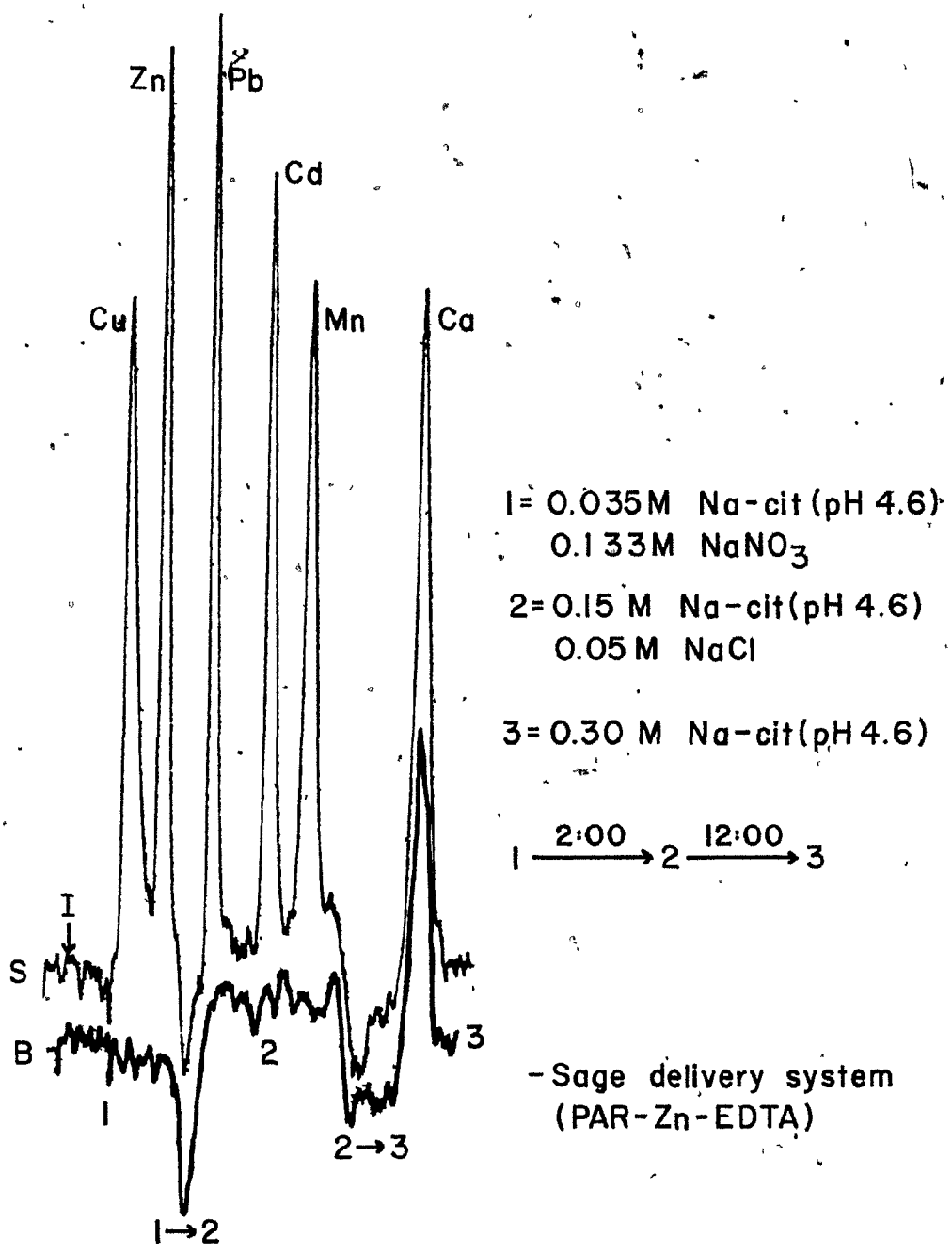
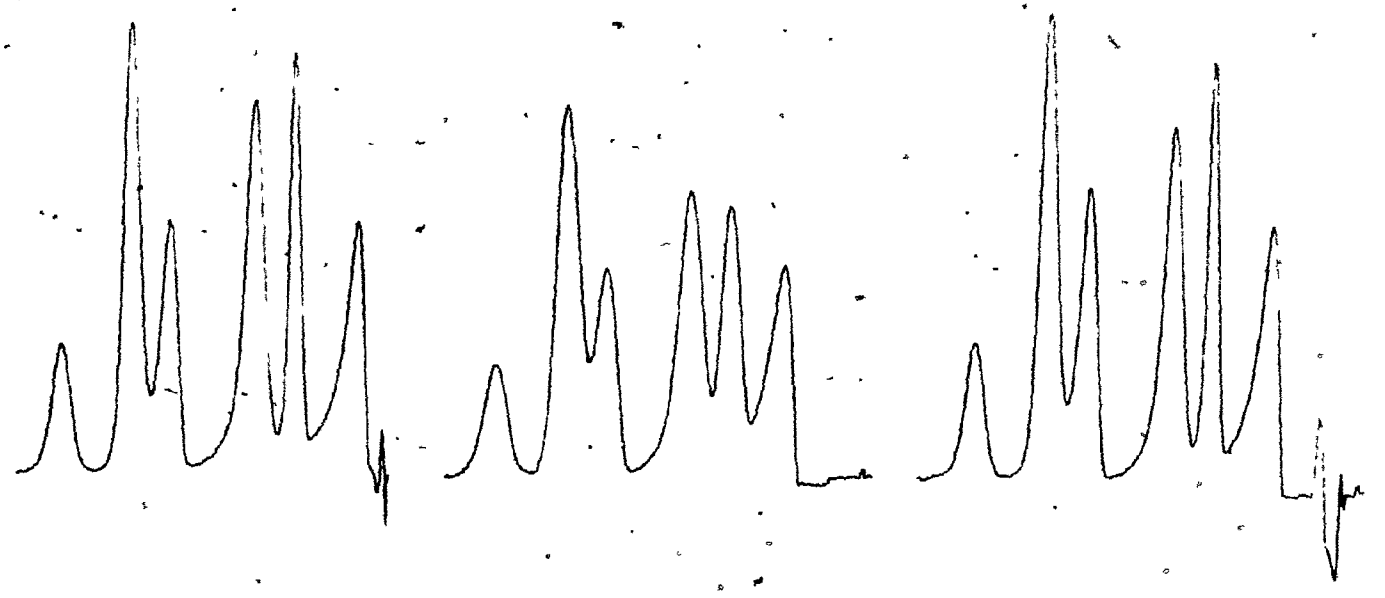


Fig.3-11: Blank and sample chromatograms with a three-step gradient procedure.

baseline indicated that it was more efficient for model studies to use the isocratic citrate system for alkaline earth analysis.

3.2.d. Injection variables: Chromatographic resolution can be affected by the characteristics of the sample solution. Figure 3-12 presents chromatograms of identical 6-metal mixtures (Cu, Zn, Ni, Pb, Co, Cd) made up in three different sample matrices. A good separation was obtained when the sample medium was acetate buffer (Figure 3-12a) but a considerable loss of resolution occurred when the sample was in the tartrate matrix (Figure 3-12b). The principal reason for the difference was the "effective" injection volume: when the sample was injected in acetate buffer, the sample ions were preconcentrated at the head of the column but, in the tartrate system, the actual injection volume was 1.0 mL with no preconcentration. When the pH of the tartrate was lowered, the tartrate eluting power decreased, a similar preconcentration occurred and the resolution was improved. This is illustrated in Figure 3-12c.

Further evidence of the effect of sample matrix on chromatographic behavior is shown in Table 3-2. A comparison of retention times indicates that samples made up in tartrate elute more quickly than those made up in



c = 0.20 M tartrate (pH 2.0) b = 0.20 M tartrate (pH 3.74) a = 0.005 M acetate (pH 4.5)

Fig. 3-12: Effect of sample matrix on chromatographic resolution.

- conditions same as in Fig. 3-2

Table 3-2

Effect of sample matrix on t_R and N

Vx C = 5 nmoles	t_R			N		
	A	B	C	A	B	C
1 mL x 5 µM Zn	6:34	6:00	6:31	749	226	769
100 µL x 50 µM Zn	5:40	5:34	5:38	441	561	469
20 µL x 250 µM Zn	5:35	5:37	5:32	467	532	516

- t_R = retention time (min:sec)
- N = # plates, calculated using $16(t_R/W_b)^2$
- A = sample in 5×10^{-3} M acetate buffer, pH = 4.5
- B = sample in tartrate eluent, pH = 3.74
- C = sample in acidified tartrate eluent, pH = 2.0

either acetate or acidified tartrate medium. This observation is most evident with 1 mL sample volumes. Furthermore, peak broadening is obtained when large volumes (i.e., 1 mL) of tartrate sample are injected, as seen by comparing values of N in Table 3-2 and in Figure 3-12b. These results suggest that for chromatographic resolution to be retained, the sample matrix must be non-eluting in nature (e.g., distilled H_2O, acetate buffer, 0.1 M $NaNO_3$). In some cases, chromatographic resolution may be restored by adjustment of sample pH.

3.2.e. Ion-exchange detector systems: Both PAR and PAR-Zn-EDTA have been used with the system 2 photometric detector to analyze multication mixtures separated by ion-exchange chromatography. As previously indicated, the PAR-Zn-EDTA reagent significantly increases sensitivities for certain metals. However, in tartrate separations where Ca contamination is present, the use of the PAR reagent is to be preferred. An example illustrating this point is shown in Figure 3-13 where 6-metal mixtures in the presence of a Ca contaminant were analyzed using both PAR and PAR-Zn-EDTA reagents. As expected, the Ca contaminant observed with the PAR-Zn-EDTA reagent was not detected when PAR was used. These calcium contamination peaks were observed either when

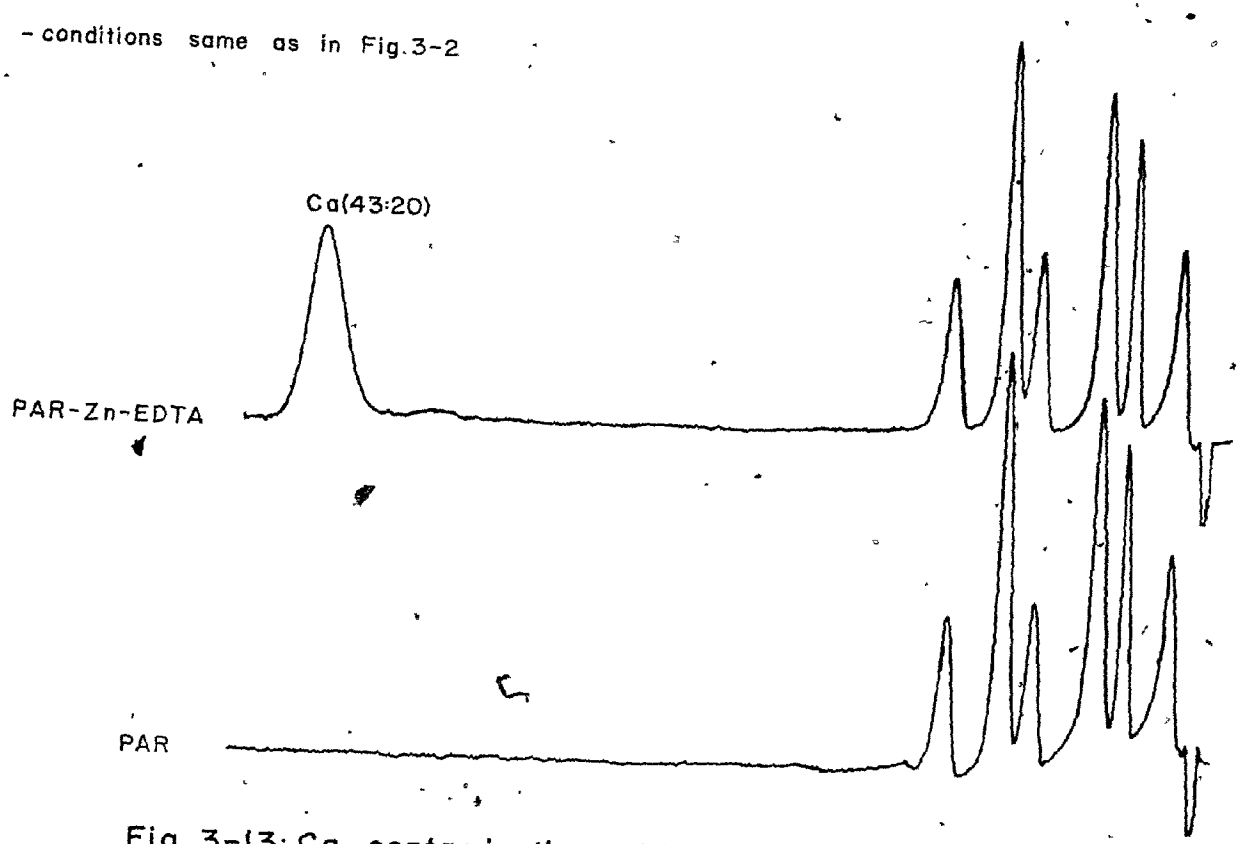


Fig. 3-13: Ca contamination with PAR and PAR-Zn-EDTA.

large injection volumes were used or when a pre-column preconcentration step had been included before analysis. This will be illustrated in Chapter 4.

An example of the same 6-metal mixture determined using the GCE/DTPA detector system is shown in Figure 3-14. When compared to the photometric detector systems, Pb and Cd responses were improved relative to those of the other four metals. It should also be noted that the Ca contamination problem discussed above will be more severe with the GCE/DTPA detector system. This is due to its greater relative sensitivity to calcium than that shown by the PAR-Zn-EDTA photometric detector system.

3.3 Conclusions

Three ion-exchange separations were found to be suitable for model speciation studies. A tartrate system permitted the analysis of 6-metal mixtures in 18 minutes. However, strongly retained metals such as Mn, Mg and Ca could not be analyzed in acceptable separation times. These metals were analyzed using a citrate system which also included two of the weakly retained metals. A third separation using a 2-step citrate system permitted the resolution of 7 metals (Cu, Zn, Pb, Cd, Mn, Mg, and Ca) but

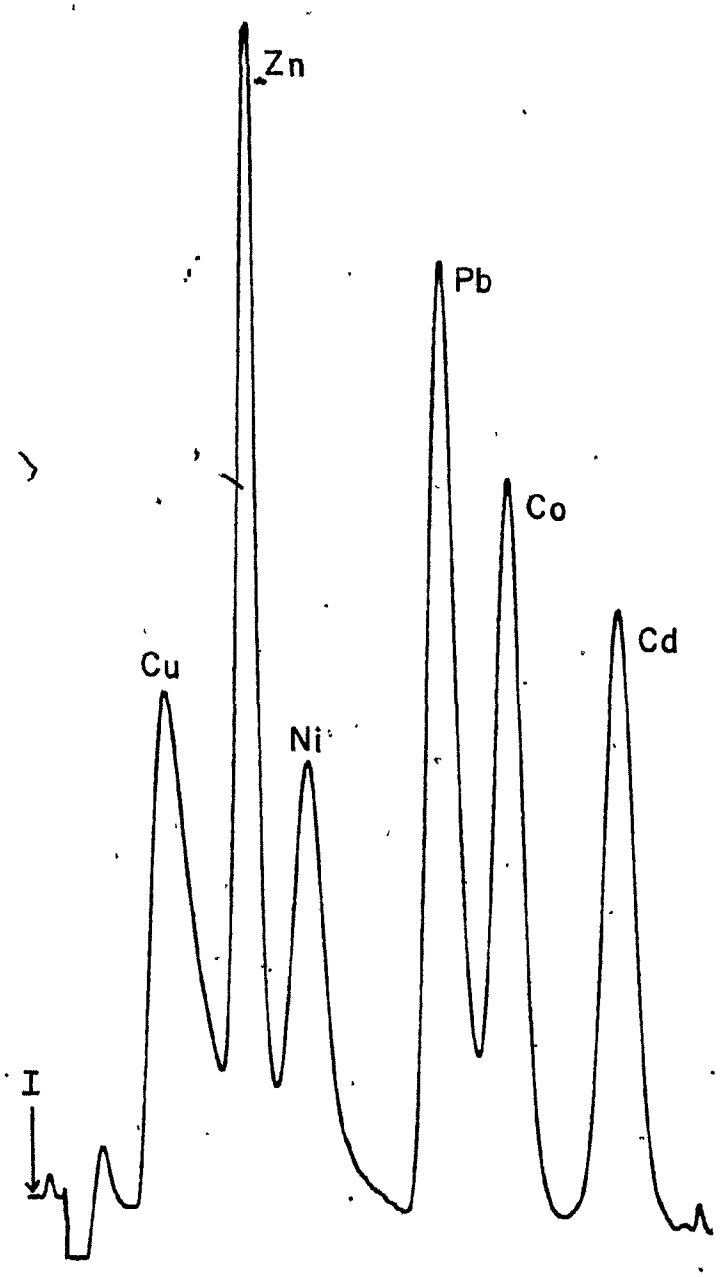


Fig. 3-14: GCE/DTPA detector system.

- 1 mL of 3.75 µM Cu, Zn, Ni, Pb, Co, Cd.

suffered from a shifting baseline. These separation systems were used to analyze various metal ion mixtures reported in Chapter 4 and Chapter 5.

CHAPTER 4

Preconcentration Methods

4.0 Introduction

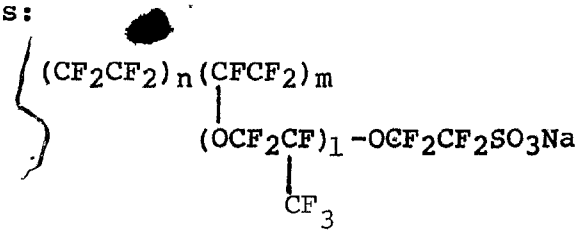
The detection limits reported in Chapters 2 and 3 for the ion-exchange/photometric detector system ranged from 0.25 nmole to 0.50 nmole for the ions Co(II) and Cd(II), respectively. If one assumes a 1 mL sample injection, these detection limits give concentration detection limits of 0.25 μM and 0.50 μM for the two ions. These detection limits are suitable for analysis of contaminated waters but not sufficient for many natural waters or for chemical speciation studies. Typical cadmium levels in natural waters are in the range of 0.1 to 20 ppb with a mean of 9.5 ppb or 0.084 μM (Manahan, 1972). These would require a preconcentration of 10 to 100 times to be greater than the detection limit. Metal speciation studies of model systems with algae (Guy and Kean, 1980) indicated that whereas the total levels of copper ranged from 1-10 μM, the "active" species (Cu^{2+}) was present at levels of 10^{-8} M. The studies of metal speciation would require a preconcentration of at least 10 times for favorable samples. A study of preconcentration methods was undertaken to evaluate the possibility of extending the use of the ion-exchange system to natural waters and speciation analysis.

One requirement imposed by metal speciation on the preconcentration technique is that the preconcentration be done after the speciation. This requirement is a result of the labile nature of the species present in a water sample -a preconcentration technique that removes free cation quantitatively will ultimately strip the metal from any labile species by shifts in binding equilibria.

A large number of preconcentration techniques have been proposed for metal ions. Solvent extraction of neutral metal complexes into organic solvents is not well-suited for polarographic or ion-exchange methods of analysis. Polarography requires a conductive medium and the Aminex ion-exchange resins and eluents require aqueous media for easy use. A back extraction of the complex into acid media would overcome these difficulties but introduce possible interferences into the analytical measurement step. A second method commonly used for preconcentration is to convert the metal dissolved in solution to a metal adsorbed onto a solid phase. This solid phase could result from co-precipitation with oxine, $Mg(OH)_2$, hydrous oxides of manganese or iron, or be an ion-exchange material. The latter method has potential applications for preconcentrating metal ions prior to ion-exchange analysis.

A preliminary study on the use of ion-exchange membranes for speciation indicated that sample volumes of 25 mL containing the free metal cation in a salt matrix would be the most probable sample requiring preconcentration. Two methods for preconcentrating these samples looked promising - a Donnan dialysis procedure using Nafion 811X cation-exchange tubing and an ion-exchange pre-column preconcentration of the metal ions prior to the analytical separation.

4.0.a. Nafion 811X ion-exchange membranes: Ion-exchange membranes have been produced as separators for industrial use in purifying process streams by electrolysis. DuPont has produced a perfluorosulfonic acid cation-exchange membrane that is sold under the trade name "Nafion". These membranes have a Teflon-like backbone with an anionic functional group as a substituent. A general formula for Nafion 811X is:



Nafion 811X is available in sheet and tubular form. The membrane has an equivalent weight (for protons) of approximately 1100 grams. This membrane exhibits the

chemical stability of Teflon and the hydrophillic sites make it suitable for use in aqueous media.

When a membrane is ion selective and the solution contains several different free ions, an unequal distribution of ions will result across the membrane. Consequently, osmotic and electrical potential differences, called "Donnan potential" or membrane potential, will result (Hwang and Kammermeyer, 1975). Suppose a charged membrane (e.g., Nafion 811X) is used to separate two electrolyte solutions I and II. The two solutions contain two counter-ions (i.e., cations), 1 of valence Z_1 and 2 of valence Z_2, and a common co-ion (anion) Y of valence Z_Y. If the solutions are sufficiently dilute ($\overline{C_R}$ (capacity of membrane)$>C_Y$), then the passage of the co-ion through the membrane is negligible due to Donnan exclusion of the co-ion. The result of the ion-exchange equilibria will be (Hwang and Kammermeyer, 1975, p138):

$$\left[\frac{a_1^I}{a_1^{II}}\right]^{1/Z_1} = \left[\frac{a_2^I}{a_2^{II}}\right]^{1/Z_2} \qquad (4\text{-}1)$$

subject to electroneutrality conditions:

$$\begin{aligned} Z_1 C_1^I + Z_2 C_2^I &= |Z_Y| C_Y^I \\ Z_1 C_1^{II} + Z_2 C_2^{II} &= |Z_Y| C_Y^{II} \end{aligned} \qquad (4\text{-}2)$$

The implications of equations 4-1 and 4-2 can be best understood if the solutions are ideal, i.e., $\gamma_i^I = \gamma_i^{II} = 1$. Then,

$$\left[\frac{C_1^I}{C_1^{II}}\right] = \left[\frac{C_2^I}{C_2^{II}}\right]^{z_1/z_2} = K \qquad (4-3)$$

The equilibrium distribution depends on the initial distribution of co-ion which does not change because of the permselectivity of the membrane. If one selects the electrolytes such that $C_Y^I/C_Y^{II} > 1$, then the counter-ion 1 will be pumped from II to I. The driving force for the diffusion of 1 is due to the electric potential generated by the distribution of the anion Y. A large driving force can be achieved by controlling the anionic concentrations in I and II and this can often exceed the force due to the difference of its own concentration between the solutions. This procedure was initially proposed by Kelley et al (1973) as a means of separating metal ions from a stream. This continuous dialysis or Donnan dialysis is often affected using exchange with acid (Hwang and Kammermeyer, 1975, p167). The metal ions permeate through the membrane to the diffusate stream and hydrogen ions transport in the opposite direction. The ion-exchange rate may be

accelerated by adding a complexing agent to the stripping (or receiver) stream.

The validity of equation 4-3 and the potential analytical use has been demonstrated for cations (Blaedel and Haupert, 1966) and anions (Blaedel and Christensen, 1967). One can envision two possible uses of equation 4-3 and the Donnan effect. If $C_{2,II} = C_{2,I}$ (and $C_{Y,II} = C_{Y,I}$), it would be possible to use the membrane as a selective speciation device for free cations. This application will be illustrated in Chapter 5. The second use is to have $C_{2,II} > C_{2,I}$ (with electrolyte I the sample, and II the receiver) and use the device for preconcentration. This application will be described in this chapter.

Blaedel and Kissel (1972) have demonstrated that the initial transfer rate across the membrane is proportional to the analyte concentration. They used a fixed enrichment time and obtained linear working curves even though the recoveries were less than the theoretical value (i.e., Donnan equilibrium was not attained).

Cox and co-workers (1975; 1977; 1978, a,b; 1980 b,c; 1981; 1982 a,b) have used flat membranes for Donnan enrichment of cations or anions in various matrices. The cation-exchange studies used a receiver electrolyte composed of 0.2 M $MgSO_4$ and 0.5 mM $Al_2(SO_4)_3$ to enhance

enrichment factors and rates. The Al^{3+} was thought to interact with strong sites on the membrane and thereby minimize the interactions between these sites and the analyte ions. Cox and Twardowski (1980a) reported that a tubular membrane enhanced the enrichment rates over flat membranes. The advantages of the tubular membrane are a greater surface area to receiver volume ratio, and the flowing receiver stream minimizes concentration polarization.

The preconcentrations described above result from the membrane being in contact with two solutions of different ionic strengths, as defined by equation 4-3. There are some disadvantages to this approach. First, enrichment is adversely affected when the sample ion is in a solution of moderate to high ionic strength. For example, Cox and DiNunzio (1977) reported a 50% decrease in enrichment factors when the ionic strength of the copper analyte solution increased from 0.01 to 0.02. Second, quantitative enrichments are not obtained. Cox and Twardowski (1980a) reported enrichment factors of 17, 38, 48, and 49 for 10, 20, 30 and 40 minutes, respectively. The enrichment factors were found to decrease if longer enrichment times were used even though the theoretical 200-fold preconcentration had not been approached. They postulated that less than ideal permselectivity may be the cause. Cox

and DiNunzio (1977) noted that poor reproducibility was a problem unless one approached the equilibrium distribution. To improve the reproducibility, they required precise temperature and stirring control and recommended the use of an internal standard.

One means to overcome these difficulties is to introduce a complexone into the receiver solution. The complexone can enhance the dialysis rate by lowering the free metal cation concentration in the receiver and also minimize adsorption problems caused by metal-membrane interactions. Blaedel and Haupert (1966) and Wallace (1967) used EDTA in the receiver to preconcentrate Zn and La or Sr, respectively. A strong complexone like EDTA would be suitable for atomic absorption and ICP analysis but would be unsuitable for an ion-exchange analysis procedure. A tartrate or citrate receiver solution would be useful for preconcentration and would also provide a medium exchange suitable for the ion-exchange procedure described in Chapter 3.

4.0.b. <u>Ion-exchange pre-column preconcentrations:</u> The ion-exchange equilibrium for a system at constant ionic strength is given by:

$$M^{n+} + nRNa \rightleftharpoons MR_n + nNa^+ \qquad (4-4)$$

where M^{n+} is an analyte cation and R the resin. The equilibrium constant K is given by:

$$K = \frac{[MR_n][Na^+]^n}{[RNa]^n[M^{n+}]} \qquad (4\text{-}5)$$

where concentrations are used instead of activities because of constant ionic strength. A distribution coefficient D can be defined as follows:

$$D = \frac{[MR_n]}{[M^{n+}]} = K\left[\frac{[RNa]}{[Na^+]}\right]^n \qquad (4\text{-}6)$$

The preconcentration could be given by:

$$\frac{\text{amount of metal on resin}}{\text{amount of metal in solution}} = \frac{[MR_n]}{[M^{n+}]} \cdot \frac{g}{V} = D \cdot \frac{g}{V} \qquad (4\text{-}7)$$

where g is the weight of resin in grams and V the volume of solution in milliliters. With typical values of K^{Cu}_{2Na} = 0.71, [RNa] = 2 meq/g and $[Na^+]$ = 0.1 meq/mL, D would have a value of 284 (assuming Cu^{2+} is present at trace levels). For a batch equilibration using 1 gram of resin and 5 mL of sample solution, the preconcentration would be:

$$\frac{\text{amount of metal on resin}}{\text{amount of metal in solution}} = 284 \times \frac{1.0\ g}{5.0\ mL} = 56.8 \qquad (4\text{-}8)$$

or 98% of the metal would be stripped from the solution. For column preconcentration procedures with trace quantities of metal ions, one can estimate the volume of solution for quantitative uptake by assuming that the sample volume must be ~20% of the elution volume. Then

$$V_{sample} = 0.20\ V_e \cong 0.20\ D \cdot g \qquad (4\text{-}9)$$

For the conditions described earlier, V_{sample} = 0.20 x 284 x 1.0 g = 56.8 mL of sample could be preconcentrated (assuming concentration of metal ions is less than 1% of the resin sites; in this case, less than 1.8×10^{-4} M). It is evident that ion-exchange preconcentration can be very effective, assuming that no species is present in solution that can decrease D. Strong complexones in high concentration can lower D (cf. Chapter 3) and prevent preconcentration.

Cantwell et al (1982) have used a short column (0.30 g of resin or 1.5 meq) and large sample volumes (1.0 L) to effect a speciation of metal cations. The column was eluted with acid to give $[MR_n]$ and equation 4-6 was used to deduce $[M^{n+}]$. The large sample volume was necessary to ensure that the column and solution were in equilibrium. It is evident that it is possible using this method to do a preconcentration of free metal ion and speciation of metal cations in the same experiment. The work also illustrates, however, that preconcentration will depend on the species present in the solution.

Trace enrichment of metal ions on pre-columns has been reported by Cassidy and Elchuk (1980, 1981a). They used a strong-acid cation-exchange resin (Aminex A-5) to preconcentrate metal ions from nuclear reactor coolant and natural waters. Near quantitative recoveries were reported for Ni, Co, Zn, Pb and Mn. However, the samples studied were present in rather simple, uncomplicated matrices. It was decided to study the use of Aminex A-9 pre-columns with the aim of characterizing recoveries under various sample conditions including sample pH, metal concentration and the presence of dissolved organic matter.

4.1 Experimental

4.1.a. Nafion 811X ion-exchange membranes: The preconcentration experiments using Nafion membranes were done using the arrangement described in Figure 4-1. A two meter length of Nafion 811X tubing (DuPont Polymer Products, Wilmington, Delaware) with dimensions of 0.025" I.D. and 0.035" O.D. was coiled in 3 cm diameter circles and tied using Teflon tape. Push-fit connections were made by inserting the Nafion tubing into the Tygon tubing (0.0315"I.D. x 0.1625" O.D.) outlet of a Masterflex #7520-00 Variable Speed Peristaltic Pump (Cole-Parmer Inst. Co., Chicago, Ill.) equipped with Masterflex Pump Head #7013. The membrane coil was placed in the sample solution and one

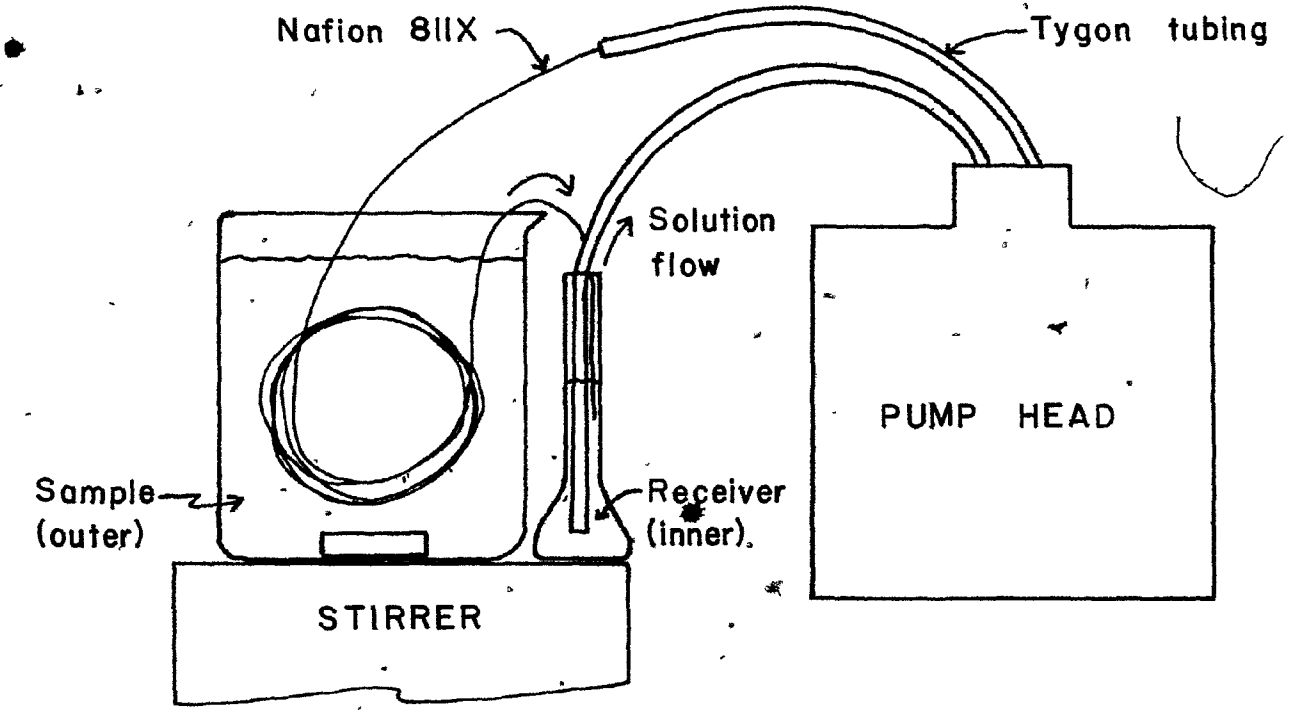


Fig. 4-1: Preconcentration by ion-exchange membranes.

end of the tubing was placed in the receiver volumetric flask. The sample solution was mixed using a magnetic stirrer and the receiver solution was pumped through the membrane at a rate of 3.0 mL/min. With receiver solution in the membrane tubing, the coil was completely immersed in the sample. The Masterflex pump was capable of driving six heads which permitted six experiments to be done simultaneously.

The receiver solutions usually had a higher ionic strength than did the sample solutions and were therefore susceptible to osmotic dilution. As a result, receiver flasks were not filled to the mark, leaving sufficient margin to compensate for this dilution (typically 0.5-1.0 mL). After prescribed enrichment times, the membranes were emptied into the volumetric flasks. If analysis was to be done by HPLC, acidification of the receiver solution (tartrate or citrate eluent) to pH 2.0-2.5 was done prior to making up to volume with distilled H_2O (i.e., 75 µL conc. HNO_3 per 5 mL eluent). Analysis by HPLC was done according to the procedures outlined in Chapter 3.

Strong complexones (EDTA, DTPA) were also used as receiver solutions and the metals analysis was done using emission spectroscopy. The analysis was done using an ISA JY48 inductively coupled plasma (ICP) spectrometer (Instruments SA Inc., Metuchen, N.J.). The analytical

conditions were as follows: RF power 1300 watts; concentric type nebulizer with 0.5 mL/min uptake rate; Ar flow rates were 12 L/min (plasma), 1.0 L/min (auxiliary), 0.5 L/min (nebulizer). The samples were spiked with chromium (Cr^{3+} as the chloride) for use as an internal standard.

To avoid metal contamination and ensure uniform membrane characteristics, the membranes were washed after each experiment. The membranes were treated with 2 M HNO_3 for 30 minutes to remove metals, 2 M NaOH for 30 minutes to convert to the sodium form, and with receiver solution for 15 minutes to ensure pH equilibration. In each washing, the acid, base or buffer was used both as sample and receiver solutions. For certain studies at high preconcentration ratios near the detection limits of the ion-exchange analysis, the unspiked samples were treated to remove metal impurities. The unspiked samples with membrane coils in place were cleaned by passing 50-75 mL of receiver solution through the membrane and to waste. This cleaning procedure reduced reagent blanks to a minimum. The samples were spiked with the metals of interest and the preconcentration experiment completed.

4.1.b. Ion-exchange pre-column preconcentrations: The ion-exchange preconcentrations were achieved using the experimental arrangement described in Figure 4-2. The HPLC pump, high pressure Rheodyne sampling valve, analytical

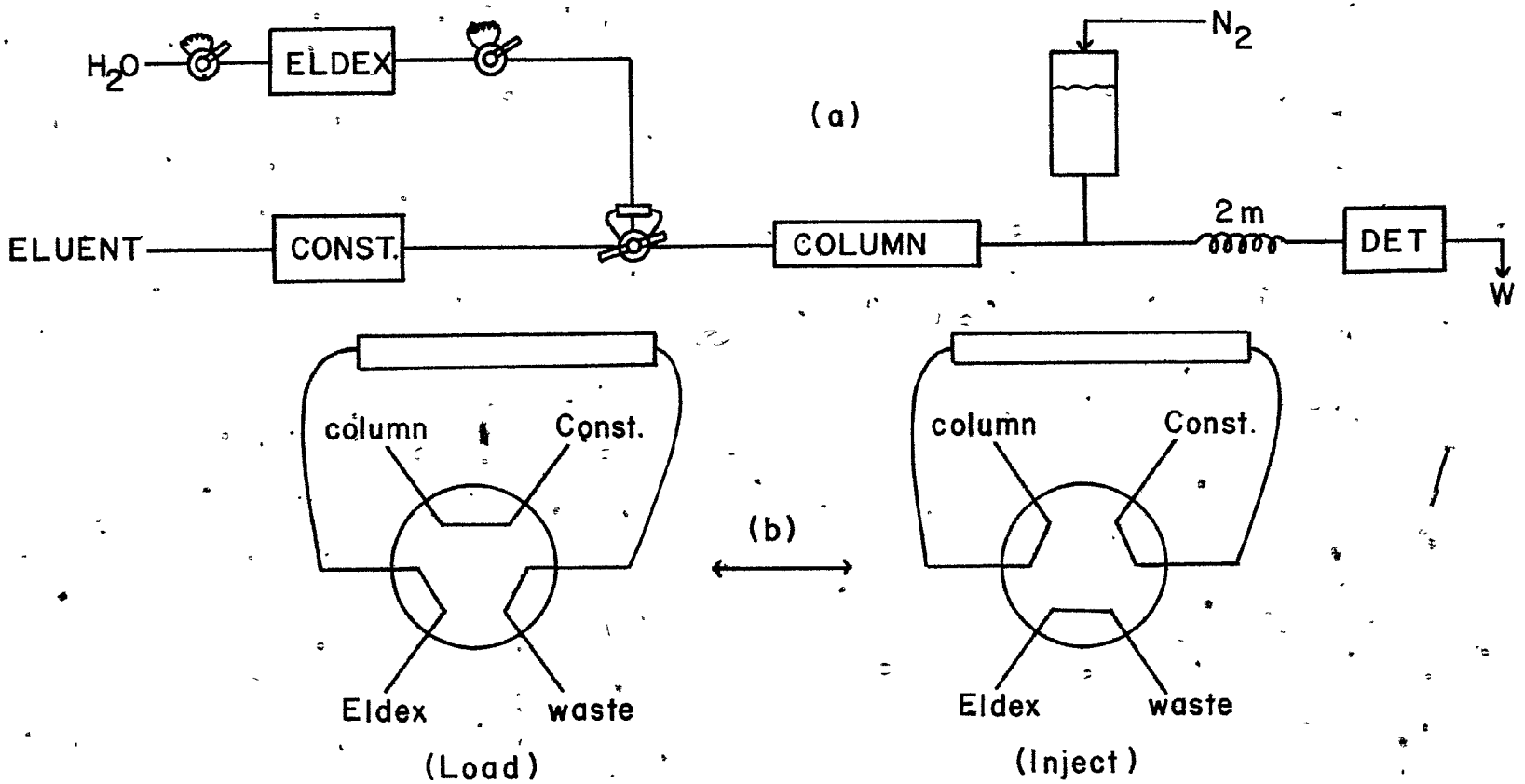


Fig. 4-2: Preconcentration by Aminex A-9 pre-column.

columns, pressurized delivery system, photometric system 2 detector and connections were the same as those described in Chapters 2 and 3. A Rheodyne 4-way Teflon Rotary Selection Valve equipped with a 5 mL Teflon sample loop was used for the injection of large volumes. Smaller sample volumes were injected using the Rheodyne 7125 valve fitted with 1 mL, 250 µL, 100 µL, or 20 µL sample loops. All injections were made by suction because the conventional "direct injection" mode produced metal contamination from the syringe components. The Aminex A-9 pre-column produced a backpressure of ~300 psi. A medium pressure single piston pump (Eldex E120S, Menlo Park, Calif.) was therefore used for solution sampling. The sampling flow rate was 1.8 mL/min. A Valco Model CV-6-UHPa-N60 6-way high pressure sample injection valve (Valco Inst. Co., Houston, Texas) was used for switching between sampling and analysis flow modes. The pre-column was 3 cm x 4.2 mm stainless steel tubing packed with Aminex A-9. The pre-column was placed in the sample loop position of the Valco valve. Figure 4-2b illustrates the flow patterns for preconcentration and analysis steps.

4.2 Results and Discussion

4.2.a. Theoretical Nafion preconcentrations: Theoretical preconcentration or enrichment factors (EF) can be

calculated using equation 4-3. If one assumes that the ionic strength is the same on both sides of the membrane, then:

$$C_{MFI} = C_{MFO} \qquad (4\text{-}10)$$

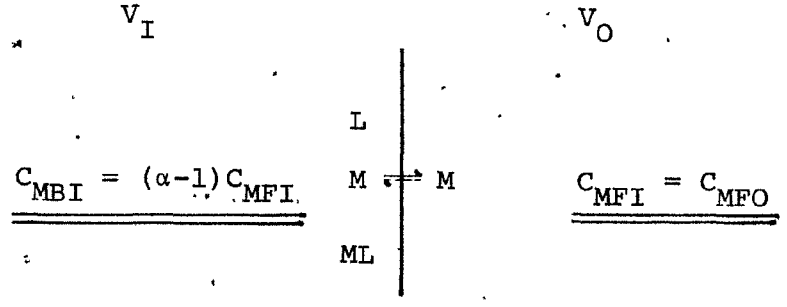
where MF represents metal free, I and O represent inner (receiver) and outer (sample) solutions, respectively. The inner receiver solution, however, contains a complexing ligand that is not dialyzable so that:

$$C_{MTI} = C_{MFI} + C_{MBI} \qquad (4\text{-}11)$$

where MT and MB represent metal total and metal bound, respectively. If one uses Ringbom's α-notation for metal complexation equilibria (Ringbom, 1979), then:

$$\alpha = \frac{C_{MBI} + C_{MFI}}{C_{MFI}} \qquad (4\text{-}12)$$

and $C_{MBI} = (\alpha-1)C_{MFI}$. The value of α can be readily calculated if one knows the pH, the concentration and binding constant for the metal complex. The experimental arrangement would be:



and the percent extraction is given by:

$$\% E = \frac{\text{metal in I}}{\text{metal in O + metal in I}} \times 100$$

$$= \frac{V_I (C_{MBI}+C_{MFI})}{V_O C_{MFO}+V_I (C_{MBI}+C_{MFI})} \times 100$$

$$= \frac{V_I ((\alpha-1) C_{MFI}+C_{MFI})}{V_O C_{MFO}+V_I ((\alpha-1) C_{MFI}+C_{MFI})} \times 100$$

$$= \frac{\alpha}{\alpha + \frac{V_O}{V_I}} \times 100 \qquad (4\text{-}13)$$

The extraction obviously depends on two factors - the α value (controlled by complexone and receiver pH) and the sample to receiver volume ratio. Two examples will illustrate the possible implications. Suppose one has a choice of two receiver solutions - a 0.20 M tartrate eluent at pH 4.0 and a 0.01 M EDTA solution at pH 5.0. Table 4-1 presents the calculated enrichment factors for copper and zinc.

The predicted recoveries for zinc are much lower in the tartrate system because of the weak complex formed with tartrate. The strong complexone should be more effective in attaining high enrichment factors.

Under practical operating conditions, the ionic strength of the receiver solution will be greater than the sample solution. The equations given earlier can be modified to give:

$$\frac{C_{MFI}}{C_{MFO}} = \left[\frac{Na_I}{Na_O}\right]^2 = F \qquad (4\text{-}14)$$

Table 4-1

Enrichment factors for sample receivers‡

	EDTA*		Tartrate*	
V_O/V_I	Cu	Zn	Cu	Zn
10	10.0	10.0	9.9	6.6
20	20.0	20.0	19.6	10.0
60	60.0	60.0	57.1	15.0
100	100.0	100.0	92.2	16.7

* $\alpha_{EDTA} = 4.0 \times 10^6$ $\alpha_{tart} = 2.51$

$\alpha_{Cu\text{-}EDTA} = 1.58 \times 10^{10}$ $\alpha_{Cu\text{-}tart} = 1.1 \times 10^3$

$\alpha_{Zn\text{-}EDTA} = 7.91 \times 10^7$ $\alpha_{Zn\text{-}tart} = 20$

‡ assuming equilibrium is attained

(assuming a divalent sample ion and a sodium salt as ionic strength adjuster). Equation 4-13 can be modified to give:

$$\% E = \frac{\alpha F}{\alpha F + \frac{V_o}{V_I}} \times 100 \qquad (4\text{-}15)$$

Table 4-2 presents enrichment factors for zinc assuming a 0.20 M tartrate receiver solution at pH 4.0 and at varying sample ionic strengths (I_o).

It is evident that ionic strength differences will play an important role in the enrichment factors. A weak complexone like tartrate will be very dependent on the ionic strength differences whereas the stronger complexone EDTA will be less dependent.

4.2.b. Experimental Nafion preconcentrations: Nafion membrane preconcentration experiments using tubular membranes have three variables suitable for study: the ionic strength ratio F, the value of α controlled by the ligand, and the ratio V_o/V_I. Figure 4-3 presents the cadmium kinetic curves for a V_o/V_I ratio of 400/10 for EDTA and tartaric acid ligands. Both ligand systems gave maximum enrichment factors of 36 ± 2 after 45 minutes and both systems showed a gradual decrease in enrichment factors at longer time intervals. The apparent difference between the two systems was not considered significant because of

Table 4-2

Calculated enrichment factors for Zn into tartrate‡

		Enrichment factor			
I_o	F	V_o/V_I = 10.0	20.0	60.0	100.0
0.002	10^4	10.0	20.0	60.0	100.0
0.02	10^2	10.0	19.8	58.1	95.3
0.05	16	9.7	18.8	50.9	76.2
0.10	4	8.8	16.0	34.2	44.1
0.20	1	6.6	10.1	15.1	16.7
0.50	0.16	2.4	2.8	3.0	3.1

‡ assuming equilibrium is attained,
α not corrected for I

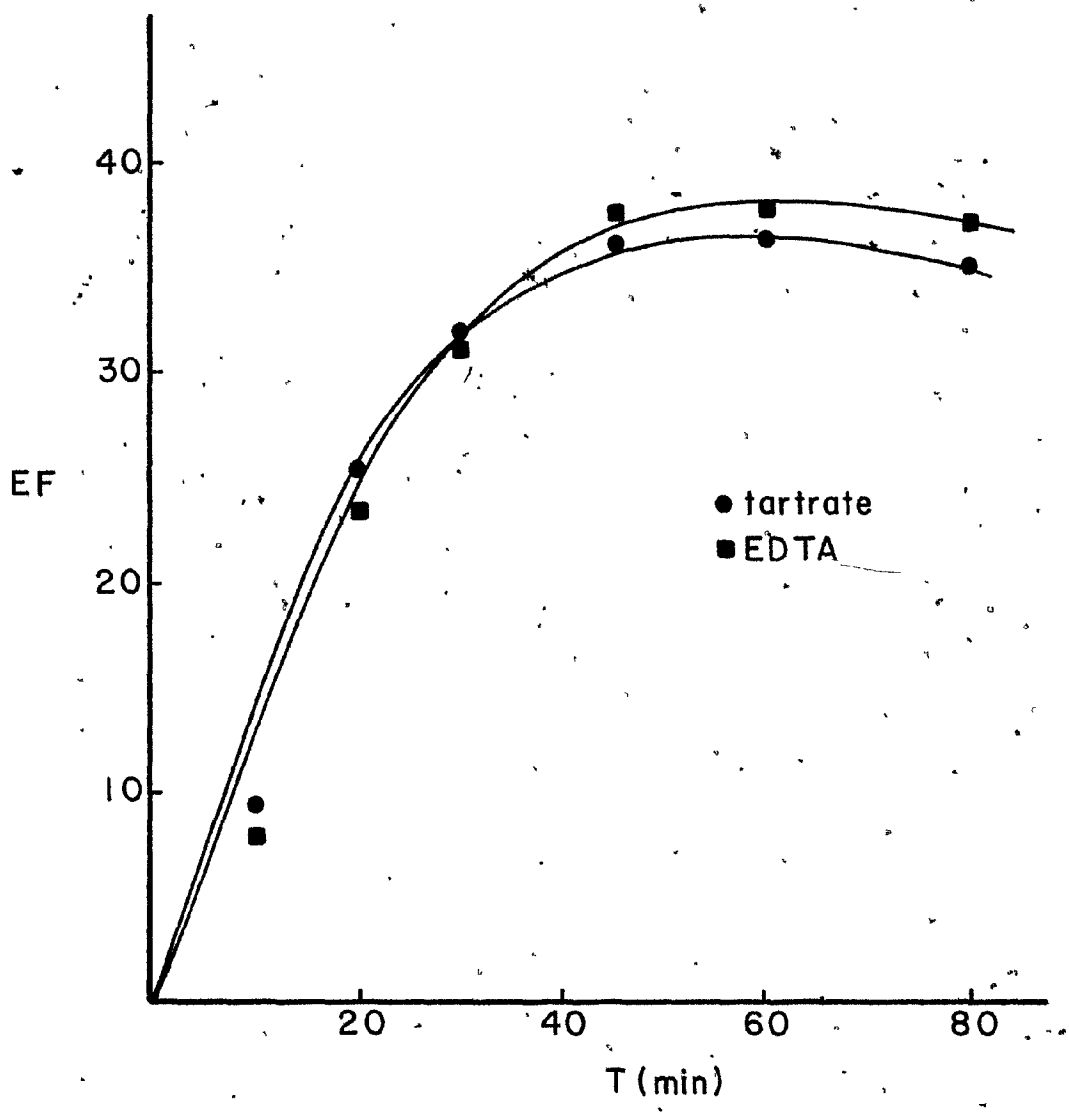


Fig. 4-3: Enrichment factors as a function of time for Cd into 0.01M EDTA (pH 6.0) or 0.20M tartrate (pH 3.74).

experimental uncertainties in the measurements at short time intervals.

Figure 4-4 presents the enrichment factors as a function of time for a tartrate inner solution at various V_O/V_I ratios. All curves attained experimental enrichment factors below the theoretical V_O/V_I ratio. The time to attain the maximum enrichment factor ranged from 20 minutes for 5X to 60 minutes for 60X theoretical ratios. One possible method to attain rapid preconcentration would be to use large V_O/V_I ratios but stop the experiment at times before equilibrium is attained. Inspection of Figure 4-4 suggests that to attain a preconcentration of 20X, one could either use V_O/V_I = 20 and 30 minutes to reach equilibrium or use V_O/V_I = 60 and 15 minutes to reach a 20X preconcentration. Table 4-3 presents the results of two experiments, the first a 40-fold preconcentration with measurements at equilibrium (60 minutes) and the second a 100-fold preconcentration measured at 45 minutes. The results indicate that it is possible to obtain rapid preconcentrations but the reproducibility suffers unless the experiment is allowed to attain equilibrium.

One means to obtain high preconcentration ratios but maintain small sample volumes is to decrease the internal volume (V_I). Preconcentration ratios after 15 minutes for V_O/V_I = 100 but with V_I values of 2, 4 and 10 mL were 53.5,

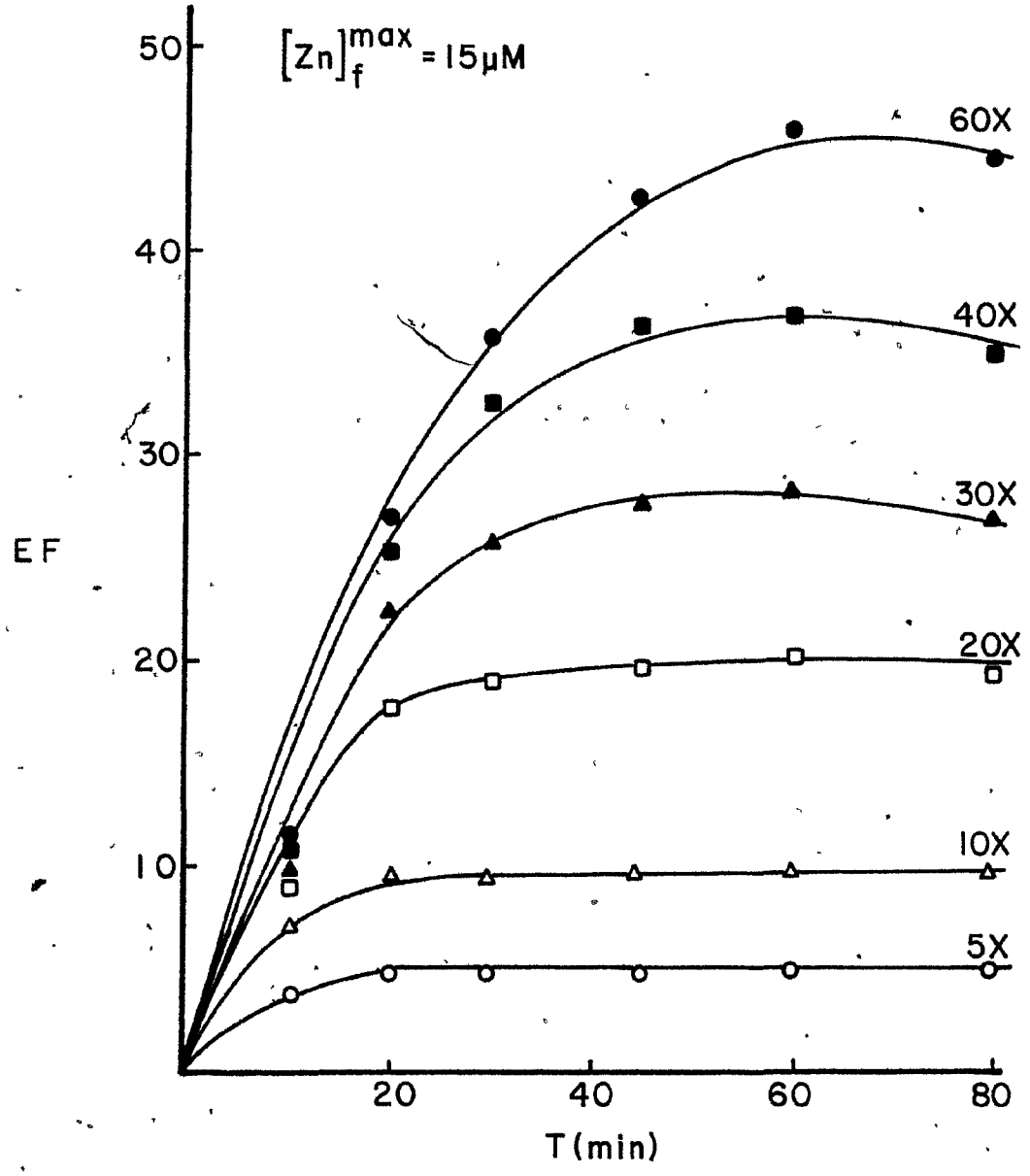


Fig. 4-4: Enrichment factors vs. time.

- V_o / V_s = 50, 100, 200, 300, 400, 600 mL to 10 mL.
- receiver = 0.20M tartrate (pH 3.74).
- sample = Zn in 0.01M acetate (pH 4.0).

Table 4-3

Reproducibility measurements for 40X preconcentrations*

Sample	Preconcentration 40X Equilibrium	100X 45 minutes
1	33.0	37.5
2	33.8	31.9
3	34.6	35.6
4	3[illegible]	32.1
5	35.6	41.6
6	34.2	34.0
$\bar{X}$	34.2	35.5
S.D.	0.86	3.7
%R.S.D.	2.5%	10.4%

* - 0.20 M tartrate, pH 3.74 receiver solution.
40X = 400/10 ratio; 100X = 1000/10 ratio.

42.4 and 15.4, respectively. Figure 4-5 presents a kinetic curve for a 200/2 mL preconcentration using tartrate as the inner solution. Equilibrium was attained after 30 minutes with EF = 68. These preconcentration values were corrected for the small changes in inner solution volume. Table 4-4 presents a reproducibility study for six samples of 200/2 mL preconcentrations of zinc. If one omits sample 6 (by Q test), the mean preconcentration factor was 67.1 and the relative standard deviation was 3.0%. This was considered to be the optimum situation for preconcentration using small sample volumes on a routine basis. Higher V_O/V_I ratios using V_I = 2.0 mL can also be used to attain high preconcentrations, but for good reproducibility one must attain equilibrium.

The data in Table 4-2 suggested that the value of F will affect the enrichment factors and equation 4-15 indicates that a stronger complexone will be less susceptible to ionic strength differences. Figure 4-6 presents the enrichment factors for cadmium into 0.01 M EDTA, 0.01 M EDTA + 0.20 M $NaNO_3$, and 0.20 M tartrate buffer as a function of sample ionic strength. The data for 0.01 M EDTA + 0.20 M $NaNO_3$ and 0.20 M tartrate fall along the same line contrary to the expectations of equation 4-15. The three systems have a decrease in EF at an outer ionic strength corresponding to 0.05 M $NaNO_3$ and are at 50% of

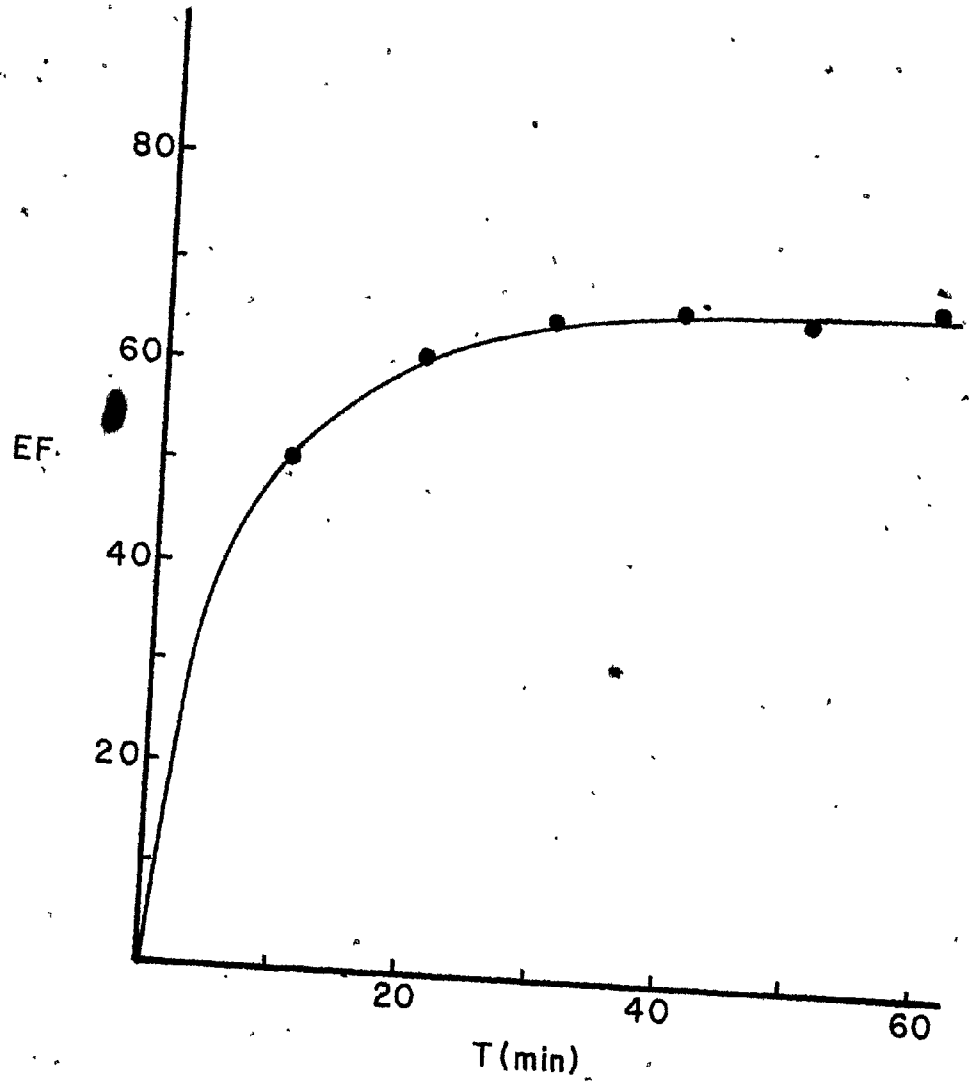


Fig. 4-5: A 200/2 preconcentration of Zn into 0.20M tartrate (pH 3.74).

- initial sample concentration = 0.125 μM

Table 4-4

200/2 reproducibility study

Sample	EF
1	63.9
2	67.0
3	67.3
4	69.3
5	68.0
6	76.7

$[Zn]_o$ = 0.25 μM and 30 minutes enrichment time:

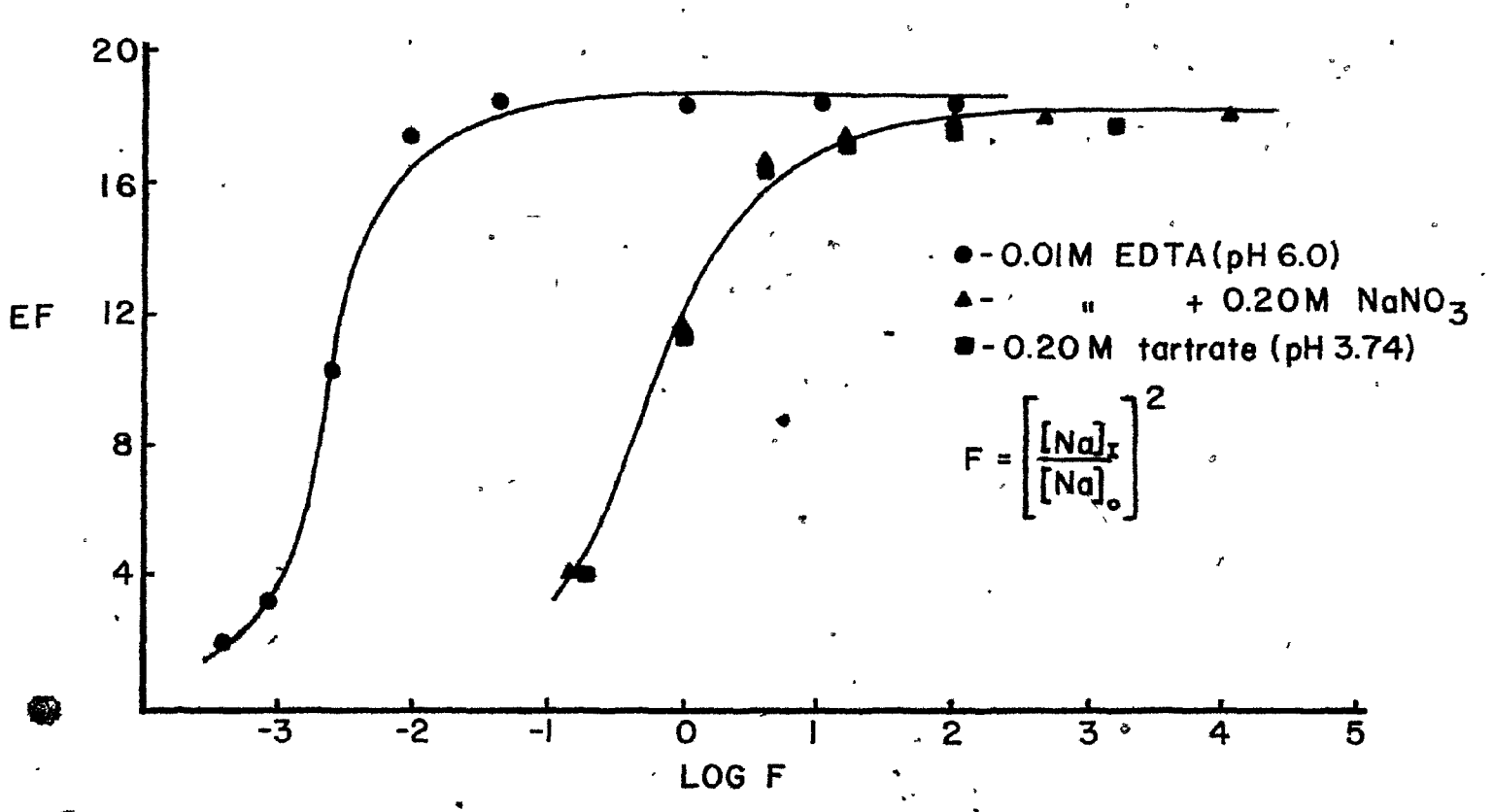


Fig. 4-6: Preconcentration of Cd vs ionic strength.

full preconcentration at 0.20 M $NaNO_3$. This value corresponds well with the calculated value for tartrate in Table 4-2 but is low for the EDTA systems. A possible explanation for this behavior is the breakdown in Donnan exclusion at high ionic strength and slow leakage of anions across the membrane. This leakage could also explain the slight decrease in enrichment factors observed after long equilibration times (cf Figure 4-3). The Donnan dialysis will not be suitable for preconcentrations of high salt matrices unless one augments the ionic strength of the inner solution.

Preliminary experiments on the use of Nafion membranes for cation speciation had indicated that a high salt matrix (0.30 M) would be necessary to prevent cation adsorption onto the membrane. The optimum inner solution volume for speciation was found to be 25.0 mL. The results reported earlier in this chapter suggest that it should be possible to effect a preconcentration using a 2.0 mL inner volume of 0.20 M tartrate + 0.30 M $NaNO_3$ and a 25.0 mL volume of outer sample solution obtained from a previous speciation experiment. The Donnan dialysis preconcentration for 30 mL of 0.30 M $NaNO_3$ outer solution and 2.0 mL of 0.20 M tartrate + 0.30 M $NaNO_3$ inner solution gave a mean enrichment factor after 60 minutes of 12.3 with a relative standard deviation of 5.2% (6 repeat experiments). The slightly higher

relative standard deviation for this run was a result of poor membrane/solution contact. A glass insert with tubing coiled around it should increase membrane/solution contact and give better experimental precision.

The enrichment factors will vary with the strength of the metal-ligand complex. Table 4-5 presents enrichment factors for both the tartrate and EDTA inner solutions. The tartrate systems were analyzed using the ion-exchange/photometric detector described in Chapter 3. The relative precision of the enrichment factors ranged from 3.9 to 6.6%. The precision of the analysis step (i.e., 6 injections of a standard) ranged from 0.7 to 1.4%. The preconcentration step contributes considerably to the uncertainty in the measurement. The EDTA experiments were determined using the ICP and the analytical measurement had a precision ranging from 1.7 to 4.4%. The two experiments have approximately the same precision when one takes into account the uncertainty introduced in the measurement step and the higher EF for the EDTA system.

Figures 4-7 and 4-8 present 20X calibration curves for tartrate and EDTA inner solutions, respectively. Both systems gave linear calibration curves and the slopes of the lines represent the relative sensitivities of the metal detectors. The chromatographic separation gave a significant nickel blank which arises from the leaching of

Table 4-5

Enrichment factors for Donnan dialysis preconcentrations

	tartrate*		EDTA*	
Metal	EF	%RSD	EF	%RSD
Cu	19.8	6.6 (1.3)	38.4	7.3 (1.9)
Zn	18.5	4.4 (1.0)	39.2	3.1 (2.1)
Ni	18.7	4.4 (0.7)	37.2	4.5 (2.2)
Pb	18.4	3.9 (1.0)	32.6	10.2 (2.2)
Co	17.9	3.9 (0.8)	32.5	6.2 (2.2)
Cd	17.6	5.3 (1.4)	36.1	6.2 (2.4)
Ca			38.8	4.8 (4.4)
Mg			13.9	16.8 (1.7)
Mn			36.2	6.1 (2.3)

* - tartrate = 20X preconcentration; $[M]_S$ = 0.20 µM Co, Ni, Zn, Cu; 0.40 µM Pb, Cd.

* - EDTA = 40X preconcentration; $[M]_S$ = 0.15 µM Cd, Mn, Ni, Co; 0.45 µM Cu; 3.00 µM Ca, Mg.

- bracketed values are for standards.

- all values of %RSD are for 6 repeat experiments.

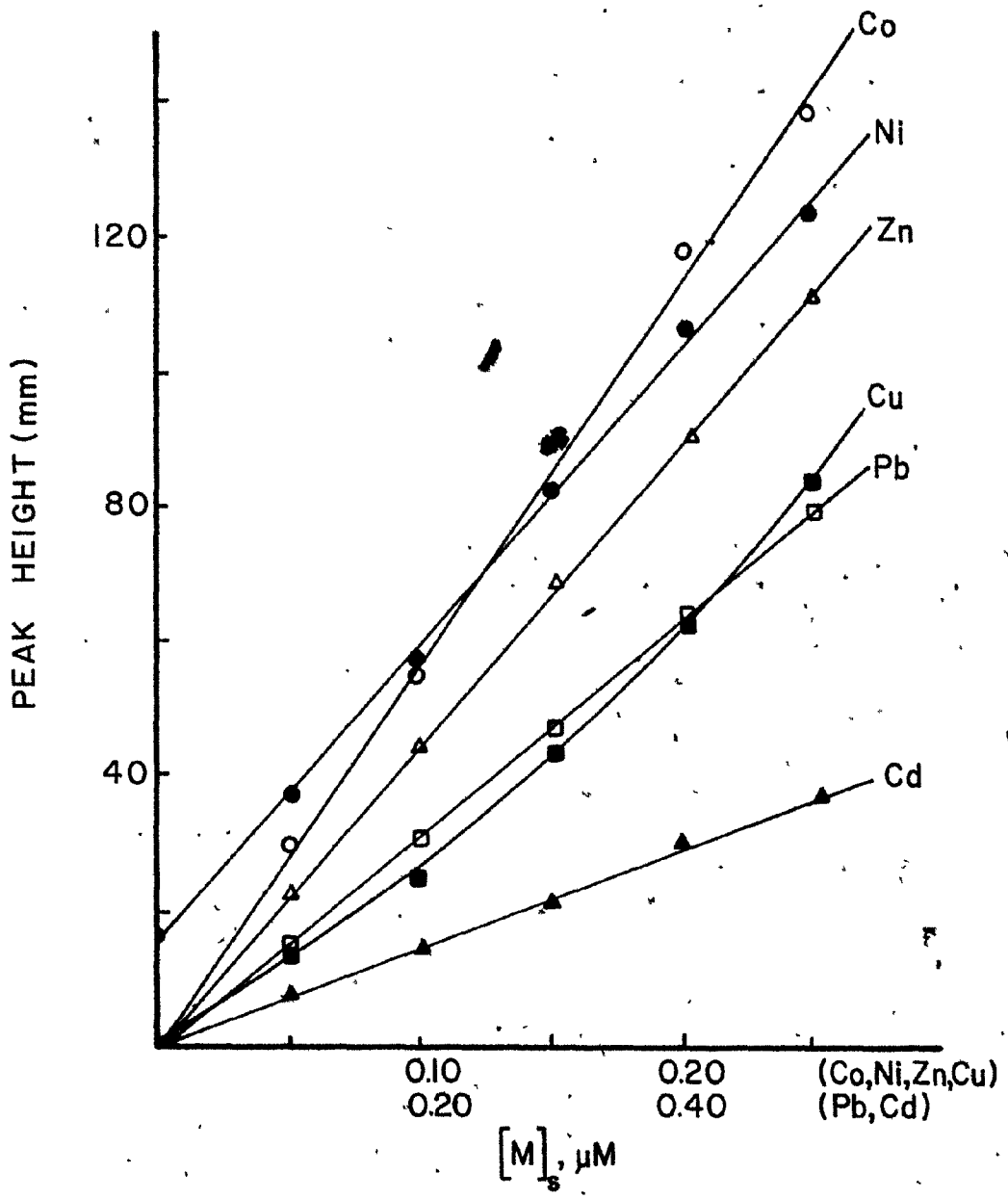


Fig. 4-7: 20x preconcentration into 0.20M tartrate.

- 200 mL sample in acetate buffer, 0.001M, (pH 4.0).
- 10 mL tartrate receiver, (pH 3.74).
- T = 60 min; analysis by HPLC.

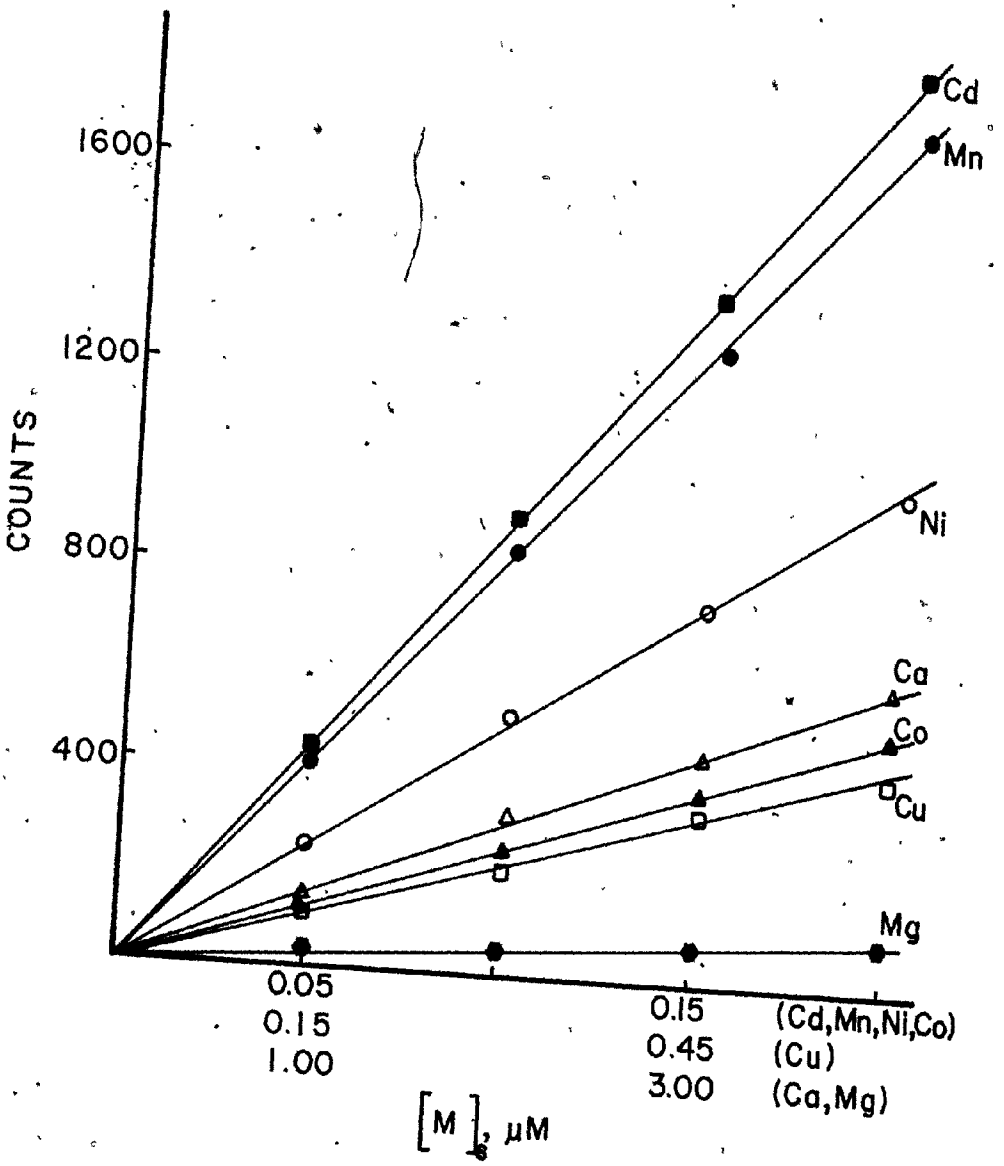


Fig. 4-8: 20x preconcentration into 0.01 M EDTA.

- conditions same as in Fig. 4-7 except pH 6.0 and analysis by ICP.

nickel from stainless steel by the acidified tartrate. The curvature of the copper calibration was discussed in Chapter 3. Using a V_O/V_I ratio of 100 and V_I of 2.0 mL, the detection limits for the chromatographic system could be lowered by a factor of 60-70.

The precision of the enrichment factors and the linear calibration curves indicate that Donnan dialysis would be suitable for preconcentration factors ranging from 10-60 with precisions of ±5%. A routine use of this method would require the inclusion of a control to determine the enrichment factor for each equilibration. For the system described in this report using a Masterflex pump with six heads, this means 5 samples could be preconcentrated with one control in 30 minutes (using 200/2 with EF ~ 68).

4.2.c. Ion-exchange pre-column preconcentrations: The preconcentration sampling system described in Figure 4-2 contains two sampling valves - a high pressure valve in between sampling pump and pre-column and a low pressure valve before the sampling pump. The latter valve used Teflon sample loops whereas the high pressure valve used stainless steel sample loops. This arrangement was not the ideal situation because the sample from the low pressure valve must pass through the Eldex pump before the pre-column. This arrangement requires a prefiltration of the

sample to remove particulates as these could interfere with check valve operation in the sample pump. An in-line sample valve would not suffer from this problem because particulates would be trapped by the inlet frit of the pre-column (this would, of course, result in a gradual increase in backpressure). The low pressure sample valve and Teflon sample loop was found to be necessary for large sample preconcentrations (greater than 1 mL) because a large 10 mL stainless steel sample loop obtained from Valco gave high nickel and zinc blanks. Passivation of this sample loop with EDTA, HNO_3, and tartaric acid was found to be ineffective.

The first experiment used the pre-column in-line with a flow injection analysis manifold. Individual metal ions were preconcentrated from 1 mL onto the pre-column and backflushed with eluent into the FIA manifold for detection. The time from switching the valve to peak maximum was determined to be 42 seconds for Cu, Zn, Ni, Co, Pb, and Cd and 52 seconds for Ca and Mg. The transfer time from valve to detector was estimated to be 35 seconds. The 7 second time difference for the transition metals was probably the time required for eluent to wash through the pre-column and restore maximum eluent strength. The 17 second time difference for the alkaline earths is probably due to the

retention of these ions by the resin in the presence of tartrate (cf the behavior of Ca and Mg on the analytical column).

Preliminary experiments suggested that reproducible blanks could be obtained only if a standardized injection procedure was used. This procedure for a 5 mL sample volume consisted of a 10 minute load cycle during which sample and distilled water were passed through the pre-column (i.e., 5 mL of sample + 13 mL of water) and a 4 minute inject cycle during which tartrate eluent passed through the pre-column. Similar cycling schemes were used for other sample volumes.

Table 4-6 presents the results of an experiment in which 50 nmoles of metal were injected in different sample volumes (C_M x V_M = 50 nmoles). Complete metal uptake was observed for volumes ranging from 0.5 to 10.0 mL. Relative standard deviations ranged from 4.0% to 7.3%. This irreproducibility was probably due to the use of different sample loops and loading cycles. Six repeat injections of 5.0 mL x 1.0 μM using the injection procedure described earlier gave much lower relative standard deviations, i.e., 0.6 - 1.6%. It is therefore advisable, whenever possible, to use the same sample loop and sample loading cycle for both samples and calibration standards.

The effect of solution ionic strength on preconcentration was studied by injecting 1 mL or 5 mL

Table 4-6

Uptake by Aminex A-9 pre-column vs sample volume

Injection	Peak heights (mm) Cu	Zn	Ni	Pb	Co	Cd
0.5 mL x 10 µM	81	101	118	59	122	49
1.0 mL x 5 µM	80	105	124	65	134	53
2.0 mL x 2.5 µM	81	103	126	66	128	53
5.0 mL x 1.0 µM	81	93	111	59	118	48
10.0 mL x 0.5 µM	88	114	114	58	120	49
$\bar{X}$ =	82.2	103.2	118.6	61.4	124.4	50.4
%RSD^A =	4.0	7.3	5.4	6.2	5.3	4.8
%RSD^B =	1.5	1.1	1.1	1.6	0.6	1.4

- samples made up in 10^{-2} M acetate buffer (pH 4.0)
- %RSD^A = for injections shown (i.e., 0.5, 1.0, 2.0, 5.0 and 10.0 mL).
- %RSD^B = for 6 injections of 5.0 mL x 1.0 µM.

samples of metal ions in the presence of increasing amounts of $NaNO_3$. The results are shown in Table 4-7. The 1.0 mL sample solutions were not affected as the sodium concentration increased but the 5.0 mL sample volume was affected at values above 0.5 M $NaNO_3$. For comparison, 5.0 mL injections with NaCl as ionic strength adjuster indicated that the presence of chloride affected the uptake even at 0.5 M NaCl. These results suggest that the ion-exchange pre-column would be suitable for the 0.30 M $NaNO_3$ salt solution proposed for speciation studies but not suitable for seawater samples.

Figure 4-9 presents the calibration curves for a 6-metal mixture using 5.0 mL injections. The chromatograms were indistinguishable from sample injection directly onto the column. For 5.0 mL injections, the blank peak heights were 2.5, 3.5, and 3.0 mm for Cu, Zn and Ni, respectively, whereas Pb, Co and Cd gave no blank peaks. The detection limits were as follows: 0.05 μM Cu, 0.02 μM Zn, 0.03 μM Ni, 0.10 μM Pb, 0.02 μM Co and 0.10 μM Cd. These limits were a factor of 5 times lower (in concentration units) than the 1.0 mL injection. This suggests that by using larger sample volumes, it would be possible to lower the detection limits further.

The principal difficulty associated with large sample volumes is the time required for sampling. The sampling pump flow rate was pressure-limited to about 2 mL/min. The

Table 4-7

Effect of salts on Aminex A-9 pre-column uptake

A - $[NaNO_3]$	Peak heights (mm) Cu	Cd	Mn	Mg	Ca
0.0 M	170	82	71	22	66
0.1 M	172	82	73	21	66
0.2 M	172	82	72	22	65
0.5 M	174	82	72	21	63
1.0 M	178	84	72	20	61

* - 1 mL injections of 10 µM Cu, Cd, Mn; 100 µM Ca; 500 µM Mg.

B - $[NaNO_3]$	Cu	Zn	Ni	Pb	Co	Cd
0.0 M	117	176	131	128	193	76
0.1 M	115	180	128	117	183	73
0.2 M	120	177	139	130	198	80
0.5 M	119	172	132	131	180	77
1.0 M	123	114	97	119	95	45

** - 5 mL injections of 1 µM Cu, Ni, Co, Zn; 3 µM Pb, Cd.

C - [NaCl]	Cu	Zn	Ni	Pb	Co	Cd
0.2 M	56	165	133	131	184	75
0.5 M	33	150	124	123	156	11

*** - 5 mL injections of 1 µM Cu, Ni, Co, Zn; 3 µM Pb, Cd.

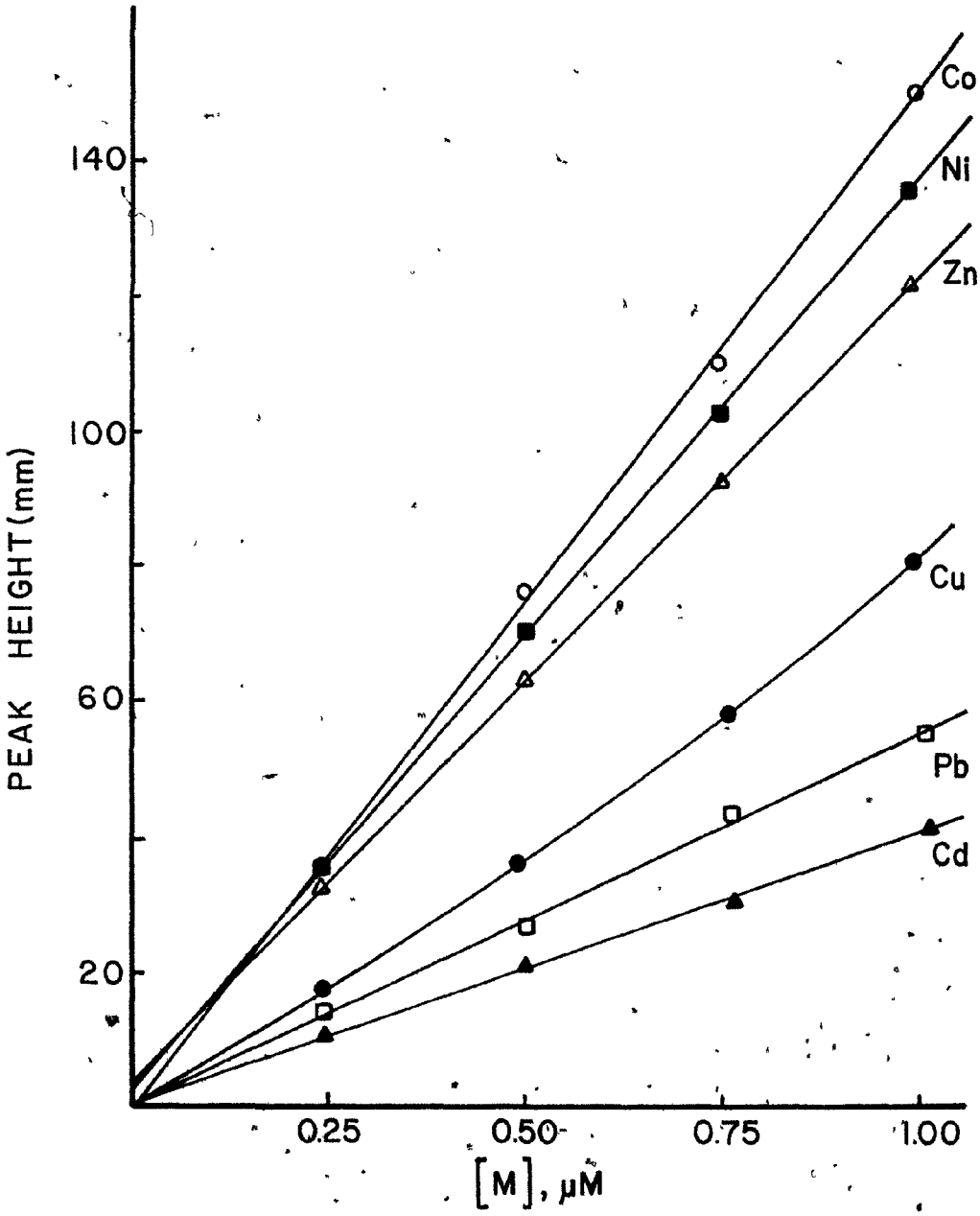


Fig. 4-9: 5 mL injections onto pre-column.

- analysis using PAR-Zn-EDTA.

dead volume of the tubing between valve and column was 2 mL and one should rinse the tubing (sample loop and dead volume) to ensure complete transfer. A 6-metal chromatogram requires 18 minutes. It should therefore be possible to complete a 25-fold preconcentration (i.e., 25 mL sample injection) on one sample during the analytical separation of a previous sample. This is not as time-efficient as the similar 25-fold preconcentration of six samples using Donnan dialysis but is more efficient in use of sample volume.

A study of the effect of complexones on metal ion uptake was undertaken for two reasons. The first reason was to evaluate the possibility of coupling the Donnan dialysis preconcentration to the ion-exchange pre-column preconcentration and the second was to determine whether organic species will affect the preconcentration of natural waters.

Figure 4-10 presents the results of metal uptake as a function of pH for solutions containing 0.30 M citric acid and 0.20 M tartaric acid. Both ligands could be used for Donnan enrichment followed by pre-column preconcentration if the sample pH was less than 2.5. Calibration curves and reproducibility studies for 1 mL acidified tartrate samples were done for Cu, Zn, Ni, Pb, Co and Cd. The results were similar to the direct injection of 1.0 mL acidified tartrate samples and gave reproducibilities on the order of 1% RSD. The injection of 5.0 mL of acidified tartrate, however, gave

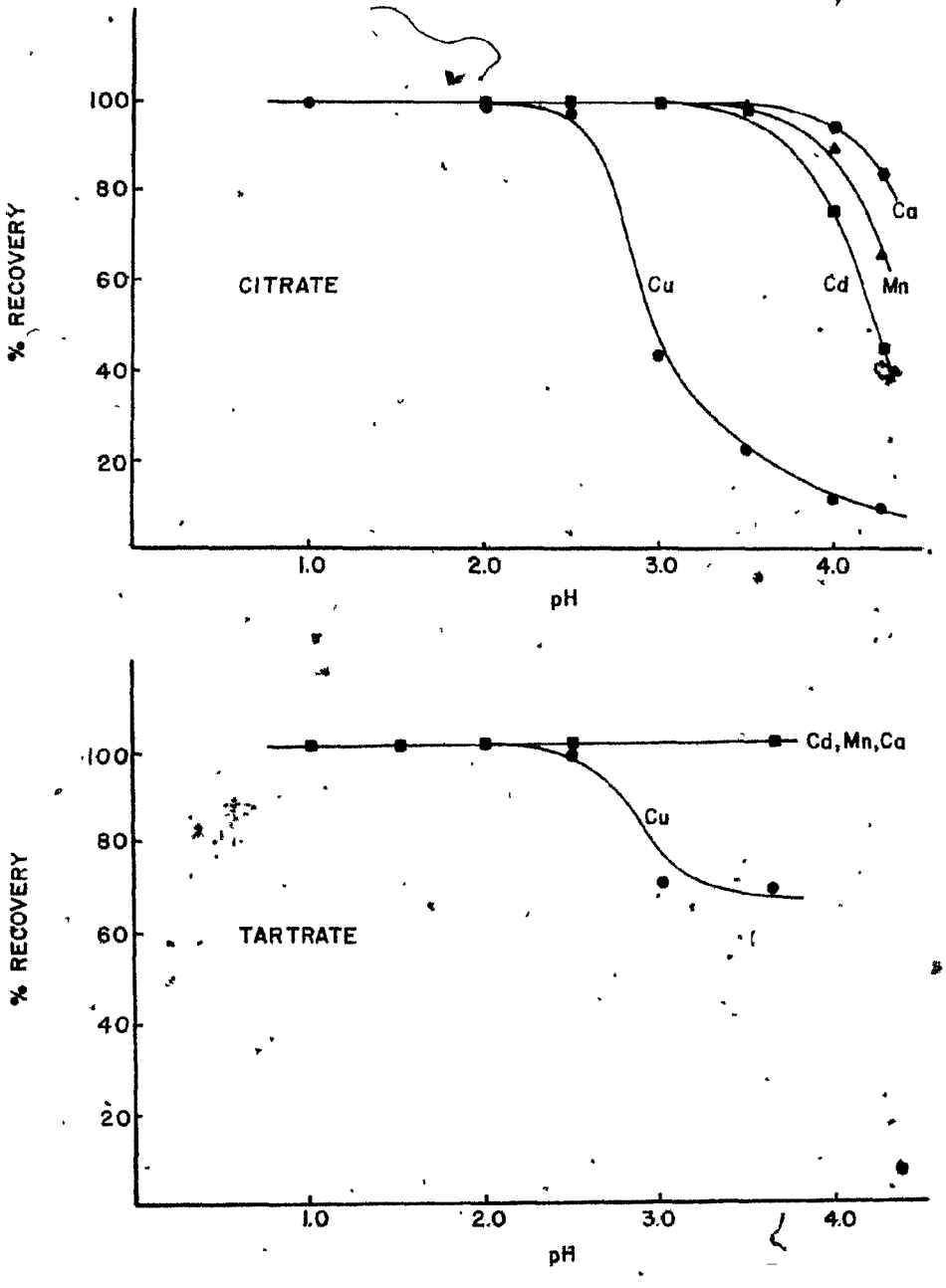


Fig. 4-10: Pre-column uptake as a function of pH.

-samples made up in 0.30M citrate or 0.20M tartrate.

high blank values - 11, 25 and 83 mm for Cu, Zn and Ni, respectively. These blanks should be compared with earlier blanks of 2.5, 3.5 and 3.0 mm for the injection of 5.0 mL of 0.01 M acetate buffer. The high nickel blank probably is a result of nickel being leached from the stainless steel components (10-14% Ni) whereas the copper and zinc contaminations may result from impurities in the acidified tartrate. Similar results were noted for stronger complexones such as EDTA and NTA where a low sample pH was required for metal uptake by the pre-column. This problem with blanks negates the use of pre-column preconcentration of Donnan dialysis samples unless one uses components less susceptible to acid leaching.

4.2.d. Effects of speciation on preconcentration: The results reported in Figure 4-10 clearly illustrate the role of complexation in the use of a pre-column for preconcentration. The speciation of metal ions in the sample will also affect the Donnan dialysis method. The equations developed earlier assumed that the metal ions in the sample were free. Equation 4-15 can be readily modified to take into account the role of sample speciation by including an α' term for binding of ions in the sample:

$$\% E = \frac{\alpha F}{\alpha' \frac{V_O}{V_I} + \alpha F} \times 100 \qquad (4\text{-}16)$$

Two examples can be used to illustrate the role of sample speciation on the enrichment factors by Donnan dialysis. Table 4-8 presents the results for the Donnan dialysis preconcentration of citric acid sample solutions at pH 5.0 using a 0.20 M tartrate receiver, also at pH 5.0. Complete preconcentration was noted for these samples even though most of the metal ions were bound in citrate complexes. A second, less encouraging, example is given in Figure 4-11 for copper, lead and cadmium in humic acid solutions. The humic acid significantly decreases the enrichment of metals by Donnan dialysis. The effect is strongest for copper and lead which also are the metal ions reported to bind most strongly with humic acid. The copper and lead speciation, for example, in a 25 ppm solution of humic acid (more details in Chapter 5) indicated that they were nearly completely bound. The tartrate receiver, however, stripped approximately 25 and 55% of the copper and lead, respectively. The ion-exchange pre-column, however, gave complete uptake for the three metal ions in all of the humic acid solutions.

The complete uptake of the metals by the ion-exchange pre-column in the presence of humic acid was quite unexpected. Earlier work by Figura and McDuffie (1979) and preliminary work to this thesis indicated that humic colloids prevent uptake of copper using Chelex-100. This is

Table 4-8

Donnan dialysis of citric acid solutions

[CA], μM	% free*			EF**		
	Cu	Pb	Cd	Cu	Pb	Cd
0	100	100	100	10.0	10.0	9.7
50	31	87	100	10.2	10.1	9.8
100	14	73	100	10.2	10.2	10.0
200	7	58	100	10.4	10.2	9.9

* - experimental speciation reported in Chapter 5, Figure 5-15.

** - V_O/V_I = 250/25 = 10.

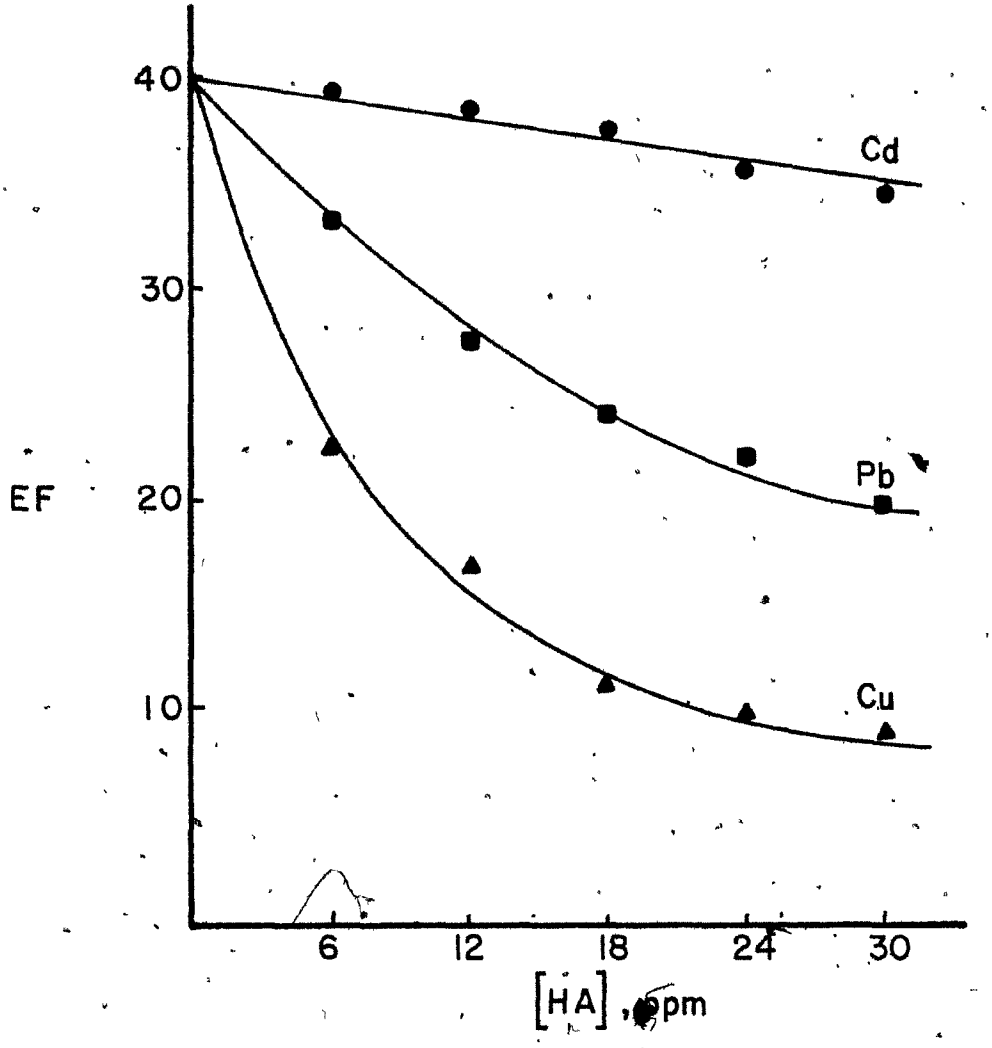


Fig. 4-11: Effect of HA on Donnan dialysis preconcentration of Cu, Cd and Pb.

- 400/10' with tartrate receiver ; pH 5.00, $[M]_s \simeq 0.5\,\mu M$.
- analysis by DPP.

usually explained by poor kinetics of metal-humic acid dissociation. It is evident that additional work is required comparing metal speciation and the preconcentration methods before one can offer a definitive explanation for the differences between Aminex A-9, Chelex-100 and Donnan dialysis. Chapter 5 reports on a dialysis procedure that may be useful in future work relating metal speciation and preconcentration.

CHAPTER 5

Metal Speciation by Nafion Dialysis

5.0 Introduction

Dialysis has been shown to be an effective method of achieving a size separation. It does, however, suffer from two drawbacks which may limit its applicability. First, it is very time consuming, often requiring 24 hours or more to attain equilibrium. Second, the dialyzable fraction does not necessarily represent that which is bioavailable. The dialyzate may consist of a small species capable of passing through a dialysis membrane but unable to interact with a biological membrane.

These limitations associated with dialysis are not necessarily inherent to the method but may instead reflect the properties of the cellulose membranes. One possible speciation procedure would be to combine the selectivity of ion-exchange with a batch dialysis separation. The advantage of a batch separation is the possibility of using small sample volumes and rapid equilibration. Cellulose dialysis bags, for example, contain residual anionic sites that retard the dialysis of anionic species. The charge density is too low, however, to provide complete exclusion of anions. DuPont produces ion-exchange membranes (e.g.,

Nafion 811X) that have an inert fluorocarbon backbone with sulfonic acid exchange sites bonded to it. One conceptual model of this membrane is a hydrophobic matrix with islands of hydrated exchange groups connected by channels in the matrix (Yeager and Kipling, 1979).

One can then envision a speciation procedure based upon the following mechanism. One uses the membrane to separate the sample solution from a receiver solution. Both the sample and receiver solutions contain the same electrolyte at the same concentration (e.g., 0.10 M $NaNO_3$). An ion-exchange equilibrium is established between the free metal cation and membrane sites on the sample side of the membrane:

$$2NaX_{(M,S)} + Cu^{2+} \rightleftharpoons CuX_{2\,(M,S)} + 2Na^{+} \qquad (5\text{-}1)$$

The adsorbed metal ions diffuse through the channels and redistribute to establish ion-exchange equilibrium with all sites:

$$CuX_{2\,(M,S)} \rightleftharpoons CuX_{2\,(M,R)} \qquad (5\text{-}2)$$

and the exchange equilibrium is finally established with the receiver solution:

$$CuX_{2\,(M,R)} + 2Na^{+} \rightleftharpoons Cu^{2+} + 2NaX_{(M,R)} \qquad (5\text{-}3)$$

In the above expressions, M, R and S represent membrane, receiver side and sample side, respectively. As illustrated in Chapter 4, it will be necessary to maintain the same electrolyte concentration in both sample and receiver. Under these conditions, the Donnan effect will be minimized and:

$$[Cu^{2+}]_S = [Cu^{2+}]_R \qquad (5\text{-}4)$$

Expression 5-4 is for free metal concentrations. The charged membrane will exclude anionic species and the "connecting channels" will be partially selective with respect to size.

This chapter will describe the application of Nafion ion-exchange membranes to the speciation of metal ions in model systems containing various ligands. The working parameters will be characterized and limitations established. In some cases, comparisons between calculated speciation and that obtained experimentally will be made in order to clarify the possible permselectivity parameters.

5.1 Experimental

The Nafion speciation dialysis experiments were done using an arrangement similar to that shown in Figure 4-1. A large volume, (2-4 L) stock solution was made up in

distilled/deionized water and contained appropriate amounts of electrolyte ($NaNO_3$) and acetate buffer. Both receiver and sample solutions were taken from the same stock solution prior to spiking the sample solution with metals and ligands. Unless otherwise stated, the sample solutions (400 mL) were placed in Nalgene beakers whereas the receiver solutions were placed in 25 mL volumetric flasks made up to volume. Sample solutions were stirred for the duration of the experiment after which the membrane contents were emptied into the volumetric flasks. These were not made up to volume as this was considered to be a dilution of an equilibrated system. With a 400 mL sample solution and a 25 mL receiver solution, the dilution effect on chemical equilibria was less than 6%. Receiver solution fractions were considered "free" (or dialyzable) whereas sample solution contents were considered "total".

Analysis of receiver solutions was done using either the HPLC/system 2 detector described in Chapters 2 and 3 or a PAR 174A Polarographic Analyzer (Princeton Applied Research, Princeton, N.J.) with dropping mercury electrode used in the differential pulse mode (DPP). Where possible, sample solutions were also measured (e.g., Cd-NTA by DPP and M-CA by both DPP and HPLC). In experiments where pH

was varied, pH measurements were made before and after dialysis with the latter being considered the equilibrium pH.

Membrane treatment was similar to that described in Chapter 4, i.e., 2 M HNO_3 for 30 minutes, 2 M NaOH for 30 minutes and receiver solution for 15 minutes. Since speciation experiments did not involve the preconcentration of contaminants as was the case in Chapter 4, the membranes were used without the sample clean-up procedure described earlier.

Dialysis experiments were usually done using one dialysis cell arrangement to represent one set of experimental conditions. For example, kinetic studies involved six individual dialysis cells run simultaneously with each being stopped at a different dialysis time. Similarly, pH and titration experiments involved six dialysis cells, each cell representing a different pH or a different concentration of metal or ligand.

Mass balance experiments were done as follows. Once the dialysis experiment was completed, the membranes were immediately removed and placed in a beaker containing 40-45 mL of 0.5 M HNO_3. The membranes were completely immersed and the acid wash circulated for 30 minutes. The wash was neutralized with NaOH and brought to pH 4.5 with acetate buffer. These washings were then transferred

quantitatively to a 50 mL volumetric flask and made up to volume. Metal analysis was done by differential pulse polarography.

The humic acid (HA) solutions were prepared by serial dilution of a stock solution made up as follows. Acid-washed technical grade humic acid (Aldrich Chemicals Co., Milwaukee, Wis.) was suspended in distilled/deionized water and NaOH pellets added to bring the pH to about 11. This solution was filtered through Whatman #42 paper with the filtrate being acidified with HNO_3 to pH 2. The humic acid precipitate was recovered by centrifugation at 3500 rpm for 30 minutes. The precipitate was redissolved in distilled/deionized water by bringing the pH to 7. This HA solution was filtered sequentially through Whatman #1, Whatman #42, Nuclepore 1 µm and Nuclepore 0.2 µm filters. The final filtrate represented the humic acid stock solution. Its concentration was determined by acid-precipitation (pH 2) of 25 mL aliquots. The precipitates were recovered on Nuclepore 0.2 µm filters, dried under vacuum for 1 week and weighed. The concentration of the stock solution was found to be 2580 ppm. The fulvic acid (FA) stock solution was prepared by dissolving an appropriate amount of freeze-dried fulvic acid (obtained

from Dr. C. Langford, Concordia University, Montreal) in 1 L of distilled/deionized water which was also made 0.30 M in $NaNO_3$. The fulvic acid stock solution was 100 ppm.

5.2 Results and Discussion

5.2.a. Characterization of speciation by Nafion dialysis:

As implied by equation 4-3, speciation using ion-exchange membranes requires that ionic strengths be the same in both sample and receiver solutions. If this is not the case, dialysis will either be reinforced or suppressed to the extent determined by equation 4-3. For example, if the receiver electrolyte is 0.11 M $NaNO_3$ and the sample electrolyte is 0.10 M $NaNO_3$, a preconcentration of sample trace metals will result amounting to an enrichment factor of 1.21 (i.e., $(0.11/0.10)^2$) or an error of 21%. This requirement can be met if the electrolyte concentration used for sample and receiver solutions is much greater than the total concentration of sample trace metals.

Initial experiments were done using 0.10 M $NaNO_3$ as electrolyte, with 200 mL sample volumes and 10 mL receiver volumes. Figure 5-1 presents the dialysis of a 10 μM Cd^{2+} sample solution as a function of time. The sample solution contained no strong ligands (i.e., 10^{-3} M acetate, pH 4.5) and therefore, at equilibrium, both sample and receiver

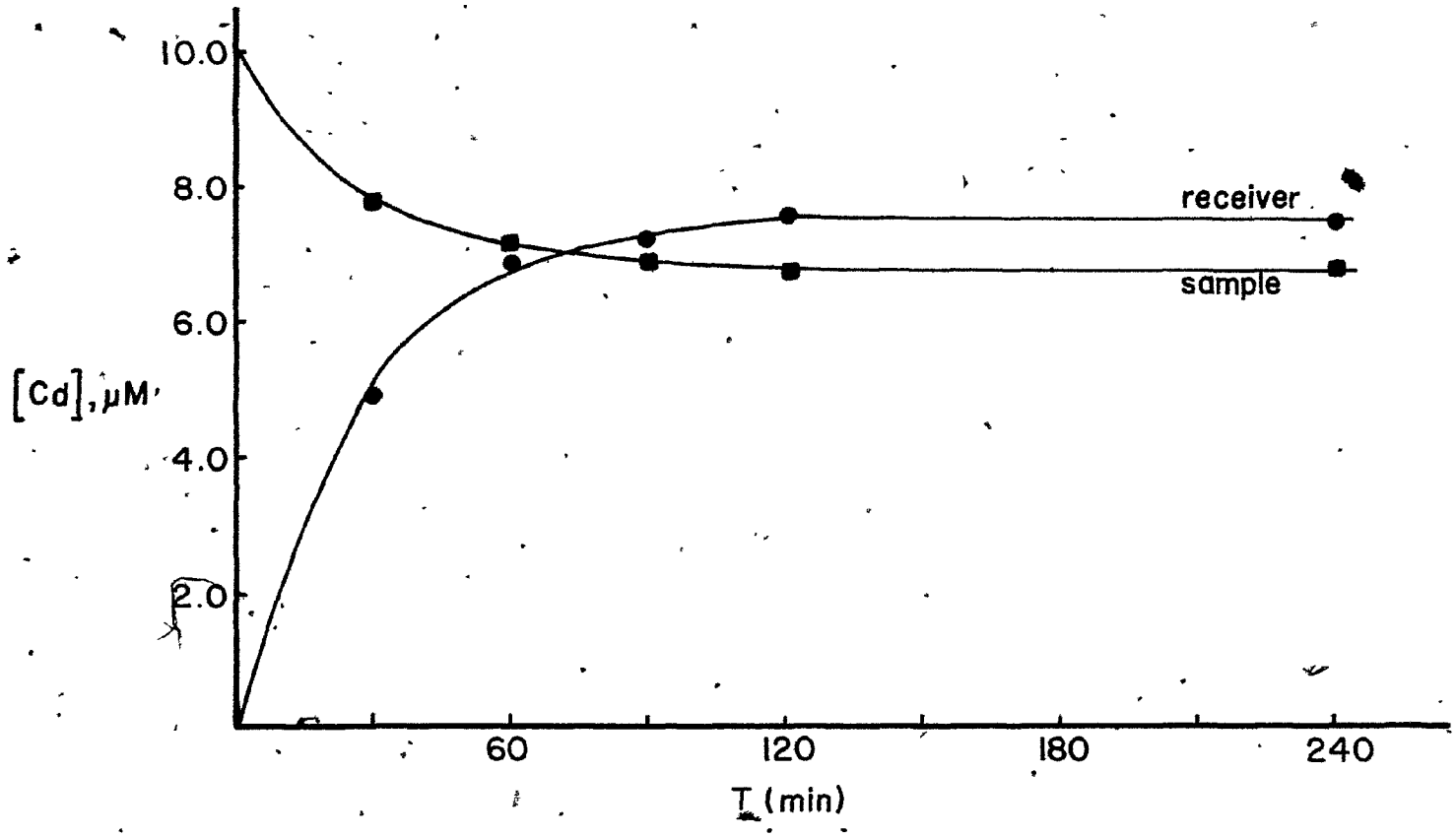


Fig. 5-1: Dialysis using 0.10M $NaNO_3$ and a 200/10 arrangement.

concentrations should be the same. For the sake of simplicity, only Cd results are shown but similar results were also obtained for Cu, Mn, Mg and Ca.

Several points should be noted. First, both receiver and sample solutions attained equilibrium concentrations after approximately 120 minutes. Second, the receiver concentration was greater than the sample concentration, typically a 5-10% enrichment. Third, losses of 20-30% were observed for both solutions.

The fact that the receiver metal concentrations were always 5-10% greater than those in the sample solution was thought to be the result of water evaporation on the receiver side. This evaporation appeared to be proportionally more significant on the receiver side, possibly due to evaporation losses from exposed dialysis tubing. Losses of several tenths of a milliliter during a 120-180 minute dialysis run were not uncommon. The result of water evaporation would be an increase in electrolyte concentration, resulting in receiver preconcentrations. For example, a decrease of receiver volume from 10.0 mL to 9.5 mL would result in a 5.2% increase in electrolyte concentration and a 10.8% increase in divalent trace metal concentration (i.e., a 10.8% enrichment with respect to sample).

A more significant problem is the 20-30% loss from both sample and receiver solutions. In the absence of any adsorption losses of metal ion, both solutions should equilibrate at ~9.5 μM (i.e., 200/210 x 10 μM). The fact that losses cannot be accounted for by sample dilution indicates that adsorption may be occurring. These 20-30% losses were independent of pH over the 3.5-6.0 range. They were also reproducible and proportional to sample concentrations. Calibration curves for Cu, Zn, Ni, Pb, Co, Cd, Mn, Mg, and Ca were all linear despite these losses. Table 5-1 presents the results of a reproducibility experiment using 0.10 M $NaNO_3$, 200/10 sample to receiver ratio and a 75 minute equilibration. The relative standard deviations for receiver concentrations ranged from 2.6 to 5.4% and sample losses were typically 20%.

Separate experiments indicated that the adsorption loss was not onto the glassware or Tygon tubing but onto the Nafion membrane. Figure 5-2 indicates the amount of cadmium adsorbed onto the membranes (as determined by the acid wash procedure) as a function of initial sample cadmium concentration. It is obvious that the amount adsorbed is directly proportional to the sample analyte concentration. Table 5-2 presents data for a similar

Table 5-1

Reproducibility of Nafion dialysis procedure

	Cu	Cd	Mn	Mg	Ca
$\bar{X}_{std}$	167	172	128	17	117
$\bar{X}_{rec}$	138(5.4)	144(4.0)	101(3.6)	14(5.4)	94(2.6)
$\bar{X}_{sam}$	128	134	97	14	92

$\bar{X}_{std}$ = mean response (mm) for 2 standard solutions.

$\bar{X}_{rec}$ = mean response (mm) for 6 receiver solutions.

$\bar{X}_{sam}$ = mean response (mm) for 2 sample solutions.

- bracketed values are %RSD for 6 determinations.
- analysis by HPLC/PD.

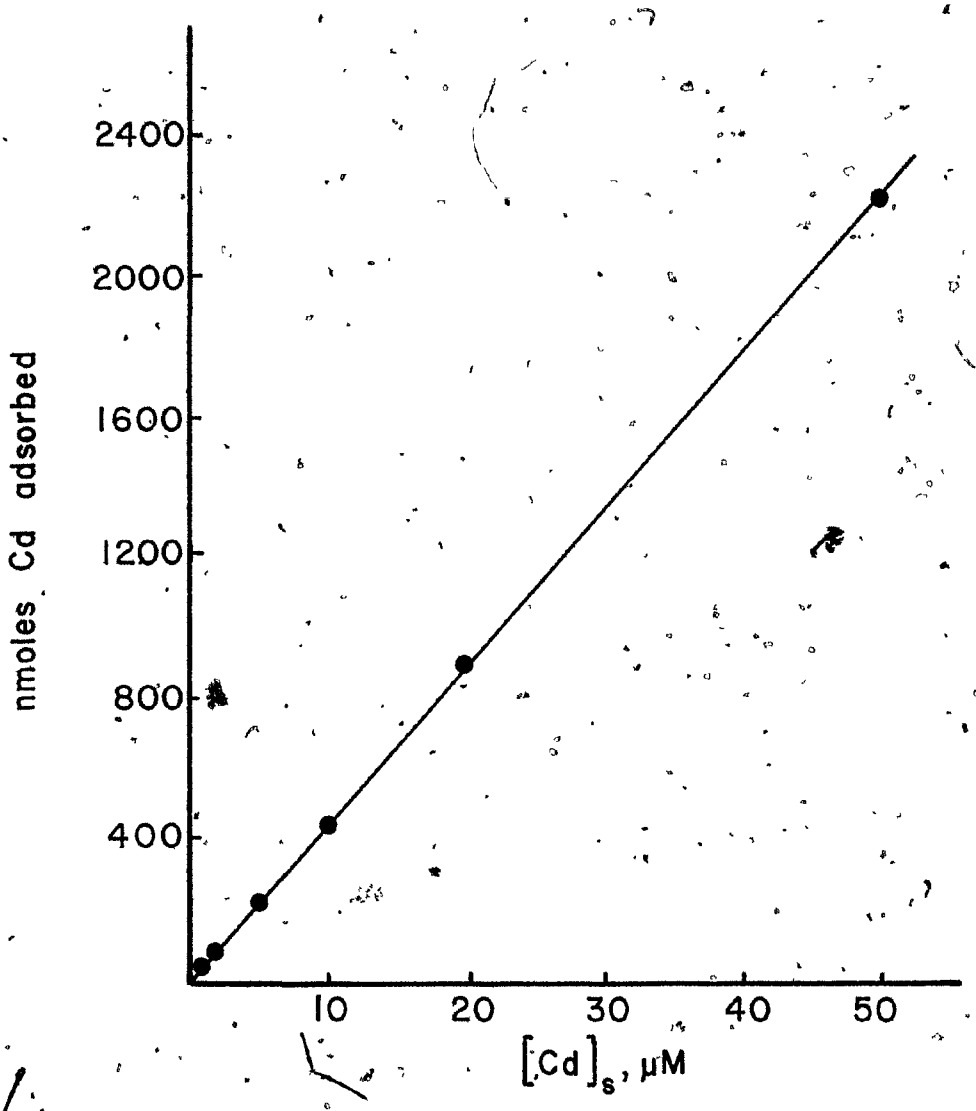


Fig. 5-2: Adsorption of Cd onto Nafion 811X

- conditions as in Fig. 5-1 with T = 120 min, pH 4.5 and analysis by DPP.

Table 5-2

Adsorption of Cu onto Nafion membranes

	Receiver (10 mL)		Sample (200 mL)		Wash (50 mL)		Σ
$[NaNO_3]$	[Cu]	nmoles	[Cu]	nmoles	[Cu]	nmoles	nmoles
0.05 M	4.32	43.2	4.26	852	20.21	1011	1906
0.07 M	6.87	68.7	5.87	1174	13.29	665	1907
0.10 M	6.87	68.7	6.76	1352	9.69	485	1905
0.20 M	10.72	107.2	8.98	1796	3.71	186	2089
0.30 M	11.74	117.4	9.58	1916	2.21	111	2144
0.50 M	11.32	113.2	9.58	1916	0.90	45	2074

T = 120 min, pH = 4.5 and analysis by DPP

Initial sample concentration = 10 μM

adsorption study but as a function of electrolyte concentration. These results are for Cu but similar results were also obtained for Cd, Pb and Zn. Two observations should be noted. First, essentially all of the metal loss could be accounted for by membrane adsorption. Within experimental error, the mass balance for all electrolyte concentrations was obtained (vs theoretical 2000 nmoles). Second, membrane uptake was dependent on electrolyte concentration. As electrolyte concentration increased, membrane uptake decreased and attained negligible values at 0.30-0.50 M $NaNO_3$.

All of our data are consistent with metal adsorption onto the membrane. Blaedel and Niemann (1975) reported 5-10% adsorption losses of copper onto ion-exchange materials when the electrolyte was 0.10 M NaCl. They investigated these adsorption losses for three ion-exchange membranes: AMF C-103, a sulfonated styrene on a polyethylene backbone; RAI P1010, a Teflon-sulfonated styrene copolymer; and Nafion XR-170, a sulfonated fluorocarbon polymer. They reported that of the three, the Nafion membrane suffered least from adsorption problems. They attributed these losses to the presence of impurities (carboxylate or olefinic groups) in the membrane structure.

Our results, however, would seem to indicate that almost all of the losses observed when using Nafion 811X tubular membranes are due to ion-exchange processes. If adsorption losses were the result of binding by impurities, we would expect greater relative losses at low metal concentrations with decreasing losses as the binding sites become saturated (i.e., similar to an adsorption isotherm). However, we obtain both linear calibration curves and a linear adsorption curve (Figure 5-2). As well, adsorption is related to $NaNO_3$ concentration. It is therefore probable that the losses observed were the result of the simple equilibrium processes described in equations 5-1 to 5-3. As electrolyte concentration increases, sodium ions are better able to compete for the sulfonate sites and lower losses are observed.

It is possible that at very low metal concentrations, non-proportional adsorption will occur as a result of metal uptake by membrane impurities. However, none was observed over the concentration range studied, i.e., 0.5-50 μM. The practical implication of the above is that experiments must either be carried out in 0.3-0.5 M $NaNO_3$ or that losses be taken into account by using calibration curves obtained under identical conditions. This latter approach might lead to greater irreproducibility and a greater perturbation of sample chemical equilibria. It was decided to

carry out future speciation experiments using 0.30 M $NaNO_3$ as the background electrolyte. Furthermore, a 400 mL sample/25 mL receiver arrangement (vs 200/10) was also incorporated with the hope of decreasing the 5-10% preconcentration that was thought to result from receiver evaporation. Unless otherwise stated, the following results were all obtained under these experimental conditions.

To examine the Nafion dialysis of this new experimental arrangement, a kinetic study was done under conditions similar to those in Figure 5-1 except that the electrolyte concentration was increased from 0.10 to 0.30 M and that a 400/25 ratio was used instead of the previous 200/10. Samples were again made up in 10^{-3} M acetate buffer, pH 4.5. Results are shown in Figure 5-3. The change in volume ratios did not appear to significantly alter kinetics as equilibrium was once again achieved within 120-180 minutes. There were, however, two important improvements when compared to the previous conditions. The preconcentration observed when the receiver volume was 10 mL was no longer discernible. The fact that an increase in receiver volume resulted in a decrease in the small preconcentrations observed previously supports the suggestion that water evaporation from the receiver solution was its cause. In addition, adsorption losses due to the membrane

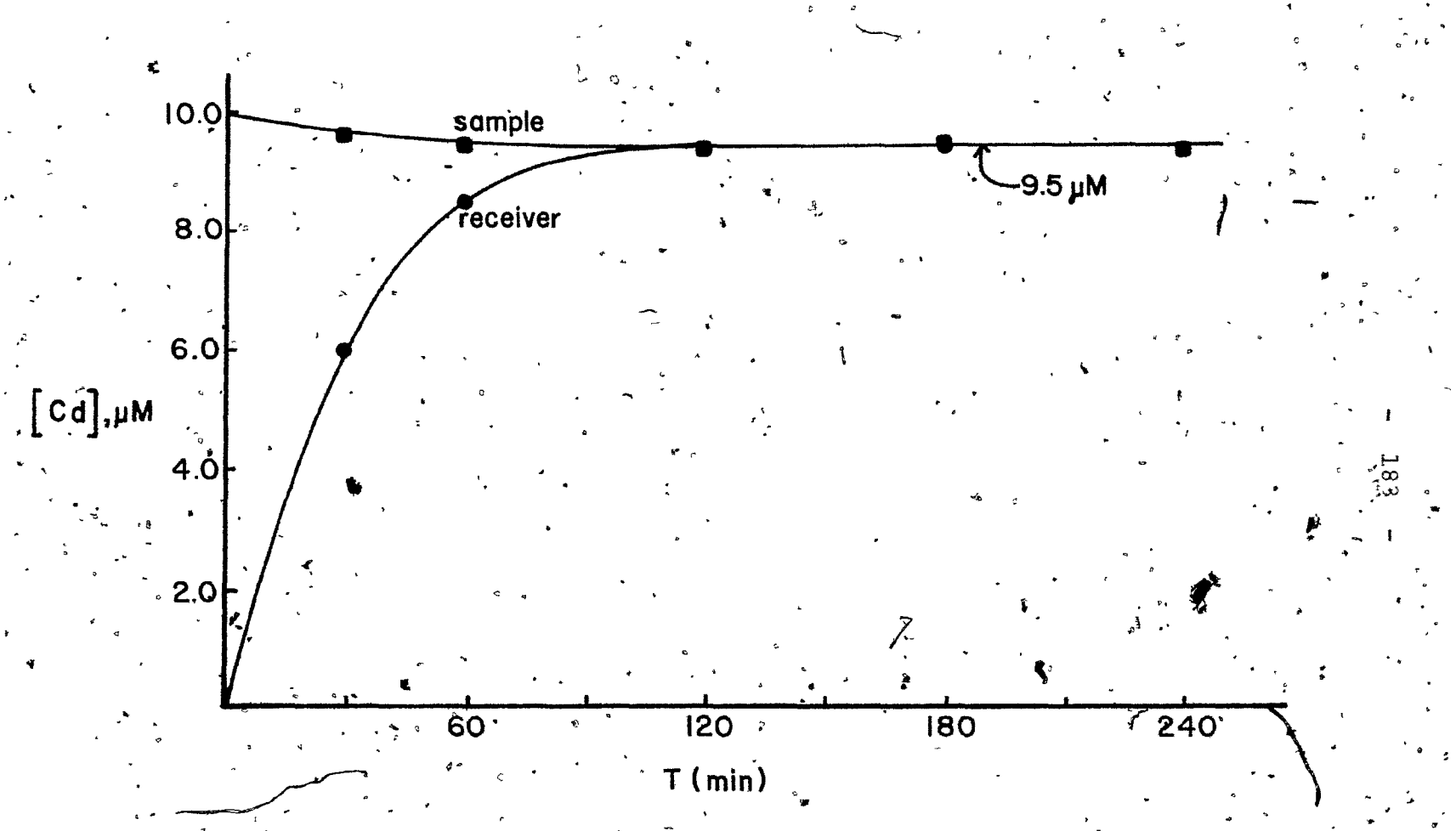


Fig. 5-3: Dialysis using 0.30M $NaNO_3$ and a 400/25 arrangement.

uptake were no longer observed. Both sample and receiver concentrations equilibrated at 95% (vs 400/425 x 100 = 94.1% theoretical). Similar results were also obtained for the other metals studied.

The Nafion dialysis speciation conditions discussed above (i.e., 0.30 M $NaNO_3$, 400/25, T = 120-180 min) appear to be giving meaningful results. In the absence of any strong ligands, measured receiver concentrations are the same as those in the sample. Calibration curves for Cu, Cd, Pb and Zn obtained under these conditions are given in Figure 5-4. Linear curves were obtained for all metals over the 0-5 μM range studied.

The Nafion membrane will have two mechanisms to effect a speciation -the Donnan exclusion of anionic species and the size exclusion of the "connecting channels". To characterize the selectivity of the membrane, an equilibrium dialysis experiment was done for a number of classes of possible species. The kinetic dialysis curves are shown in Figure 5-5 (i.e., percent dialysis as a function of time). The first species, the aquo complex of cadmium, attained dialysis equilibrium after 120 minutes. A second cationic species, triphenyl tin (ϕ_3Sn^+), did not dialyze over the 240 minute duration of the experiment. The anionic species, Cd-NTA^-, was also completely excluded by the membrane. This is in contrast with conventional

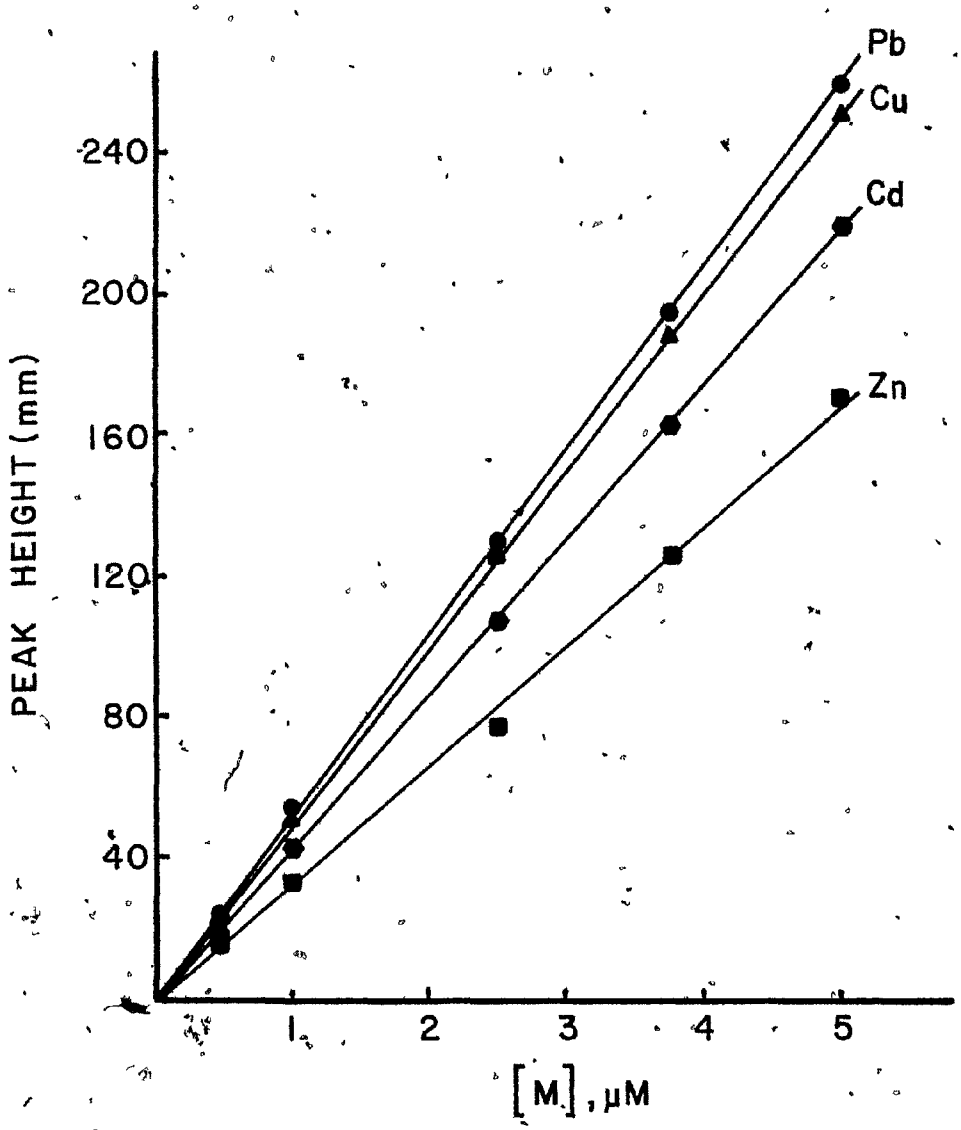


Fig. 5-4: Calibration curves using 0.30M $NaNO_3$ and a 400/25 system.

- T=180 min, pH=4.5, analysis by DPP.

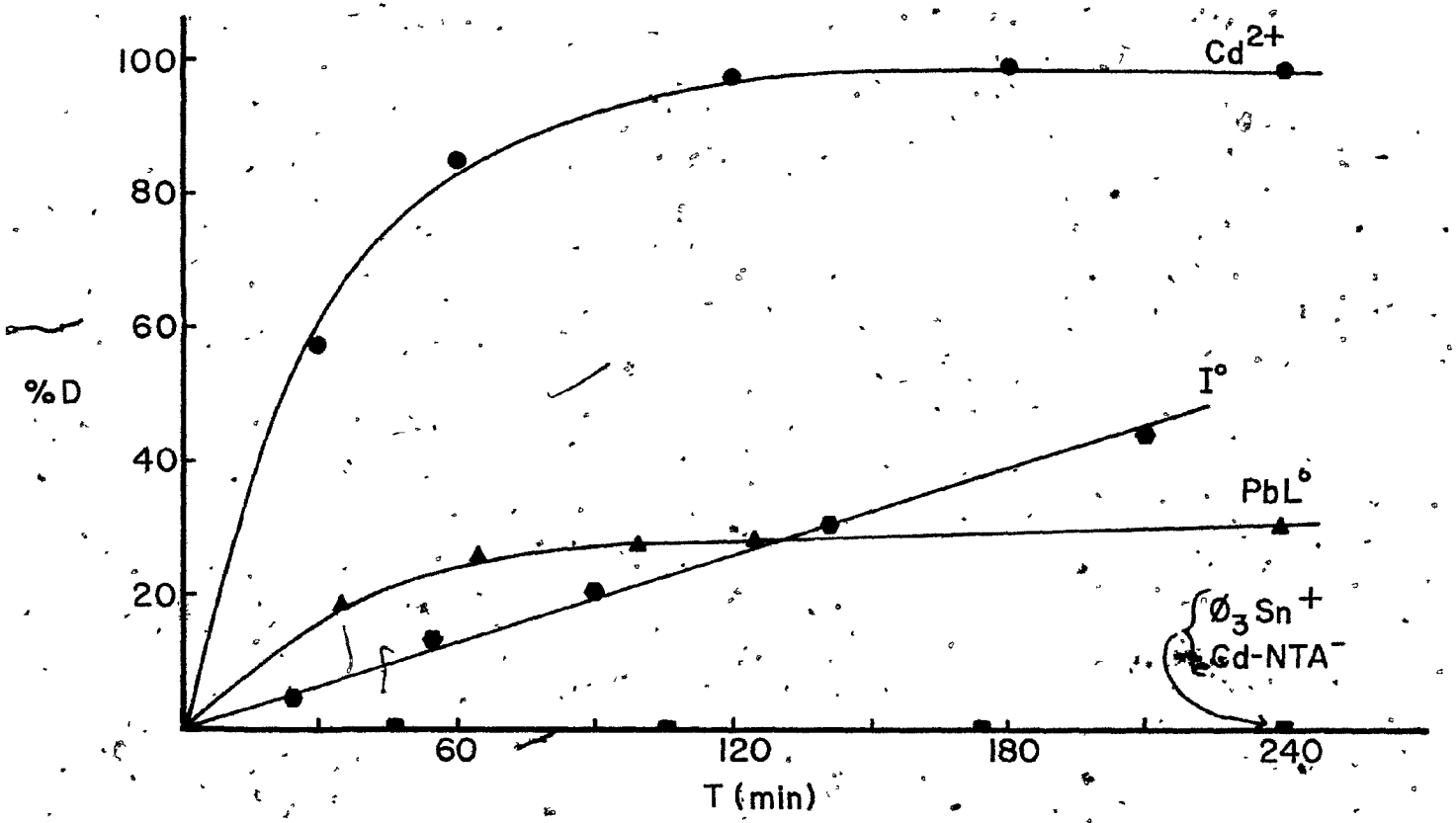


Fig. 5-5 : Permselectivity of Nafion 811X membranes.

- conditions as in Fig. 5-3 with analysis by DPP.

dialysis procedures where the anionic species Fe-EDTA$^-$ (Benes and Steinnes, 1974) and Cu-EDTA^{2-} (Guy and Chakrabarti, 1976a) dialyzed slowly but did attain 100% dialysis.

A neutral species, 2-methyl-5-nitroimidazole (I^o), dialyzed slowly and was 26% dialyzed when the free cation was 100% dialyzed at 120 minutes. The fifth system studied was the lead cation in the presence of a large excess of phthalic acid at pH 5.00. Equilibrium calculations using conditional constants (Ringbom, 1979, p325) showed a species distribution of 9.1% Pb^{2+}, 90.6% PbL^o, and 0.4% PbL_2^{2-}. The experimental dialysis attained equilibrium after 120 minutes and showed that 28.5 ±1.0% of the lead was dialyzable. At 240 minutes, 30.9% of the lead was dialyzed. These results suggest that the rapid dialysis up to 120 minutes was for the free cation and the very slight increase at 240 minutes might be dialysis of neutral species. The Ringbom conditional constant for PbL^o was 1000 whereas the experimental results suggest a constant of 250.

This simple study suggests that anionic species are definitely excluded. The selectivity with respect to size, however, would require further characterization for neutral species. It is thought that the ionic strength of the aqueous medium may play a role in determining the degree of

swelling of these membranes and consequently the size of the diffusion pathways. It would appear that these pathways would be less than 4 nm in diameter and possibly as low as 1 nm (Yeager and Kipling, 1979). It is therefore reasonable to assume that such large species as humic acids and inorganic colloids will be size excluded. Smaller species, such as neutral compounds and simple inorganic complexes (e.g., $CuCO_3$) should be characterized individually to determine their rate of dialysis. For Group 3 type speciation studies, an error estimate of the contribution by neutral species could be easily obtained by comparing the dialysis at 120 minutes (simple cations) and 240 minutes (complex cations and neutral species).

5.2.b. <u>One metal-one ligand speciation models:</u> Two procedures are commonly used to characterize a water sample. The first is a binding capacity experiment in which the sample is titrated with the metal ion of interest. The second method is to adjust the sample pH and monitor the speciation changes. The first method gives a measure of the ability of the ligands in the sample to bind the metal of interest whereas the second method allows one to estimate the "available" metal in the sample. The latter method, for example, often uses a pH of 2 or less to

give "total available" metal. The Nafion speciation method should permit one to monitor the free cation species during both of these types of experiments.

Figure 5-6 illustrates the use of the Nafion membranes for the measurement of binding capacities of a simple solution containing one ligand at a total concentration of 10 μM. Three separate titrations are given: EDTA ($\log K_{cond}' = 9.9$), NTA ($\log K_{cond} = 5.7$) and EGTA ($\log K_{cond} = 4.7$). The three titrations each provide a binding capacity of 10 μM but the EGTA result is best described as fortuitous. Extrapolation of the binding capacity curves to the abscissa or drawing tangents to the curves will give an accurate potential binding capacity when the conditional binding constant is large and one has a significant number of data points. The EGTA titration, for example, has such a low binding constant that it is difficult to graphically determine an unbiased binding capacity. One can fit, for example, the experimental data to the expression:

$$K = \frac{[M]_B}{[M]_F[L]_F} = \frac{(C_T - M_F)}{(M_F)(L_T - C_T + M_F)} \quad (5\text{-}5)$$

where K, C_T, M_F and L_T are the conditional stability constant, total metal added, metal free and binding

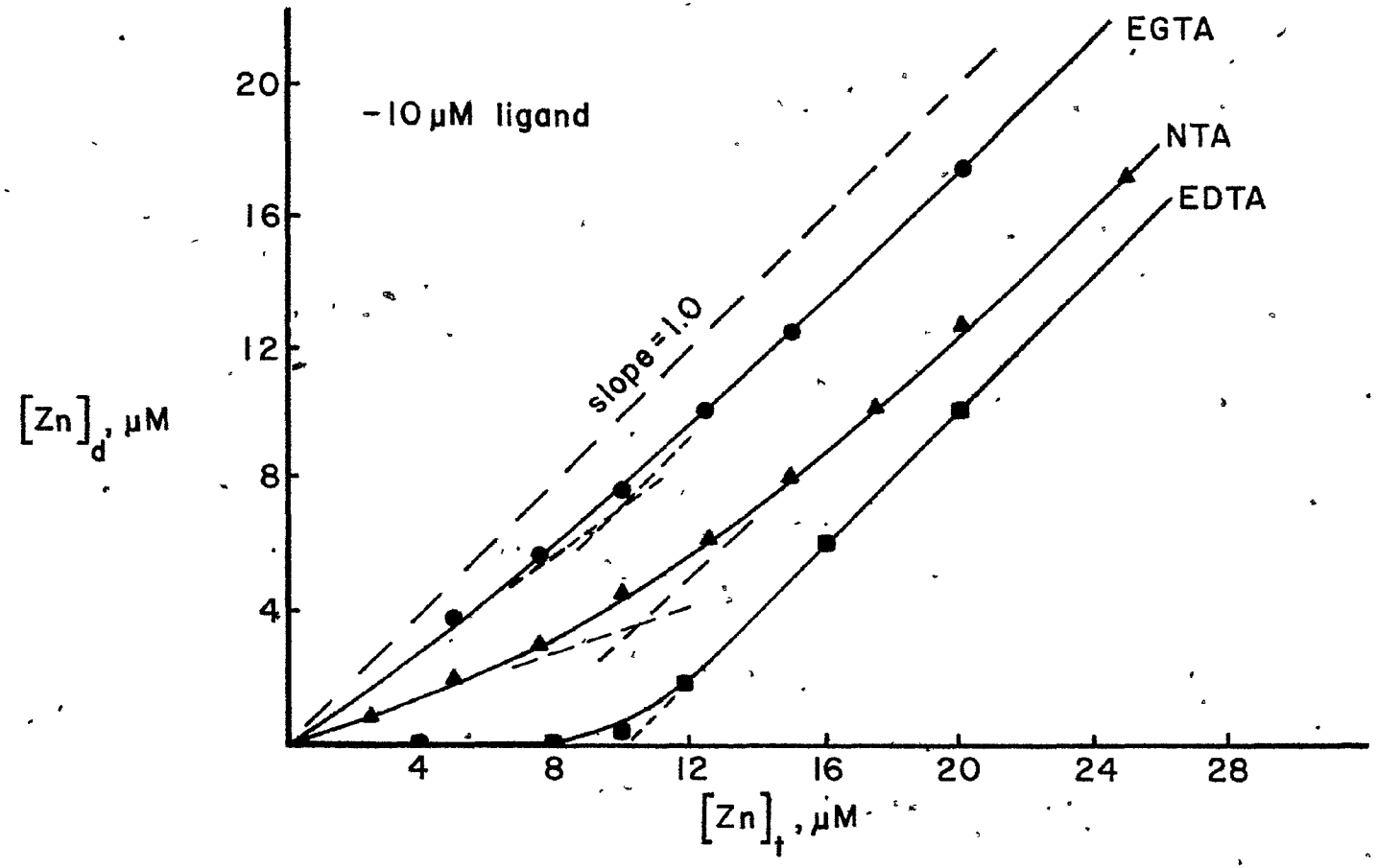


Fig.5-6: Zn binding capacity titrations for EDTA, NTA, EGTA.

- conditions as in Fig.5-3, T=180 min, analysis by HPLC/PD, pH=5.00.

capacity, respectively. This expression assumes no secondary complexing agents and the formation of 1:1 complexes. The experimental data - C_T and M_F - can be used to obtain a best estimate of K and L_T.

A simple non-linear regression program for micro-computers (Duggleby, 1981) was used to fit the Zn-NTA and Zn-EGTA titrations. The Zn-NTA titration gave a value of, $2.16 \times 10^5 \pm 1.8 \times 10^4$ for K and $1.05 \times 10^{-5} \pm 5 \times 10^{-7}$ M for L_T. The Zn-EGTA titration gave a value of $4.71 \times 10^4 \pm 2.5 \times 10^4$ for K and $7.6 \times 10^{-6} \pm 3.1 \times 10^{-6}$ M for L_T. The greater uncertainty in the Zn-EGTA data is a result of a lower binding constant, hence the amount bound (calculated as the difference between C_T and M_F) has a greater relative uncertainty. This approach of fitting experimental titration data to obtain binding constants and binding capacities of real water samples or humic acid suspensions, however, must be applied with caution (MacCarthy and Smith, 1979).

If a metal is added to a ligand solution under conditions where the ligand is in considerable excess, a constant fraction of the metal is bound (Glass, 1977). This is illustrated for a cadmium titration into 5.0 mM citric acid in Figure 5-7. A constant fraction, 38.7 ±0.8%, of the added cadmium was found to be free. The

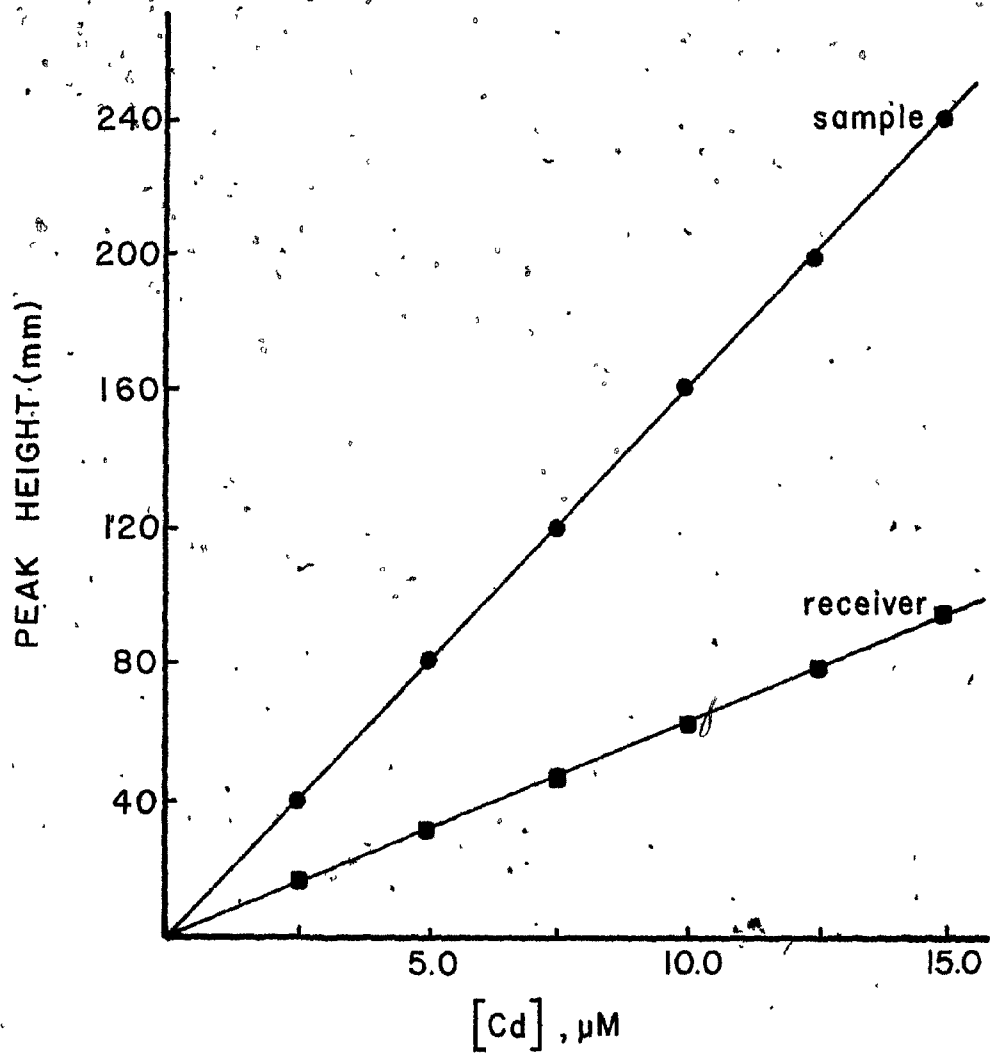


Fig.5-7: Speciation of Cd with 5 mM C.A., pH 5.00.

- conditions as in Fig.5-3 with T=180 min and analysis by DPP.

fraction of free metal depends on the conditional binding constant and the ligand concentration. Figure 5-8 shows three curves for the individual titrations of copper, nickel and zinc into 100 μM citric acid. The results showed that 22.5%, 41.5% and 66.5% of the added copper, nickel and zinc, respectively, was free over the metal concentration range of 0-20 μM. These percentages reflect the decreasing conditional constants, i.e., log K = 4.9, 3.6 and 3.3 for copper, nickel and zinc, respectively. The effect of concentration is illustrated in Figure 5-9 for cadmium in the presence of increasing amounts of citric acid. A calculated speciation using citric acid binding and protonation constants corrected to ionic strength of 0.30 (Ringbom, 1979) was included for comparison. Excellent agreement was observed between the experimental Nafion and calculated speciation.

The first example of a speciation as a function of sample pH is given in Figure 5-10. The sample contained 10 μM Cd^{2+} in a 10-fold excess of NTA. At the dropping mercury electrode, the reduction of Cd^{2+} and Cd-NTA^{-} are sufficiently well-resolved in differential pulse polarography to allow one to estimate the concentration of the free metal ion. The speciation done using DPP on the outer sample is compared with the DPP determination of free cation after Nafion dialysis. The two methods were found

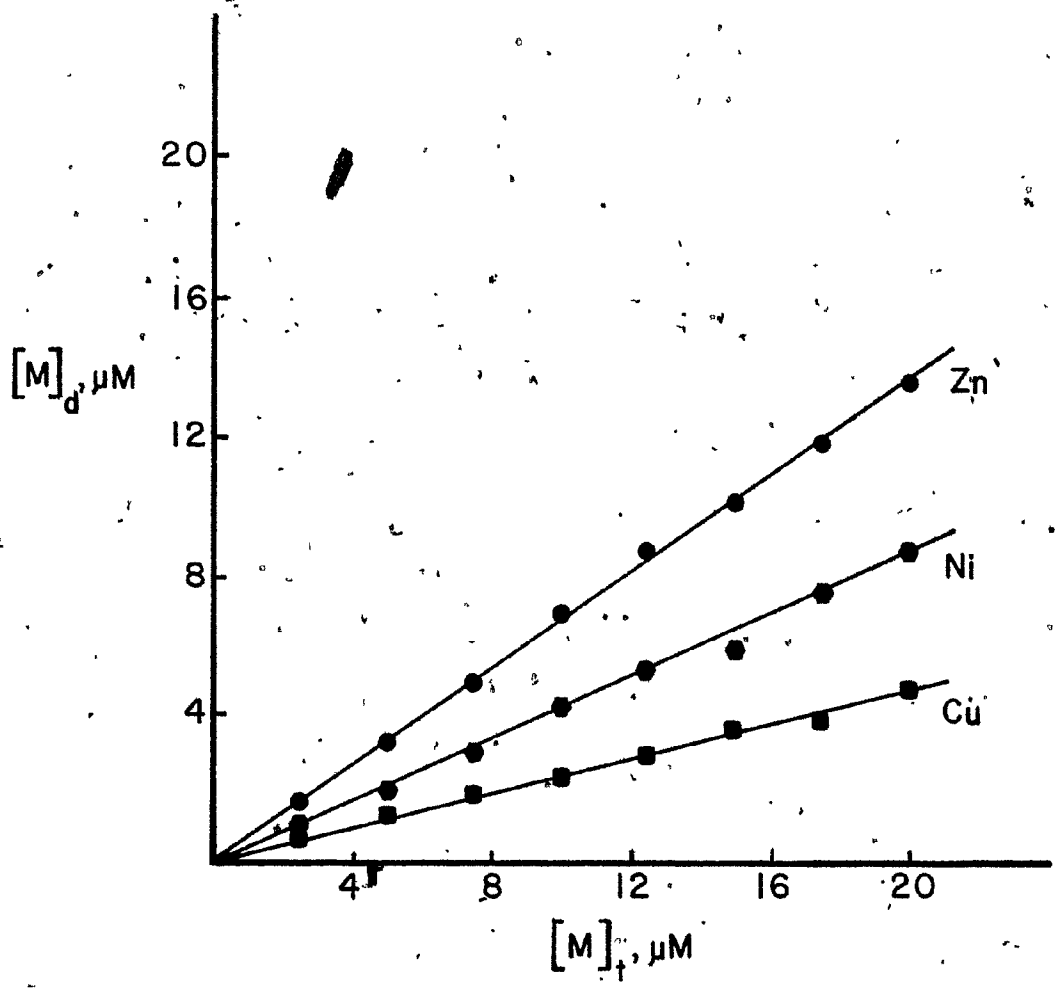


Fig. 5-8 : Speciation of Cu, Zn and Ni with 100 µM C.A. at pH 5.00.

- conditions as in Fig. 5-3 with T = 180 min and analysis by HPLC/PD.

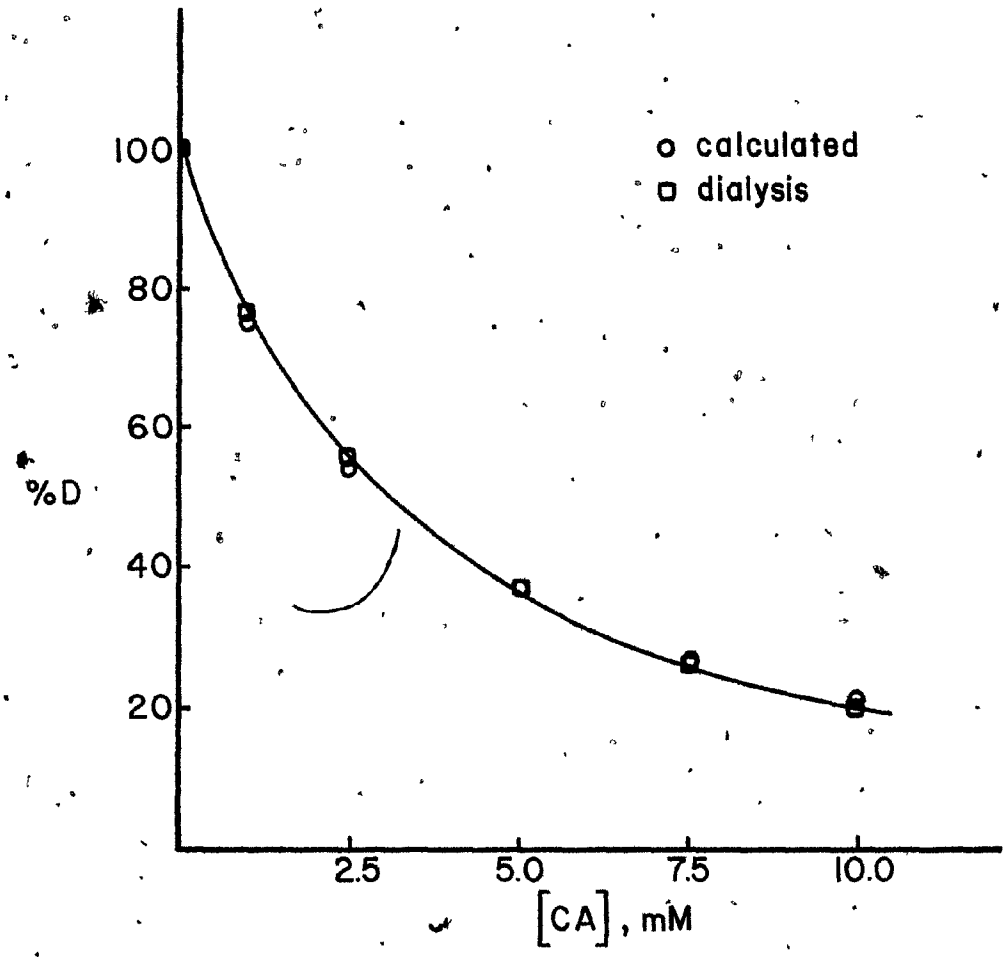


Fig. 5-9: Speciation of Cd-CA vs [CA].

- conditions as in Fig. 5-3 with pH= 4.95, T=180 min, 10μM Cd and analysis by DPP.

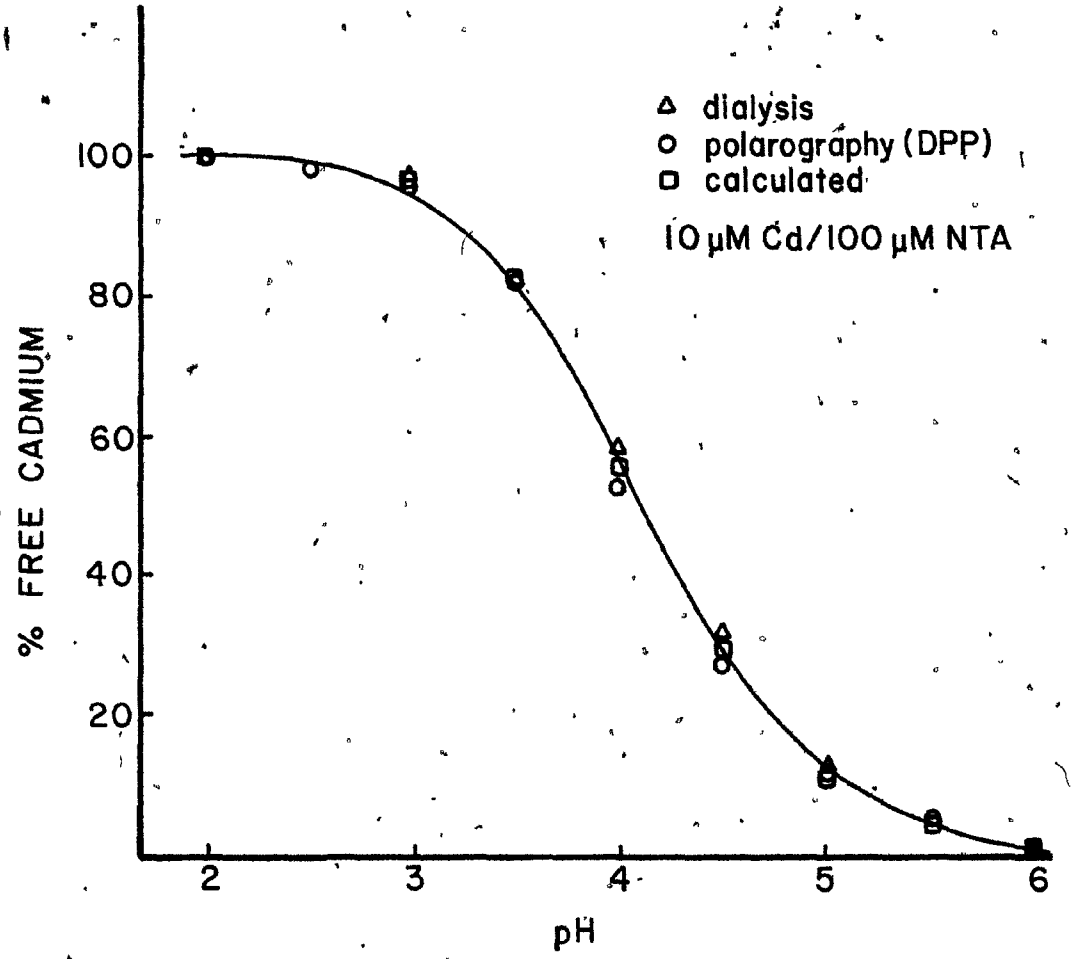


Fig. 5-10: Speciation of $Cd\text{-}NTA^-$ vs pH.

- conditions as in Fig. 5-3 with T = 180 min.

to agree within experimental error. It should also be noted that no Cd-NTA reduction peak was found in the Nafion receiver polarogram. The theoretical speciation for this system was calculated using binding and protonation constants taken from the literature (Martell and Smith, 1977) and corrected for ionic strength changes (Ringbom, 1979, p22-28). There was good agreement between the two analytical speciation methods and the calculated speciation.

A second example of pH-type speciation is given in Figure 5-11 for cadmium in the presence of citric acid. The calculated speciation using corrected constants is also included for comparison. The calculated and experimental speciation curves do not coincide as well as the Cd-NTA example shown earlier. The experimental uncertainty from repeated experiments indicates that for the CA-Cd-pH titration, the error would be on the order of ±2%, but the uncertainty in the calculated curve may be much larger because of the uncertainty in literature stability constants. The difference between the calculated and experimental curves at low and high pH regions is probably not due to changes in the membrane characteristics. Further evidence for this is provided later in this chapter.

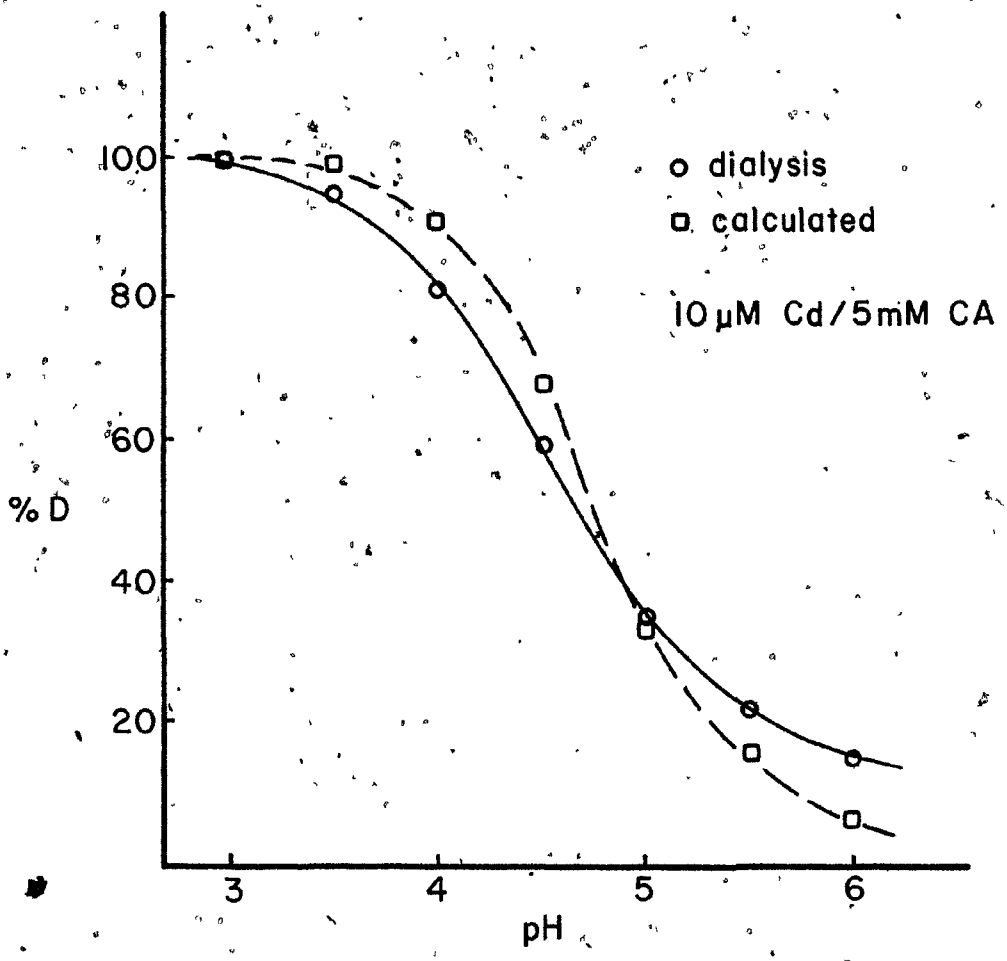


Fig.5-11: Speciation of Cd-CA vs pH.

- conditions as in Fig.5-3 with T=180 min and analysis by DPP.

5.2.c. Multimetal speciation models: The Nafion speciation studies described earlier indicate that the membrane is suitable for determining free metal ion in a complexing medium. One advantage of coupling a multication analysis method to this Nafion speciation is the ability to monitor metal competition for binding sites.

A simple 2-metal competition study is described in Figure 5-12. A 10 μM EDTA solution was titrated with aliquots of metal ion containing equimolar concentrations of copper and zinc. The conditional stability constants for copper and zinc are log K = 12.2 and log K = 9.9, respectively. The 200-fold difference in stability constants indicates that copper should displace zinc from the Zn-EDTA complex. The data illustrated in Figure 5-12 shows that below the potential binding capacity (i.e., 10 μM total metal), no competition occurs but that after the ligand is saturated, zinc is displaced from binding sites by copper. The slope of the curve in this region is 2 (1 for the added zinc and 1 for the displaced zinc). After copper has occupied all of the binding sites, the slopes for both metals are nearly one.

A typical binding capacity titration of real water samples is potentially a competition experiment. Studies reported in the literature suggest that binding capacities are only 1-5 times greater than the trace metal content.

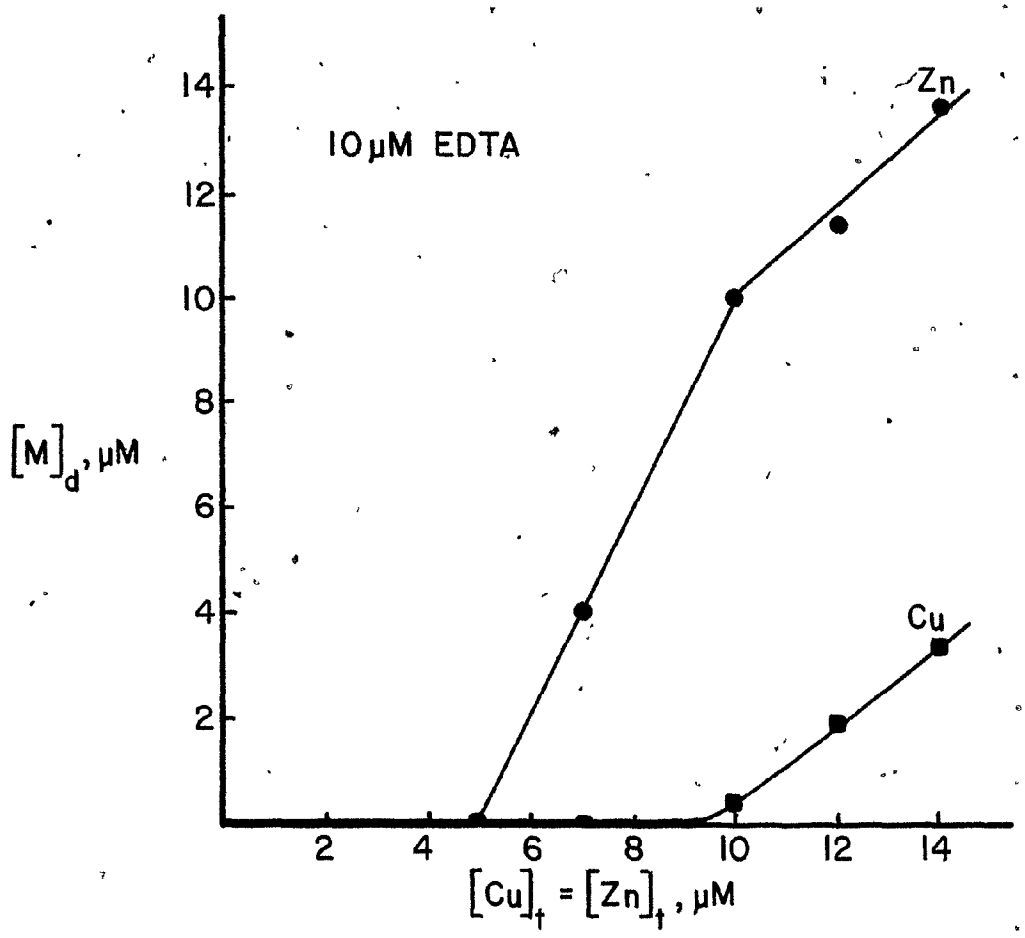


Fig.5-12: Cu/Zn competition for EDTA at pH 5.0.

- conditions as in Fig.5-3 with T = 180 min and analysis by HPLC/PD.

When a titrant metal is added to the water sample, the metal will bind available free sites and will compete with bound metal ions for the already occupied sites. The degree of the displacement of bound metals will depend on the titrant metal ion. For example, cupric ion will displace most metal ions whereas zinc or cadmium will displace smaller fractions of the bound metals.

Three examples of a binding capacity experiment are illustrated in Figure 5-13. The first and simplest case is the titration of one complexing ligand in the absence of competing metal ions. The example in Figure 5-13, curve a, is for the titration of 10 μM NTA with Zn. The potential binding capacity was found to be 10.5 ± 0.5 μM (as previously reported). Curve b is the titration of a mixture of 10 μM NTA + 10 μM Ni. Curve b in the inset shows the free nickel concentration present in the system during the Zn titration. At the start of the titration, the concentration of free Ni is 2 μM; hence one has 2 μM of available NTA sites. During the titration, the Zn and Ni compete for binding sites and some Ni is displaced from the NTA by the added Zn. The competition favors the Ni and at a 2.5-fold excess added Zn (i.e., 25 μM Zn), only 37.5% of the bound Ni had been displaced. Curve c shows the same titration but with an additional 100 μM citric acid added as a secondary complexing agent. The citric acid (CA)

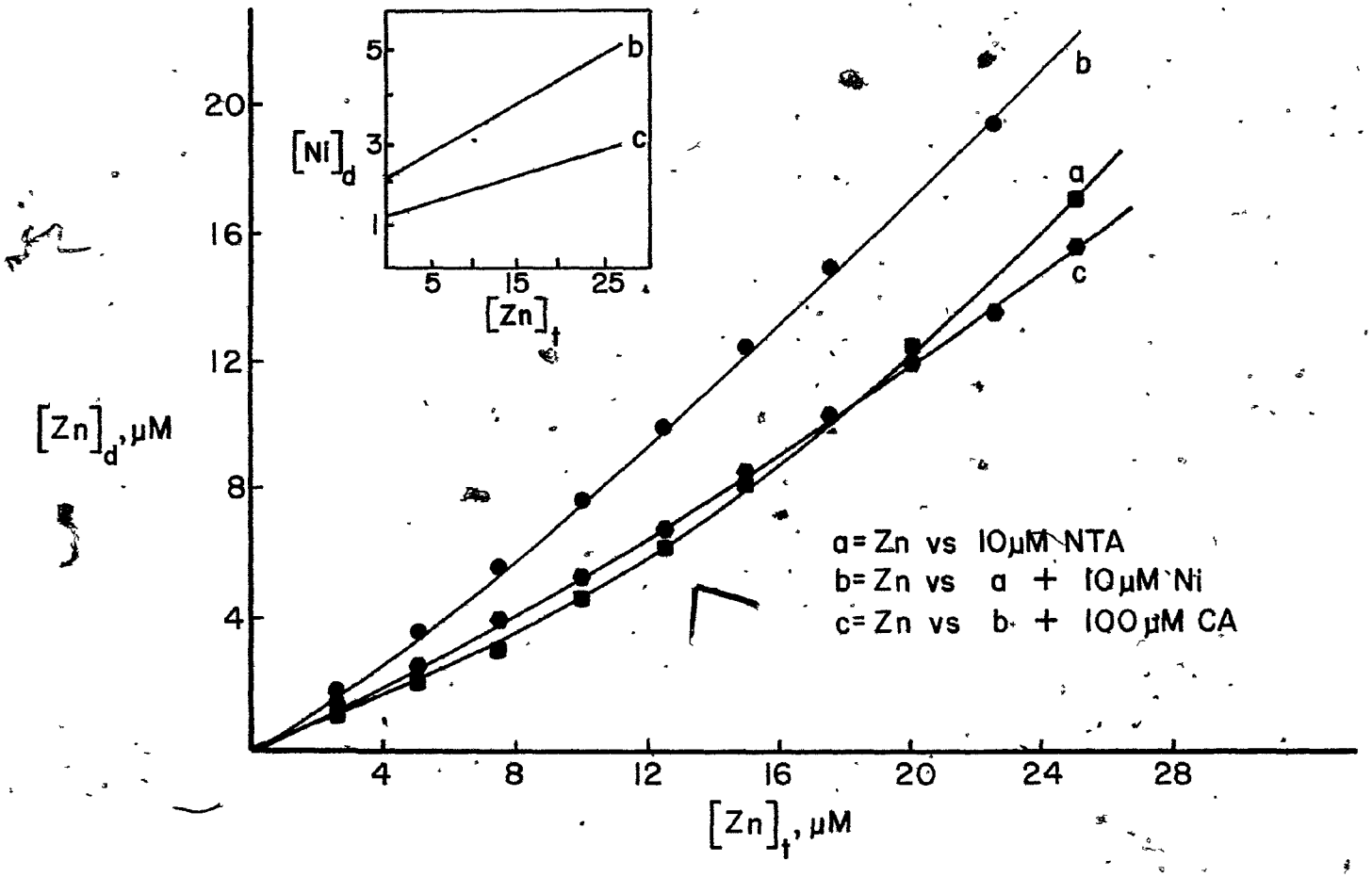


Fig.5-13: Zn/Ni competition for NTA/CA at pH 5.00:

- conditions as in Fig.5-12.

binds an additional fraction of the free Ni and Zn. The effect of the secondary complexing agent is best seen in the inset where one has plotted the free Ni concentration as a function of added Zn. The slopes of the two curves can be used to approximate the interaction intensity parameter described in Chapter 1. In the absence of the secondary complexing agent $d[Ni]/d[Zn]_T$ is 0.122 and in the presence of citric acid $d[Ni]/d[Zn]_T$ is 0.060. In natural waters the principal binding agents are humic colloids which contain multiple sites of varying conditional binding constants. The presence of a large number of weak sites on the colloids will tend to lower the interaction intensity parameters. A measurement of these parameters may be useful in characterizing binding capacities of natural waters.

A final illustration of the potential of coupling the Nafion speciation procedure with multielement analysis is presented in Figures 5-14 and 5-15 a,b. Figure 5-14 shows the results of a pH-type speciation for six metals in the presence of 5 mM citric acid. The high ligand to metal ratio in this solution results in the six metals interacting independently. For example, the curves for Cd in Figures 5-14 and 5-11 overlap. The curves in Figure 5-14 suggest that the conditional stability constants for

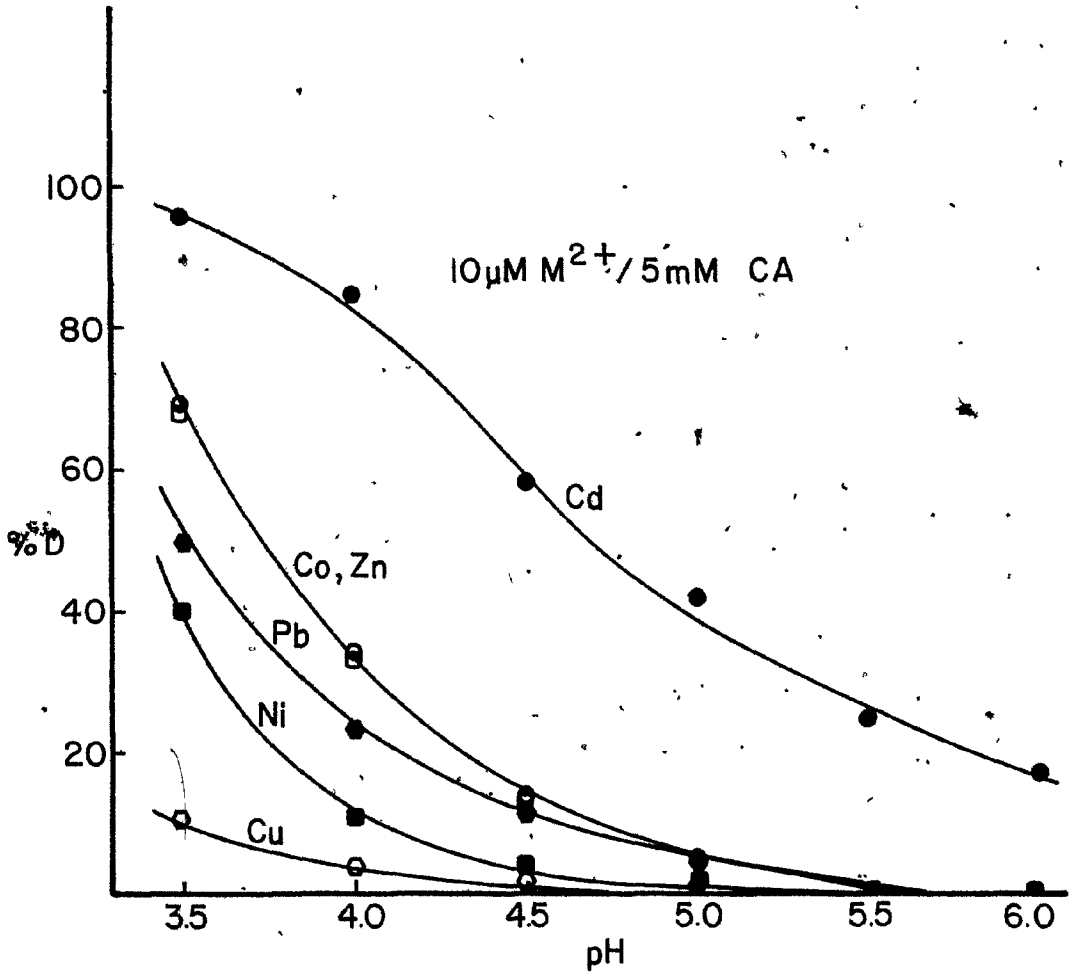


Fig.5-14: Metal-citrate speciation vs pH.

- conditions as in Fig.5-3 with T=180 min. and analysis by HPLC/PD.

the six metals should decrease in the order: Cu > Ni > Pb > Co ~ Zn > Cd. Literature values (Ringbom, 1979) indicate that lead and nickel should be reversed.

The problem of the uncertainty in literature stability constants is best illustrated in Figure 5-15 a,b. The diagrams compare the experimental and calculated speciation using stability constants from two sources and the computer program by Perrin and Sayce (1967). Calculated speciation 1 used constants from Ringbom (1979) and calculated speciation 2 used constants from Martell and Smith (1977). The latter reference listed the "best selected constant" as judged by the compilers. The relative order of conditional constants can be summarized as follows:

1 - Expt: Cu > Ni > Pb > Co ~ Zn > Cd

2 - Ringbom: Pb > Cu > Ni > Co ~ Zn > Cd

3 - Martell & Smith: Cu > Ni > Co ~ Zn > Pb > Cd

Both compilations appear to have selected a poor lead value. The use of calculated speciation data for metal toxicity studies must be examined with care, especially where there exist discrepancies in reported stability constants. This will be of particular importance in studies where one uses a low concentration of complexing agent and a 2-metal mixture to study relative toxicities of metals.

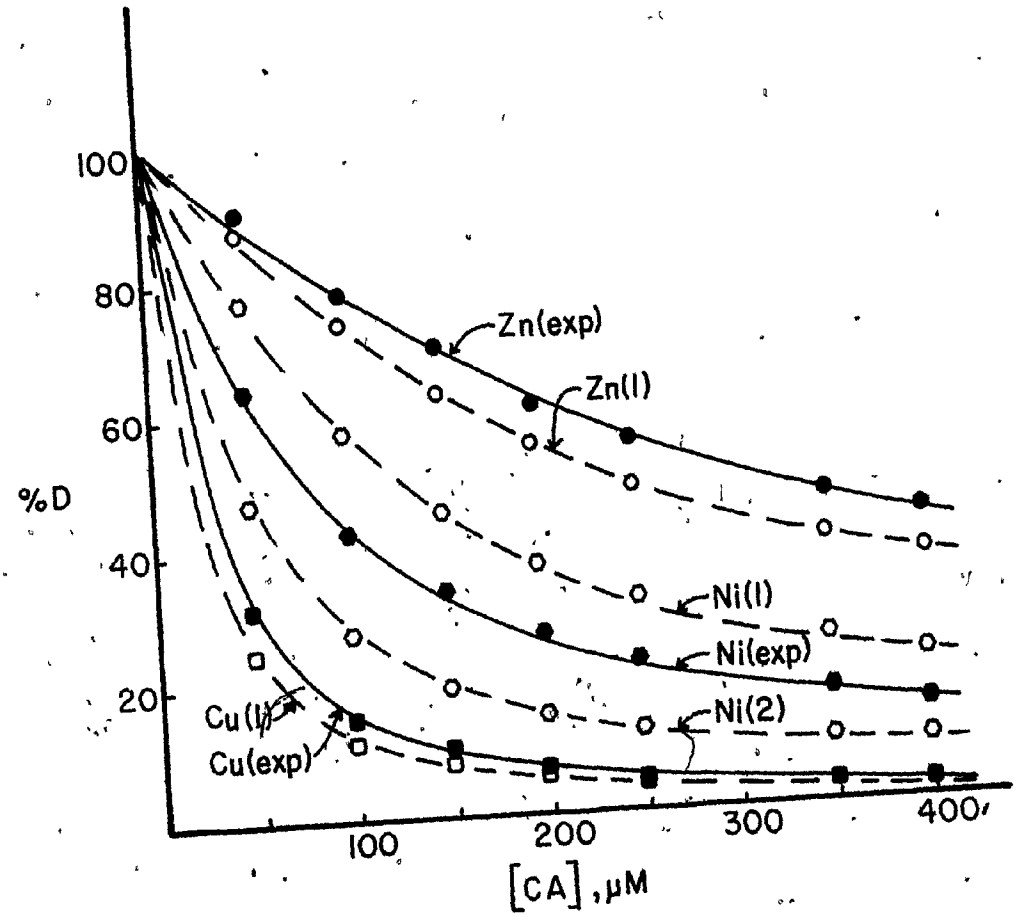


Fig.5-15a: Metal-citrate speciation vs [CA].

- conditions as in Fig.5-3 with pH = 5.00, T = 180 min and analysis by HPLC/PD.

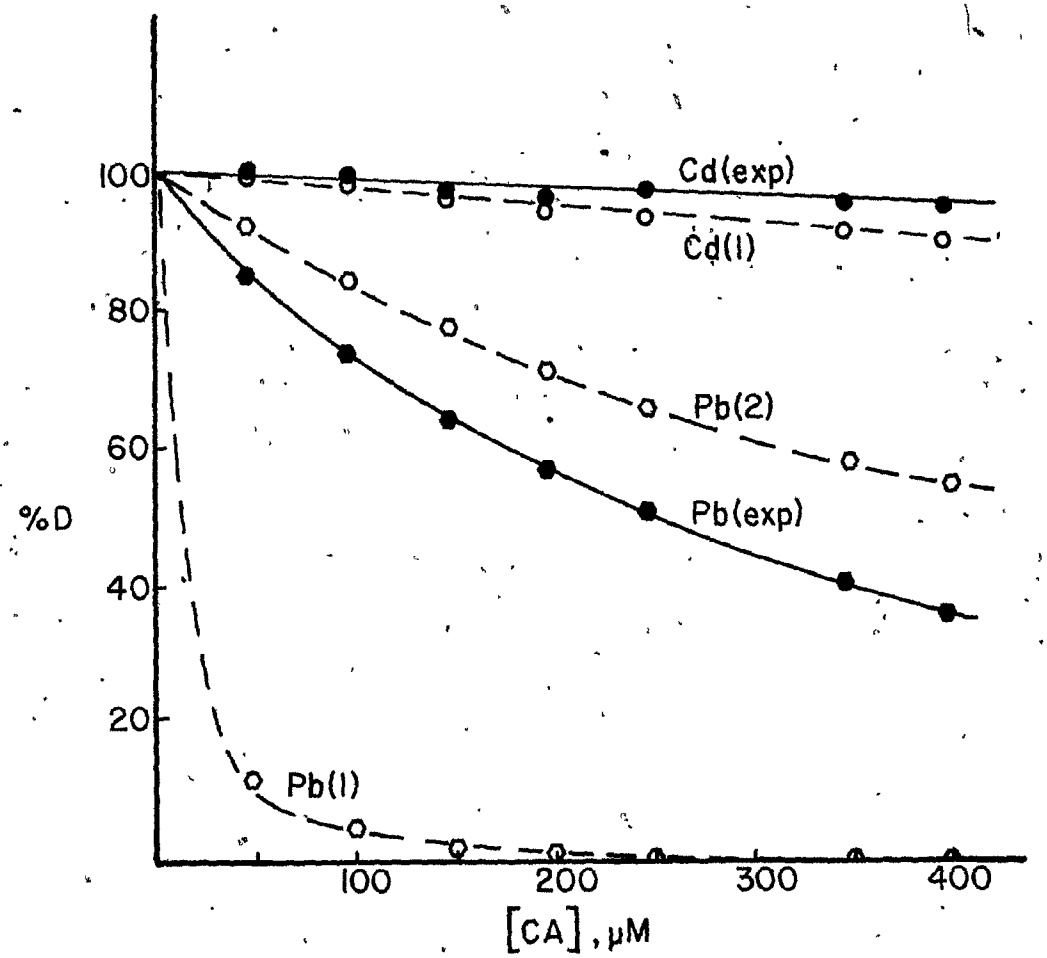


Fig. 5-15b: Metal-citrate speciation vs [CA].

- conditions as in Fig. 5-3 with pH = 5.00, T = 180 min and analysis by HPLC/PD.

5.2.d. Metal speciation in humic colloid systems: The speciation studies reported so far involved ligands of known structure possessing well-established stability constants with most of the metals of interest. These ligands represented one or possibly two types of binding sites. However, metal complexation by humic and fulvic acids differs from conventional complexation in that these ligands do not represent well defined systems.

The structures and binding characteristics of these natural ligands will depend on such variables as their source, age, past history and present environment. These ligands are usually viewed as having a continuum of binding sites. Metals will be bound by the strongest sites first and occupy sites in order of decreasing strength. Various models have been used to describe the complexation of humic and fulvic substances; for example, the "weighted average stability constants" of Gamble et al (1980) and the "stability surface concept" of MacCarthy and Smith (1979). This section will describe the application of the Nafion dialysis speciation technique to a few model systems containing humic and fulvic acids.

A binding capacity experiment is illustrated in Figure 5-16 for a 10 ppm fulvic acid solution at pH 5.0. Extrapolation of the binding curve to the abscissa gives a potential capacity of 4.6 μM for cupric ion. The titration

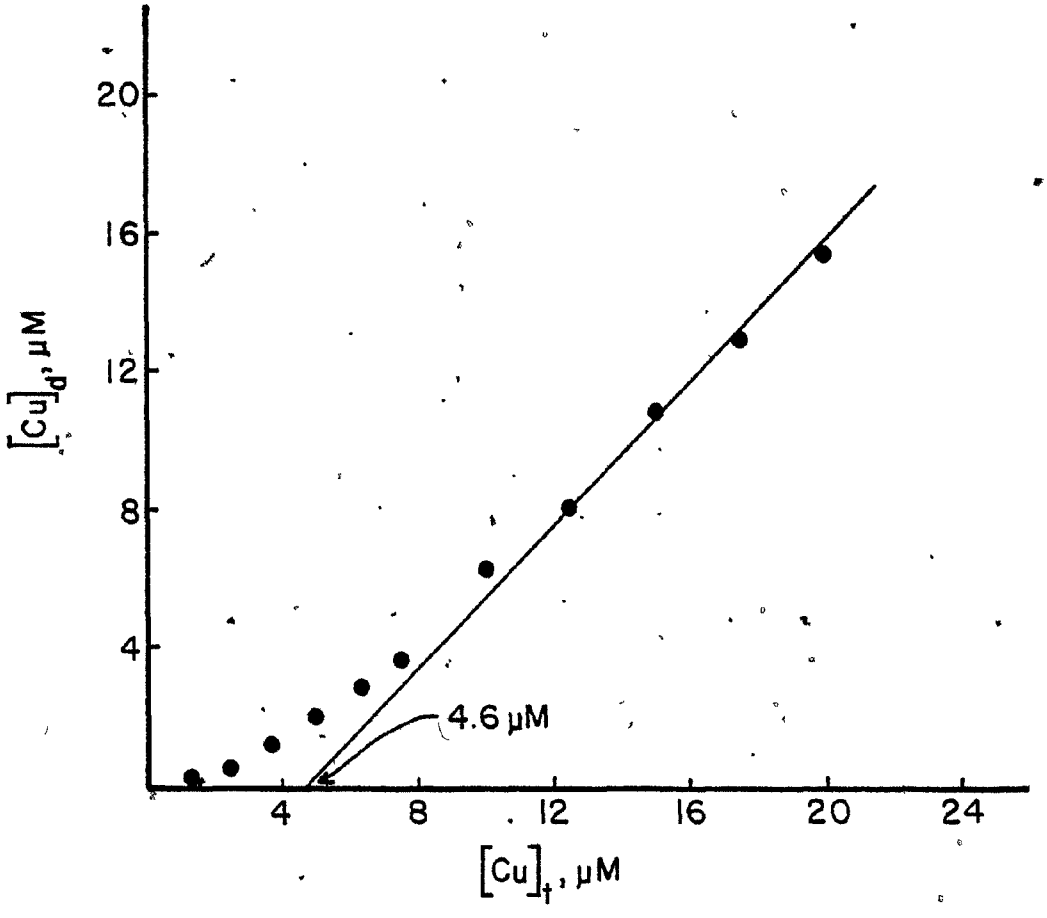


Fig. 5-16: Cu binding capacity titration for 10 ppm FA at pH 5.00.

- condifions as in Fig. 5-3 with T = 180 min and analysis by HPLC/PD.

curve can be used to obtain a binding isotherm which is shown in Figure 5-17. The binding isotherms for cupric ion onto both humic and fulvic acids attain a plateau similar to a Langmuir isotherm. The maximum capacities were 0.46 mmoles/g and 0.33 mmoles/g of fulvic and humic acid, respectively.

The value of 4.6 μM capacity for fulvic acid was low when compared to reported values obtained using various instrumental techniques (Ryan and Weber, 1982). These values of copper complexing capacity measured for 10 ppm FA in 0.1 M KNO_3 and pH 5.0 ranged from 5.2 μM to 17 μM. An additional value of 22.7 μM was given for an ion selective electrode determination made in 0.01 M KNO_3. It is possible that our low value was at least partially due to the high ionic strength medium (0.30 M $NaNO_3$) in which the determination was made. Another possibility was the fulvic acid used, its source and its mode of preparation. For example, in measuring the "maximum binding ability" of several humic substances obtained from various sources, Zunino and Martin (1977) reported values ranging from 0.29 to 2.26 atom-micrograms of Cu/mg of HA. These determinations were all made using the same dialysis technique, indicating that variations were entirely due to the HA source.

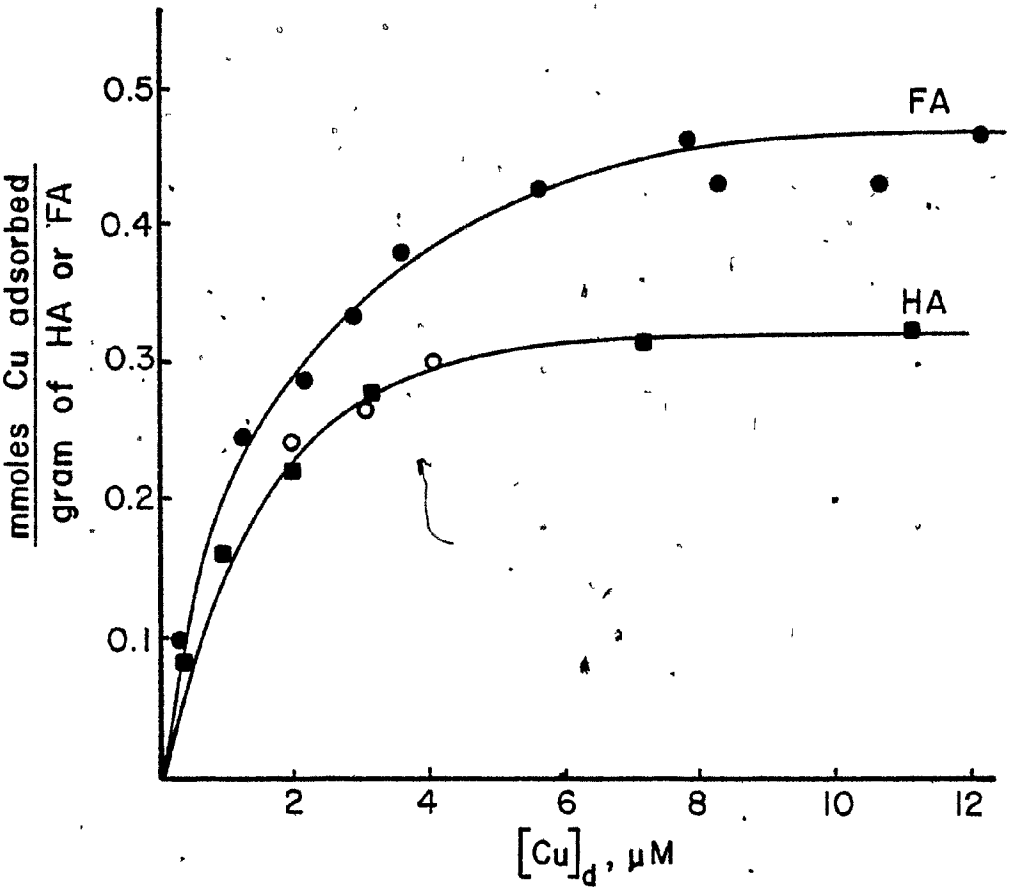


Fig. 5-17: Cu adsorption isotherms for HA and FA at pH 5.00 and 0.30M $NaNO_3$.

- conditions as in Fig. 5-3 with T=180 min and analysis by HPLC/PD.

o - from Table 5-3.

The complex interactions relating ionic strength effects and metal complexation can best be illustrated by the results of the following experiment. A 20 ppm humic acid solution at pH 5.0 was equilibrated with a 6-metal mixture (i.e., 10 μM of Cu, Zn, Ni, Pb, Co, Cd) at three ionic strengths - 0.05, 0.10 and 0.30 set with $NaNO_3$. A control dialysis at each ionic strength was used to determine the amount of metal adsorbed by the Nafion membrane and, hence, the total metal in solution. Zinc, cobalt and cadmium did not interact with the humic acid and the results for copper, lead and nickel are given in Table 5-3. The results for the copper interactions clearly illustrate that the principal effect of increasing ionic strength is to decrease the adsorption of copper onto the Nafion membrane. The total available copper increases from 6.7 to 10.0 μM when one raises the ionic strength from 0.05 to 0.30. This would suggest, therefore, that in 0.05 M $NaNO_3$, the copper species distribution will be a result of the competition between the Nafion membrane and the humic acid for available copper whereas in 0.30 M $NaNO_3$ the membrane has no effect. In comparing the three copper adsorption values (i.e., M_A/g) with the humic acid adsorption isotherm given in Figure 5-17, one can see that the three adsorption values fall on the curve. This would suggest (within experimental error) that ion-exchange

Table 5-3

Effect of $NaNO_3$ concentration on M-HA speciation

M	$[NaNO_3]$	M_T, μM	M_F, μM	M_A/g*
	0.05 M	6.7	1.9	0.24 ± 0.01
Cu	0.10 M	8.1	3.0	0.26 ± 0.01
	0.30 M	10.0	4.0	0.30 ± 0.01
	0.05 M	6.7	3.8	0.14 ± 0.02
Pb	0.10 M	8.5	6.1	0.12 ± 0.02
	0.30 M	10.0	7.9	0.10 ± 0.02
	0.05 M	6.8	5.9	0.04 ± 0.02
Ni	0.10 M	8.9	8.1	0.04 ± 0.02
	0.30 M	10.0	9.4	0.03 ± 0.02

M_T = total metal; M_F = free metal;

M_A/g = mmoles metal adsorbed/g HA.

* ± precision obtained from triplicate measurements.

reactions play little role in the adsorption of copper onto humic acid in solutions containing sodium above 0.05 M. The relative uncertainties in the adsorption values for lead and nickel are large enough to mask any small dependence on ionic strength or competition for sites with cupric ion. More extensive measurements over greater concentration and ionic strength ranges are required before one can reliably assess the applicability of Nafion speciation in humic colloidal systems.

A pH-speciation profile for lead and copper in a 20 ppm humic acid solution is given in Figure 5-18. The trends for these ions are similar to ones reported in the literature.

5.3 Conclusions

In this chapter, dialysis using ion-exchange membranes was shown to be an effective method of measuring a fraction consisting almost entirely of free cationic metal ions. This speciation method was applicable to a wide variety of metals, thereby offering the distinct advantage of a multi-element approach. Metals such as Ca, Mg, Mn, Co and Ni could be studied using this speciation procedure coupled with analysis by ion-exchange/photometric detection. This is of importance since the speciation of one metal may

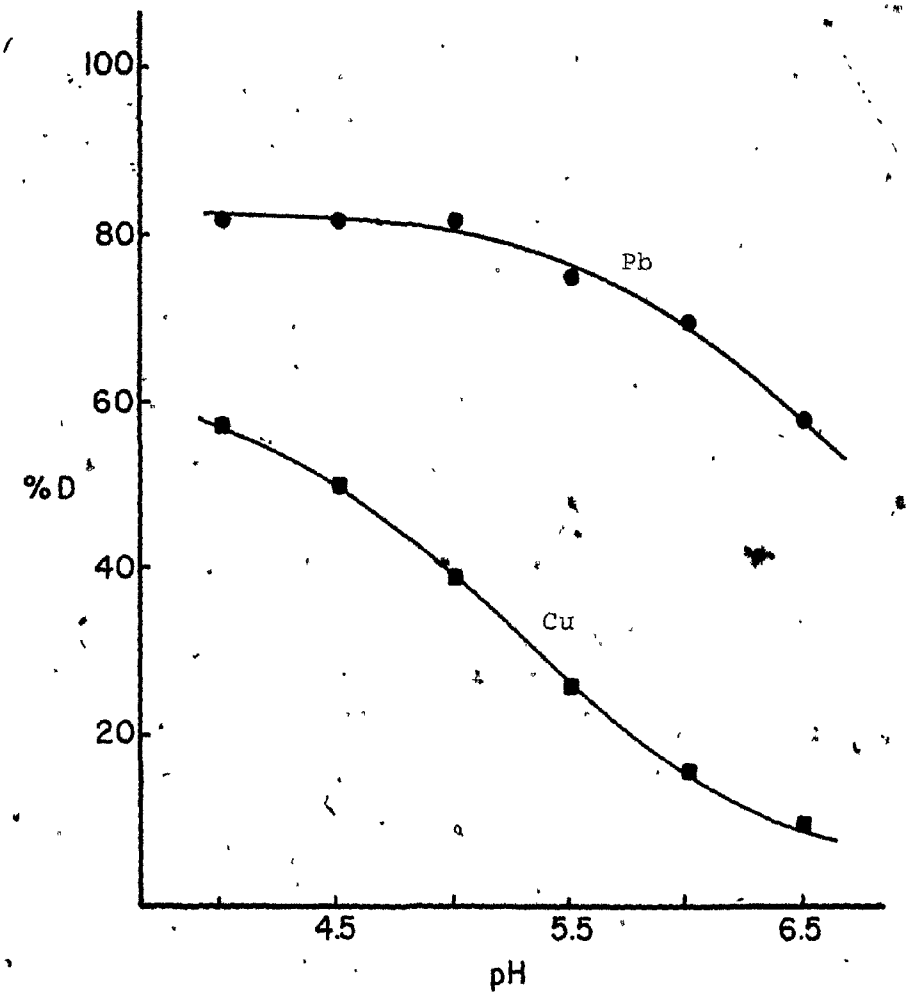


Fig. 5-18 : Effect of pH on M-HA speciation.

- conditions as in Fig. 5-3 with T = 180 min, 20 ppm HA/10µM metal and analysis by HPLC/PD.

significantly alter the speciation of a second and more toxic metal. For example, the speciation of Ca may play a role in the bioavailability of a toxic metal such as Cu.

A second advantage of this speciation method is that it measures a fraction which may be more representative of that which is bioavailable. Techniques such as anodic stripping voltammetry and Chelex-100 ion-exchange are known to measure free metal but in addition metal which is bound in a labile complex. However, these labile complexes may represent metal not available to aquatic organisms. Similarly, size separation techniques such as dialysis and ultrafiltration measure small neutral and anionic complexes which may also represent metal not available to biota. In the Nafion dialysis method, anionic complexes are charge-excluded and neutral species dialyze but at a much slower rate than cationic species. Their contribution to the measured fraction is therefore secondary. As a result, the dialyzate contains mostly free aquated metal ions.

A third advantage of this technique is that, when compared to conventional dialysis, much less time is required to attain equilibrium. Dialysis techniques using cellulose membranes usually require 24 hours or more to reach equilibrium. Dialysis using Nafion tubular membranes (811X) reached equilibrium in 120 minutes.

The main limitation to the method is the requirement of a high ionic strength sample matrix, e.g., 0.30 M $NaNO_3$ or 0.50 M NaCl. Samples present in a low ionic strength medium and where ion-exchange is a principal mechanism of analyte binding may not be amenable to this type of speciation analysis. It should be noted that some of the other speciation methods also require higher ionic strength media than those found in fresh water systems, e.g., ASV requires an ionic strength of 0.02 or greater. This Nafion speciation method may offer its greatest potential in the study of trace metals in seawater.

CHAPTER 6

Conclusions

The Nafion 811X tubular membrane has been found useful for the preconcentration of trace metal ions prior to multielement analysis and for the speciation of free metal ions in solution. The optimum preconcentration procedure employed a complexing agent as receiver solution and a high sample to receiver volume ratio. This set of conditions permitted one to attain dialysis equilibrium rapidly for maximum reproducibility. A 200 mL/2 mL sample to receiver ratio gave equilibrium preconcentrations of 68-fold after 30 minutes. The principal limitation of this procedure is the effect on the enrichment of sample matrix. High sample ionic strengths must be matched by high receiver ionic strengths to effect any preconcentration. The studies on Nafion preconcentration of humic colloidal solutions suggested that complexones in the sample solution lowered the enrichment factors. Additional studies are required to determine the relationship between enrichment factors and the conditional stability constants of the receiver and sample complexones. This relationship will play an important role in the potential application of the Nafion preconcentration mode as a means of purifying reagents.

The Nafion speciation mode was found to be selective for metal cations and small neutral species. The Nafion 811X membrane was found to exclude anions (e.g., Cd-NTA$^-$), large cations (e.g., triphenyltin) and the neutral complex lead phthalate. A further refinement of the size separation mechanism of the membrane is necessary to determine whether small cationic complexes or ion-pairs are capable of dialysis. Tracer studies using labelled neutral molecules and anions may prove useful for such a study. A high salt content in both sample and receiver solutions was required to minimize adsorption by ion-exchange. This limits the application to systems that do not use ion-exchange as a principal binding mechanism. The membrane was found to be useful in solutions containing trace metals at total concentrations of 1-10 μM. A further characterization of the membrane is required to determine whether a secondary adsorption mechanism is important at sub-micromolar concentrations.

Appendix 1

Species Distribution Calculation

For a metal-ligand complexation reaction where only one complex is formed, we have:

$$M + L \rightleftharpoons ML \qquad (1)$$

and

$$K = \frac{[ML]}{[M][L]} \qquad (2)$$

If the ligand is present in large excess (e.g., $\geq$ 10-fold), its concentration can be considered constant. The total metal concentration is given by:

$$[M_T] = [M] + [ML] \qquad (3)$$

and therefore

$$\%M = \frac{[M]}{[M] + [ML]} \times 100$$

$$= \frac{[M]}{[M] + K[M][L]} \times 100$$

$$= \frac{1}{1 + K[L]} \times 100 \qquad (4)$$

Introducing the α term for ligand protonation yields:

$$\%M = \frac{1}{1 + K\frac{[L]}{\alpha}} \times 100 \qquad (5)$$

Similarly,

$$\%ML = \frac{K\frac{[L]}{\alpha}}{1 + K\frac{[L]}{\alpha}} \times 100 \qquad (6)$$

The formation of secondary complexes (e.g., ML_2, ML_3, MOHL, MHL, MOH, etc.) can be treated in the same way.

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