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Characterization and Implementation of a Continuos Flow Trace Metal Preconcentration System for Inductively Couples Plasma Atomic Emission Spectrometry

Robert N. Sibley
Colby College

CHARACTERIZATION AND IMPLEMENTATION OF A CONTINUOUS FLOW TRACE METAL PRE-CONCENTRATION SYSTEM FOR INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY

by

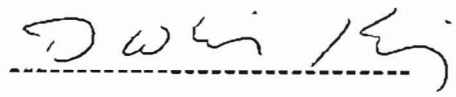
Robert N. Sibley

Submitted in Partial Fulfillment of the Requirements of the Senior Scholars' Program

COLBY COLLEGE

1992

APPROVED:



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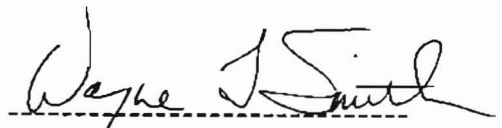

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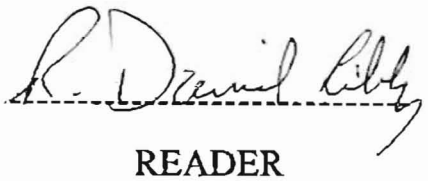

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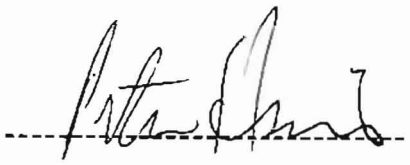

CHAIR, COMMITTEE

DEDICATION

I would like to dedicate this thesis to my parents, whose love and support over twenty-one years have made it possible for me to excel in my interests.

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ABSTRACT

A continuous flow pre-concentration system for analysis of trace metals in natural waters was characterized and used to determine the copper(II) and lead(II) concentrations in seawater from Penobscot Bay, Maine. Pre-concentrated samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). System characterization involved elucidation of the thermodynamic complexation constants, $K_{Cu(II)}$ and $K_{Pb(II)}$, for Cu(II) and Pb(II) with the pre-concentration column chromatographic material. This was done in aqueous media of known pH and ionic strength.

The column contained 8-hydroxyquinoline immobilized on Toyopearl HW-75 fractogel chromatographic resin. The buffer system used for metal binding experiments was an equimolar mixture of acetic acid (HOAc) and tris(hydroxymethyl)aminomethane (TRIS). This combination ensured maximum buffer capacity in the pH region in which the column was being characterized. Scatchard plots were generated to determine $K_{Cu(II)}$ and $K_{Pb(II)}$. These were 9.17 and 9.68, respectively, at 25.0° C and at ionic strengths between 0.01 and 0.001 M. A computer based interactive model for metal speciation in aqueous electrolyte solutions was developed and employed for these determinations.

Incorporating the new thermodynamic complexation constants into the speciation model allowed prediction of the optimal pH for pre-concentration of Cu(II) and Pb(II) in seawater. Two pK values for aqueous $PbCO_3$ in seawater are available in the literature. If carbonate ($6e^{-4}$ M) is treated as the predominant ligand of Cu(II) and Pb(II) in seawater, then predicted metal recoveries for the system at pH 7.553

(about 71% for Cu and 47% for Pb) are consistent with the observed values (70.5% for Cu and 48.5% for Pb). If Cl^- (0.54 M) is considered to be the most important ligand of Pb(II) in seawater, then the predicted recovery of Pb from the system is 100%.

In Penobscot Bay, Cu(II) concentrations were found to be between 30 and 60 nM near the surface and sediment, and about 10 nM at mid depth. Pb(II) concentrations were found to decrease from about 32 nM at the surface to less than 5 nM near the sediment. The detection limit for Cu and Pb by this method is in the low nanomolar to high picomolar range.

INTRODUCTION

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a powerful analytical tool that can measure as many as 70 different elements in a range of samples. The detection limit of ICP-AES, however, is inadequate to measure trace levels of metal contamination in drinking water and other natural samples such as seawater. The objective of my research was to provide a fast reproducible method of analyzing metals in natural water samples by automated pre-concentration followed by ICP-AES analysis. The method is shown to work for seawater taken from Penobscot Bay, Maine.

The metals investigated here, Cu(II) and Pb(II), are representative of a range of metals that could potentially be analyzed in the pre-concentration / ICP-AES system. Cu(II) and Pb(II) have unique oceanic distributions [1], which make them interesting subjects of study. In addition, Cu is a biologically important element in the ocean, and Pb, of course, is also of interest because of its importance as a pollutant.

Because both Cu(II) and Pb(II) form stable species in aqueous electrolyte solutions, the complete speciation of these metals had to be understood in order to characterize the system. This meant a suitable and well understood buffer system had to be chosen for the pre-concentration process. Several buffers were considered, but only an equimolar mixture of tris(hydroxymethyl)aminomethane (TRIS) and acetic acid (HOAc) had an ideal combined buffer capacity for the metal binding experiments. The speciation calculations were performed using a computer speciation model. A molecular modelling program was used to identify the steric limitations of binding between the column and metal complexes.

The stability constants between the fractogel immobilized 8-hydroxyquinoline chelating material (F8HQ) and Cu(II) and Pb(II), $K_{Cu(II)}$ and $K_{Pb(II)}$, respectively, also had to be determined so that accurate speciations could be generated for any natural system being studied by this method. This was a lengthy process, but a suitable method was finally elucidated - the Scatchard Plot method. This method is both quick and easy to perform so that many other metals could be investigated in the same manner for a range of different systems.

This research is important in that it has yielded new understanding as to the capabilities and chemistry of trace metal pre-concentration and analysis. Now concentrations of Pb(II), Cu(II), and potentially many more metals can be determined in natural water samples with relative ease.

CHAPTER 1

In order to represent the chemical systems being studied in this research accurately, the speciation of the metals and F8HQ ligand had to be quantified as a function of pH. This was done by constructing a computer model which was used to form "alpha" (α) plots, which show the fraction of a species theoretically present relative to the total concentration of analyte. The model is based on the tabulated critical stability constants of the chemical species in question, and the known bulk concentrations of chemicals making up the solution. A computer spread sheet allows one to generate the model with ease. The general approach to this calculation presented below can be adapted to a number of aqueous chemical systems.

Given a divalent metal, M(II), in an aqueous solution containing a buffer, BH, the following equilibria with constants K and $\beta 1$ through $\beta 4$ can be defined:

$$[BH] \leftrightarrow [B^-] + [H^+] \qquad K \qquad (1)$$

$$[B^-] + [M^{+2}] \leftrightarrow [MB^+] \qquad \beta_1 \qquad (2)$$

$$2[B^-] + [M^{+2}] \leftrightarrow [MB_2] \qquad \beta_2 \qquad (3)$$

$$[H_2O] + [M^{+2}] \leftrightarrow [MOH^+] + [H^+] \qquad \beta_3 \qquad (4)$$

$$2[H_2O] + [M^{+2}] \leftrightarrow [M(OH)_2] + [H^+] \qquad \beta_4 \qquad (5)$$

Equations 2 and 3 represent buffer ligand complexation and equations 4 and 5 represent metal hydrolysis. The total buffer in the system, TB, will have a mass balance:

$$TB = [B^-] + [BH] \tag{6}$$

Defining α_B as the ratio of uncomplexed B to total B results in equation 7:

$$\alpha_B = \frac{[B^-]}{TB} = \frac{[B^-]}{[BH] + [B^-]} \tag{7}$$

Solving equilibrium 1 for [BH], and substituting [BH] into 7 we get,

$$\alpha_B = \frac{[B^-]}{(([B^-][H^+])/K) + [B^-]} = \frac{K}{[H^+] + K} \tag{8}$$

Since $[B^-] = (TB)(\alpha_{B^-})$, then we can use 8 to determine $[B^-]$ as a function of pH.

Defining MB as the metal-buffer complex (for b number of ligands B), and MJ as any metal hydrolysis species (for j number of OH^- ligands), rearrangement of equilibria 2 through 5 in the following general way provides equations for aqueous metal species:

$$MB = \beta_b[B]^b[M^{+2}] \tag{9}$$

$$MJ = \frac{\beta_j[M^{+2}]}{[H^+]j} \tag{10}$$

The total M concentration, TM, in the system can be defined:

$$TM = \sum_{1}^{b}(\beta_b[B]^b[M^{+2}]) + \sum_{1}^{j}\left(\frac{\beta_j[M^{+2}]}{[H^+]^j}\right) \qquad (11)$$

The $\alpha_{[M+2]}$ is defined as the ratio of the free M^{+2} concentration to the total metal present.

$$\alpha_{[M+2]} = \frac{[M^{+2}]}{TM} \qquad (12)$$

Substituting 9 and 10 into 11, and substituting 11 into 12 gives the α for the free M^{+2} species.

It is now simple to determine the α of any other species MB or MJ. Recognizing that α_{MB} and α_{MJ} are the ratios of specific metal-ligand complexes to total metal present in the solution, then for a buffer-metal species:

$$\alpha_{MB} = \frac{MB}{TM} = \frac{\beta_b[B]^b[M^{+2}]}{TM} = \beta_b[B]^b\alpha_{(M+2)} \qquad (13)$$

and for a hydroxide-metal species:

$$\alpha_{MJ} = \frac{MJ}{TM} = \frac{(\beta_j[M^{+2}])/[H^+]^j}{TM} = \frac{\beta_j\alpha_{(M+2)}}{[H^+]^j} \qquad (14)$$

If all K's, β's, and bulk chemical concentrations are known, a plot of α as a function of pH for all species will show the fraction of each chemical species in the solution being studied. The representative

calculation above can be applied to any number of metal-ligand species provided suitable thermodynamic data are available.

Values of $\alpha_{(M(II))}$ become necessary when one tries to calculate the thermodynamic stability constants between the F8HQ ligand on the column and any metal M. Experimentally determined constants are based on measurements of total metal concentrations. The only way this constant can be determined is if the amount of free M(II) available to bind the column is known. Since this information is provided by $\alpha_{M(II)}$ for a specific pH, then we can calculate K for the column and any metal, M(II). The reverse process allows metal-ligand thermodynamic equilibrium constants to be converted to apparent constants for a range of natural samples.

The speciation of 8-hydroxyquinoline (8HQ), or any other weak acid, can be calculated as a function of pH using equations 1, 6, and 7. Again, all concentrations and dissociation constants must be known. Figures 1-3 contain log(α) plots which were derived by the above method. The thermodynamic data used in these calculations are listed in appendix 1 (2). Some K's for certain metals and ligands were estimated based on trends in like metals. These estimated values have parentheses around them. The log(α) plot for zinc species is included in appendix 2. Zn is another metal with interesting oceanic distributions, but was not studied due to time constraints.

The affinity of the column for these metals depends on several factors: the structure and stereochemistry of the F8HQ chelating site; the structures of the various ligand-metal species; and the charges on these various species. Since F8HQ has an affinity for positively charged metal

Figure 1.
log(α) of aqueous Cu species vs. pH.
$1e^{-4}$ M $Cu(NO_3)_2$ in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc.

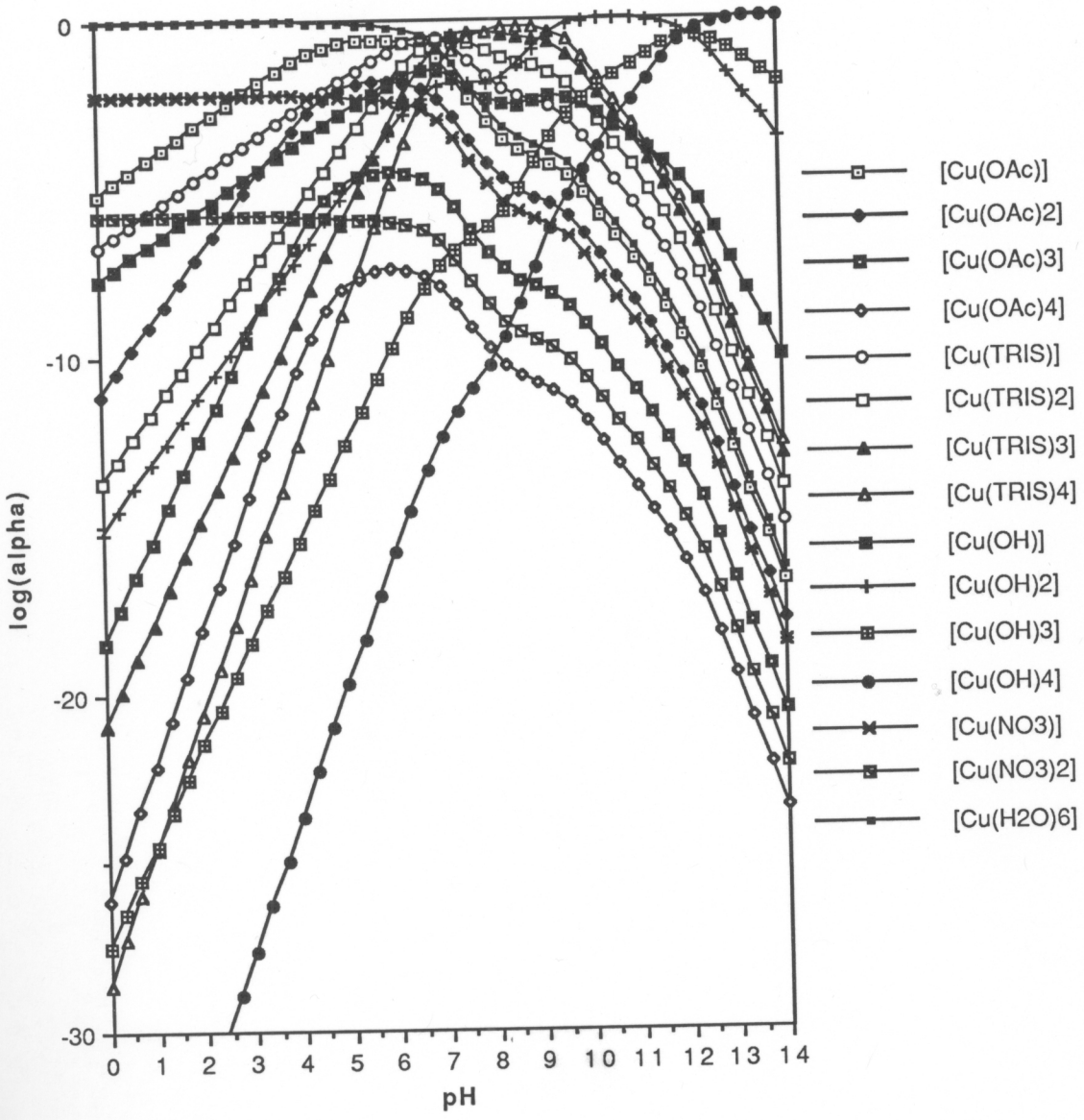

0
-10
-20
-30
log(alpha)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH
[Cu(OAc)]
[Cu(OAc)2]
[Cu(OAc)3]
[Cu(OAc)4]
[Cu(TRIS)]
[Cu(TRIS)2]
[Cu(TRIS)3]
[Cu(TRIS)4]
[Cu(OH)]
[Cu(OH)2]
[Cu(OH)3]
[Cu(OH)4]
[Cu(NO3)]
[Cu(NO3)2]
[Cu(H2O)6]

Figure 2.
log(α) of aqueous Pb species vs. pH
$1e^{-4}$ M $Pb(NO_3)_2$ in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc.

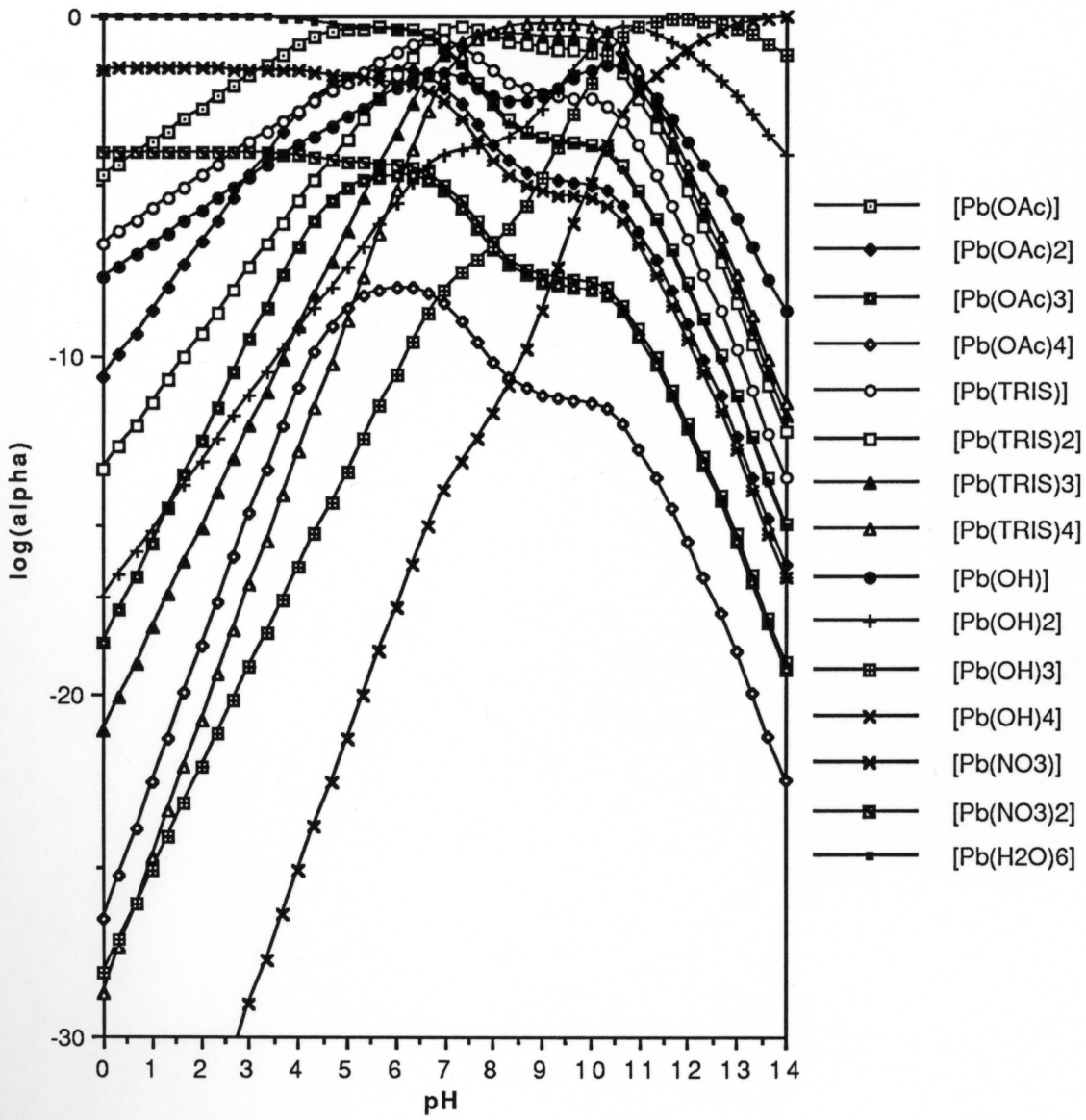
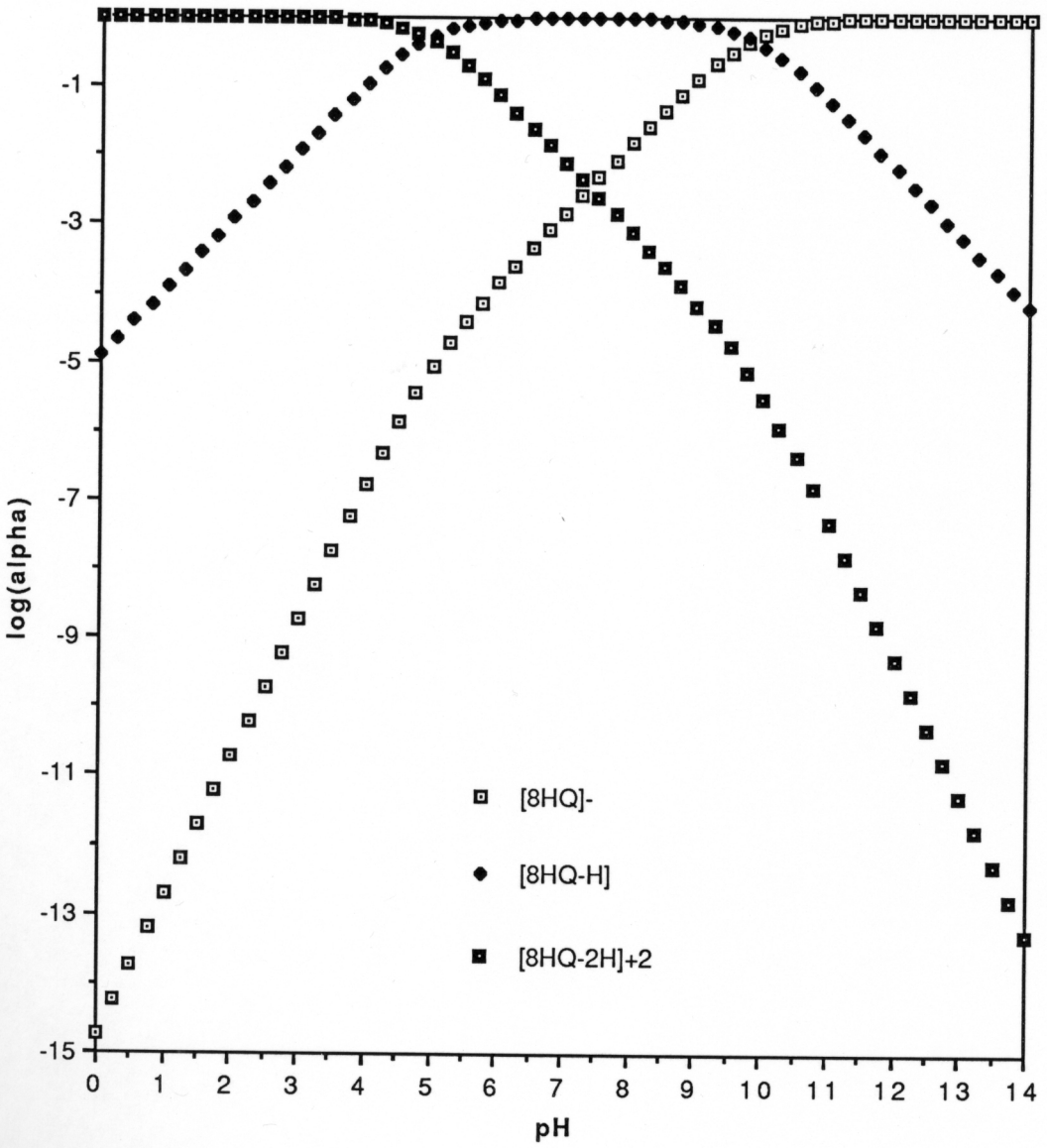
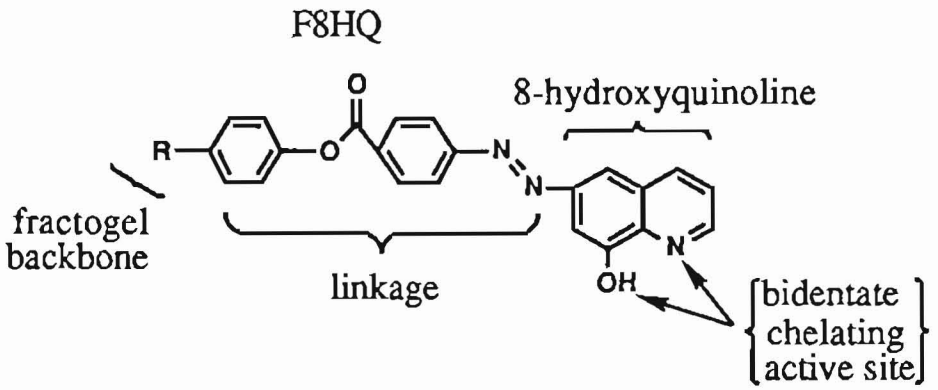

0
-10
-20
-30
log(alpha)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH
[Pb(OAc)]
[Pb(OAc)2]
[Pb(OAc)3]
[Pb(OAc)4]
[Pb(TRIS)]
[Pb(TRIS)2]
[Pb(TRIS)3]
[Pb(TRIS)4]
[Pb(OH)]
[Pb(OH)2]
[Pb(OH)3]
[Pb(OH)4]
[Pb(NO3)]
[Pb(NO3)2]
[Pb(H2O)6]

Figure 3.
log(α) of 8-hydroxyquinoline species vs. pH.
No other species are in solution.

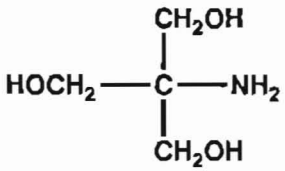

log(alpha)
-1
-3
-5
-7
-9
-11
-13
-15
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pH
[8HQ]-
[8HQ-H]
[8HQ-2H]+2

species, but not for those that are neutral or negatively charged [3], then only positively charged complexes should be chelated by the column. This is true provided that the metal center of the cationic species are sterically allowed to approach the active site of the F8HQ. Thus, the complexation capacity of the column for Cu(II) and Pb(II) will depend upon which positively charged species are chelated by the column without steric hindrance. For speciation calculations, anionic, neutral, and sterically hindered cationic species are assumed to be unavailable for column complexation.

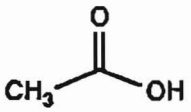
Before determining which category each of the metal species fall into, some chemistry of the molecules and metals involved has to be investigated. The MMX energy minimization routine in the molecular modelling program PCmodel was used to elucidate the conformations of F8HQ and various metal species. Aqueous ligands binding to Cu(II) and Pb(II) are OH^-, H_2O, TRIS, and OAc^-.



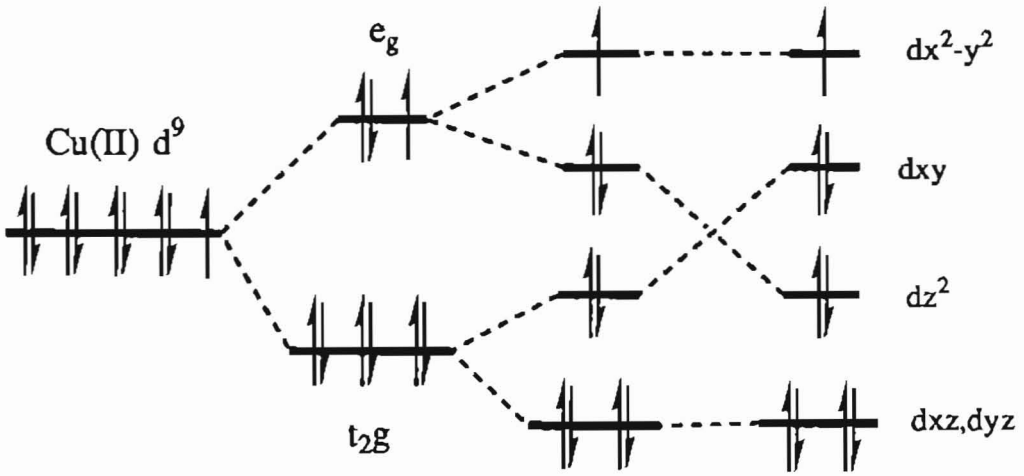
tris(hydroxymethyl)aminomethane (TRIS)



acetic acid (HOAc)



Since each 8-hydroxyquinoline molecule is attached to the fractogel backbone via a single diazo linkage, no more than one F8HQ arm can attach to a single Cu or Pb atom to form the five membered ring chelate. The Cu(II) and Pb(II) atoms will be coordinatively saturated with the above ligands depending on the solution pH and each metal's affinity for the ligand. Cu(II) is a d^9 transition metal and could have 4, 5, or 6 coordination, but most complexes are 6 coordinate resulting in octahedral geometries (e.g., $[Cu(H_2O)_6]^{+2}$, $[Cu(OH)_2(H_2O)_4]$, $[Cu(OAc)_3(H_2O)_3]^-$, $[Cu(TRIS)_4(H_2O)_2]^{+2}$, etc.). Octahedral d^9 Cu(II) often undergoes tetragonal distortion, which elongates the z-axis bonds and constricts the equatorial bonds. This is explained by the Jahn-Teller theorem, which states that if the ground state configuration of a molecule is degenerate, distortions will occur to remove degeneracies and increase its stability [4].



This is important in the binding of Cu(II) species to F8HQ; any ligands reacting with $[Cu(H_2O)_6]^{+2}$ will first displace the axial waters, which have lower energy bonds to Cu. This means that Cu(II) species even with three large ligands such as TRIS can bind to the F8HQ active site by sliding into the plane of 8-hydroxyquinoline with the bulky axial ligands above and below the plane.

Pb is not a transition metal like Cu and the Jahn-Teller theorem does not apply. But Pb is about 50% larger than Cu and it thus has a larger surface on which to spread ligands. It seems plausible that this larger surface area is similar to the axial elongation effect of Cu(II) since the ligands will have more room to spread out. Pb may in fact have coordination up to 12, but most coordinatively saturated Pb systems don't exceed 9 ligands, and for this research a maximum of 6 ligands are assumed to be present. The importance of different Pb(II) species may or may not be analogous to the importance of similar Cu(II) species, but the initial assumption will be that both have $(TRIS)_4$ complexes that are sterically unavailable to bind F8HQ.

Assuming the above, the major cationic species for both Cu(II) and Pb(II) will include $[M(NO_3)]^+$, $[M(OAc)]^+$, $[M(TRIS)]^{+2}$, $[M(TRIS)_2]^{+2}$, $[M(TRIS)_3]^{+2}$, and $[M(TRIS)_4]^{+2}$. The ligands of these complexes will be first directed axially on Cu, and spread sufficiently far apart on Pb. Equatorial positions will then start to be occupied. The only species which won't be chelated by bidentate F8HQ is the last complex, which has four bulky TRIS ligands. In this case, two equatorial and two axial sites will be occupied such that steric energy is at a minimum, and there will be no place the metal can form a bidentate chelate. In addition there is no way the metal can even approach the F8HQ active site because the bulky TRIS ligands on $[M(TRIS)_4]^{+2}$ sterically hinder this interaction.

The aqueous metal-hydroxide species formed in solution are purely a function of pH, and they are thus treated as intrinsic parts of the aqueous system. Consequently, these species are ignored in the Cu(II) and Pb(II) speciation calculations. This assumption was made by Fresco and Freiser [5] when they determined solubilities and stability constants for various metal-8HQ complexes.

All other species present and not yet accounted for are neutral or negatively charged complexes with acetate and nitrate ligands. Since none of them is chelated by F8HQ, they should be treated as unreactive Cu(II) and Pb(II) species. However, for reasons of simplification, only those species contributing more than 1% to the metal speciation will be included in subsequent models. As seen in the previous α plots (figures 1 and 2), the $[M(TRIS)_4]^{+2}$ is the most important species after about pH 7. In addition, only the $[M(OAc)_2]$ species is significant at low pH. Thus, the simplified speciation of Cu(II) and Pb(II) will include $[M(TRIS)_4]^{+2}$, $[M(OAc)_2]$, and the bulk M(II) cations which can be chelated by F8HQ.

These species were ultimately used in the characterization of the F8HQ column for Cu(II) and Pb(II) (see figures 4 and 5). Table I summarizes the speciation information described above.

The next adjustment should plausibly be made to the α plot for aqueous 8HQ species. Since the system being studied does not involve 8HQ, but rather fractogel immobilized 8HQ, the speciation plot presented in figure 3 may not apply to this research. The linkage connecting the 8HQ to the fractogel resin backbone is electron-withdrawing by resonance with respect to 8HQ. This would likely cause a shift in the two acid pK's of 8HQ (pK NH = 4.91, pK OH = 9.81). If the change in pK's is significant, it might result in inaccurate treatment of the experimental data. Determination of the real acid pK's for F8HQ is therefore necessary.

Dr. D. W. King performed a manual titration of a slurry of F8HQ brought to pH 12 with 1 M NaOH. The titrant, 0.05 M HCl, was added in 50 μL increments. All pH's were monitored using an Orion combination pH electrode with an Orion model SA 720 pH meter. The buffer capacity, β, of the solution was calculated from the experimental data as was the β due to OH^- and H^+ (6). These two values were subtracted to give β of F8HQ as a function pH. Maximum β's are observed in the approximate regions of the acid pK's of 8HQ. Figure 6 shows the β of F8HQ vs. pH, and figure 7 shows the titrant volume vs. pH curve. Even using both plots, the endpoints are not very obvious. This is due to noise created by a large ratio of aqueous solvent to F8HQ solute/slurry. Since the β peak at pH 6.39 could be spurious, and since a large amount of noise obscures the region approaching pH 10, no conclusive evidence that the pK's of F8HQ and 8HQ are significantly different exists. The literature values of pK1 and pK2 for 8HQ are thus used in treatment of data in this research.

Table I.

Aqueous Cu(II) and Pb(II) species in TRIS and HOAc buffer which will bind or not bind to F8HQ. The α plots for these metals will be determined from these categories of metal species. Pb(II) is assumed to have the same speciation chemistry as Cu(II) for the $(TRIS)_4$ complex.

Aqueous species which are intrinsic to the system and therefore ignored.

$[M(OH)]^{+}$, $[M(OH)_2]$, $[M(OH)_3]^{-}$, $[M(OH)_4]^{-2}$

Aqueous cationic metal species which can chelate to F8HQ.

$[M(NO_3)]^{+}$, $[M(OAc)]^{+}$, $[M(TRIS)]^{+2}$, $[M(TRIS)_2]^{+2}$, $[M(TRIS)_3]^{+2}$, $[M(H_2O)_6]^{+2}$

Aqueous species which do not chelate to F8HQ due to zero charge or steric hindrances, and which are prevalent enough to include in the metal speciation models.

$[M(TRIS)_4]^{+2}$, $[M(OAc)_2]$

Aqueous species which do not chelate to F8HQ due to zero or negative charge and/or steric hindrances, and which are not prevalent enough to include in the metal speciation.

$[M(OAc)_3]^{-}$, $[M(OAc)_4]^{-2}$, $[M(NO_3)_2]$

Figure 4.
log(α) of aqueous Cu species complexing F8HQ vs. pH.
$1e^{-4}$ M $Cu(NO_3)_2$ in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc.

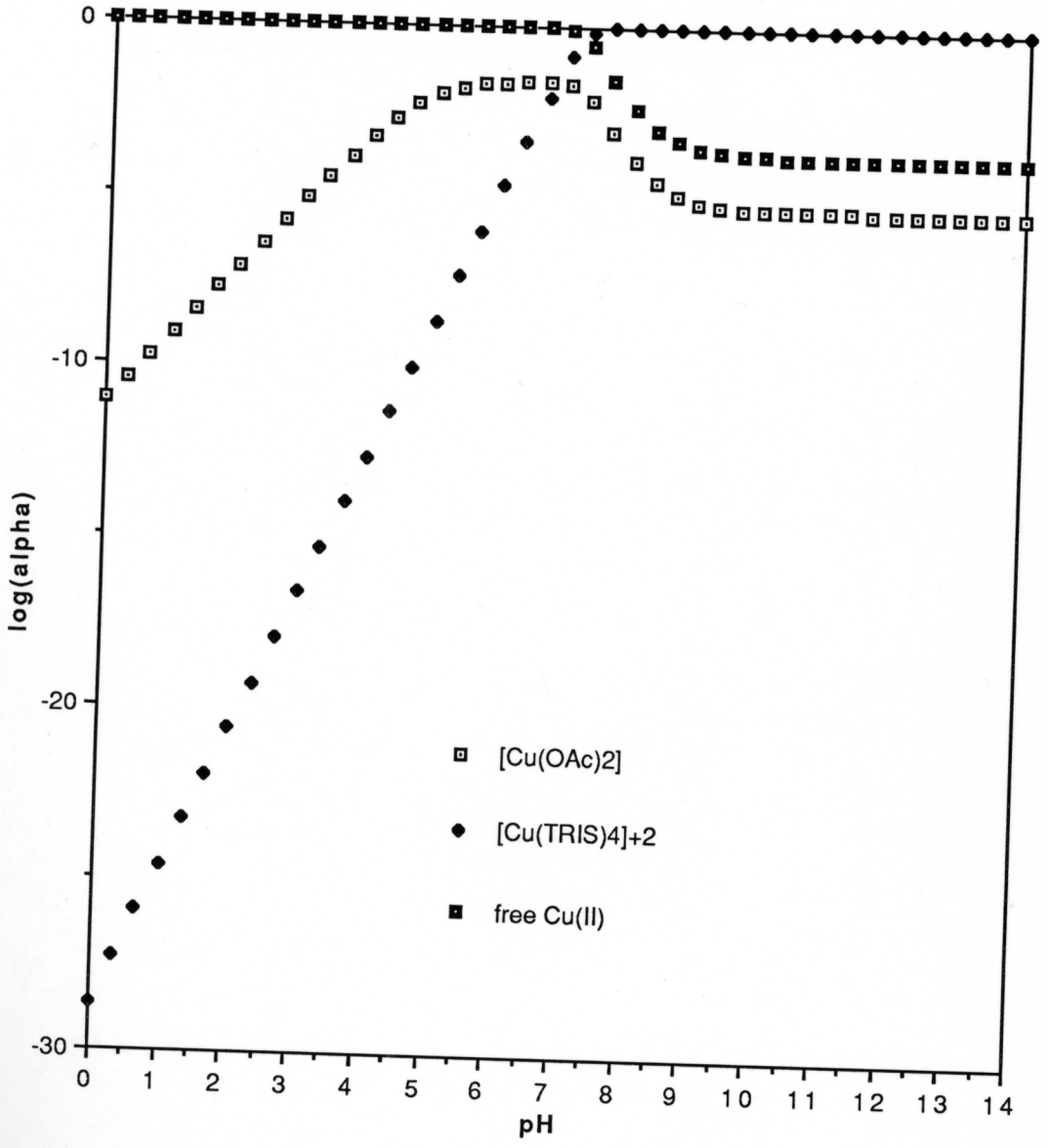

0
-10
-20
-30
log(alpha)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pH
[Cu(OAc)2]
[Cu(TRIS)4]+2
free Cu(II)

Figure 5.
log(α) of aqueous Pb(II) species complexing F8HQ vs. pH.
$1e^{-4}$ M $Pb(NO_3)_2$ in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc.

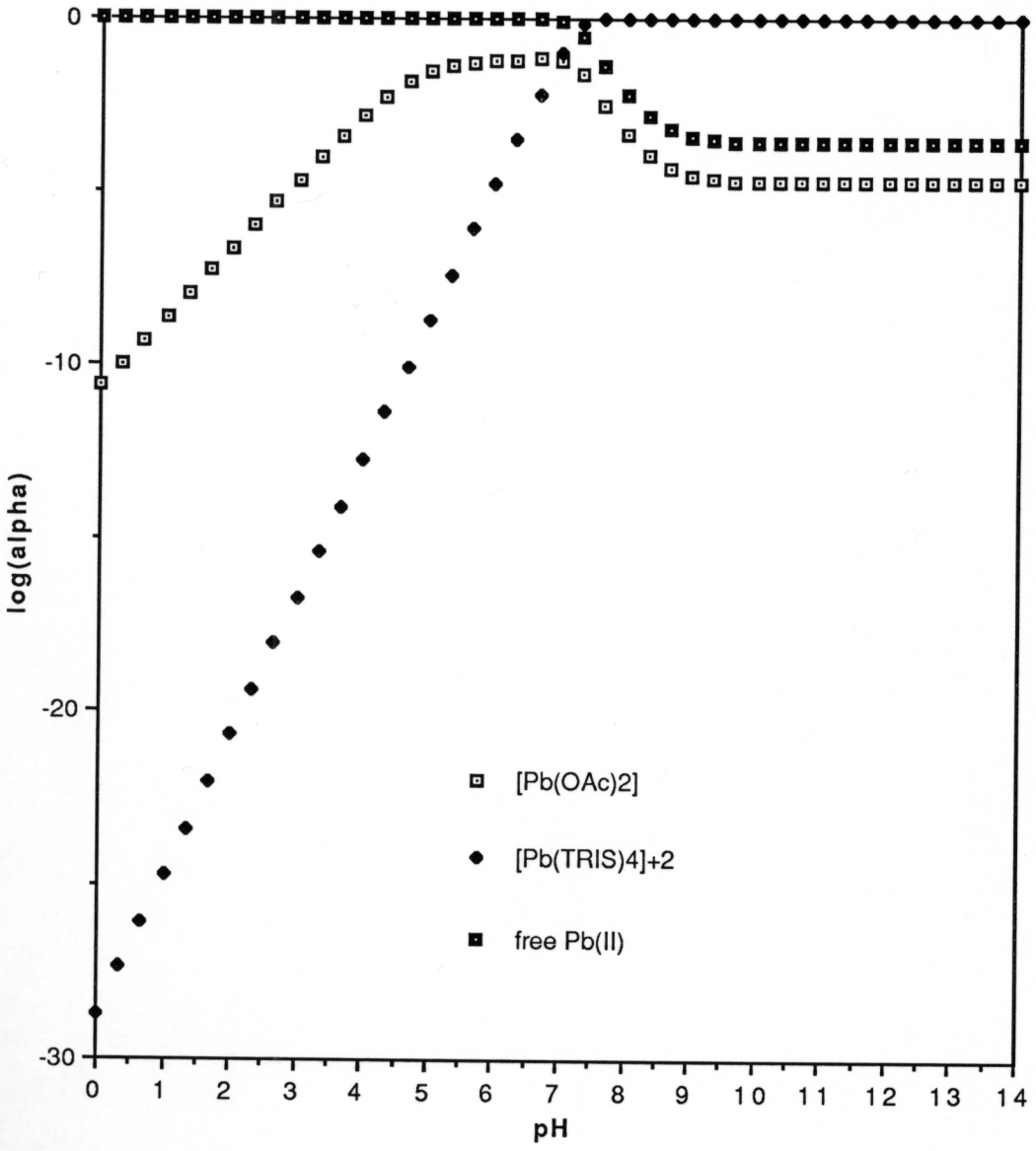

0
-10
-20
-30
log(alpha)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH
[Pb(OAc)2]
[Pb(TRIS)4]+2
free Pb(II)

Figure 6.
β of F8HQ vs. pH.
27 ml slurry of F8HQ
titrated with 0.05 M HCl.

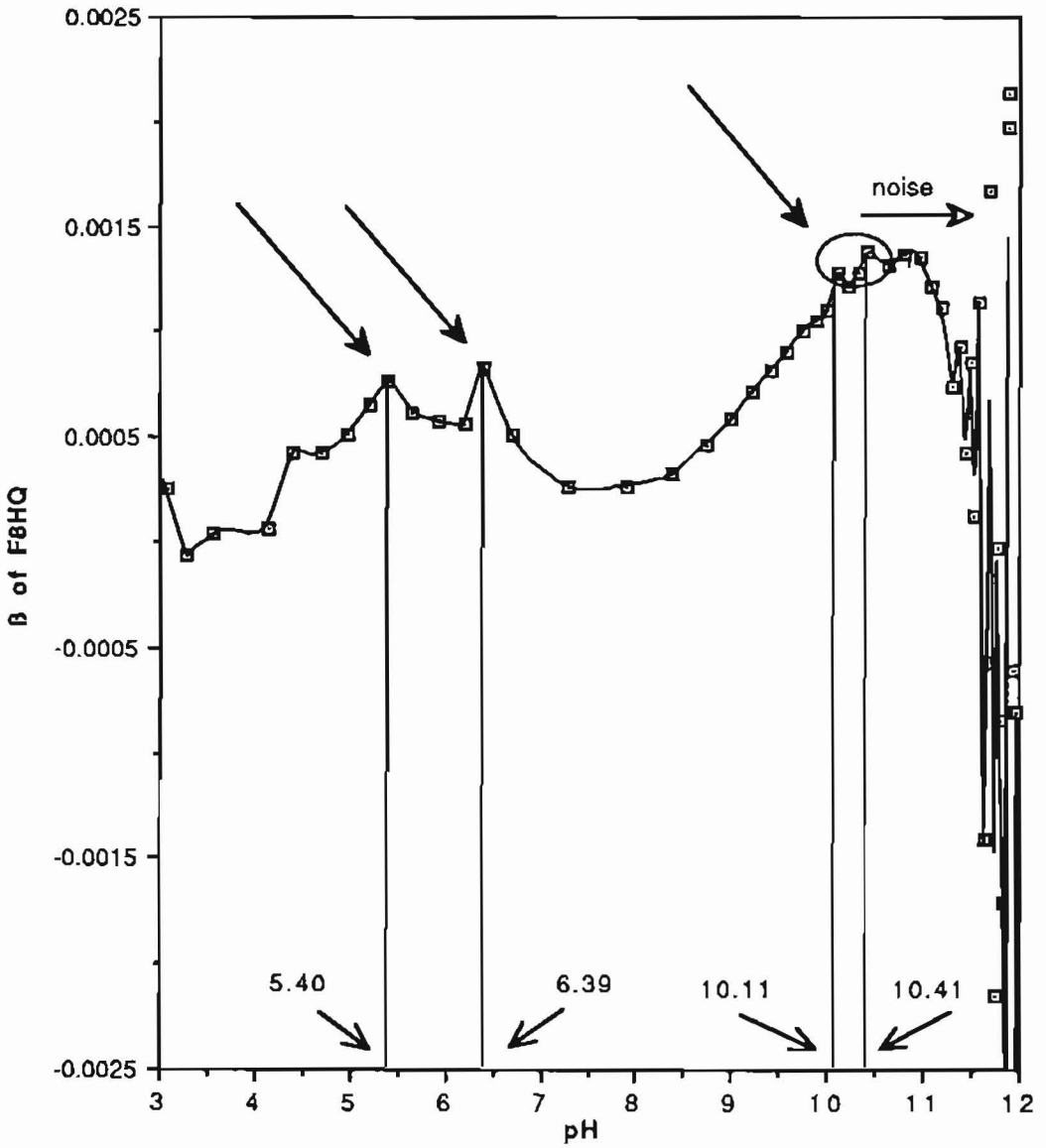
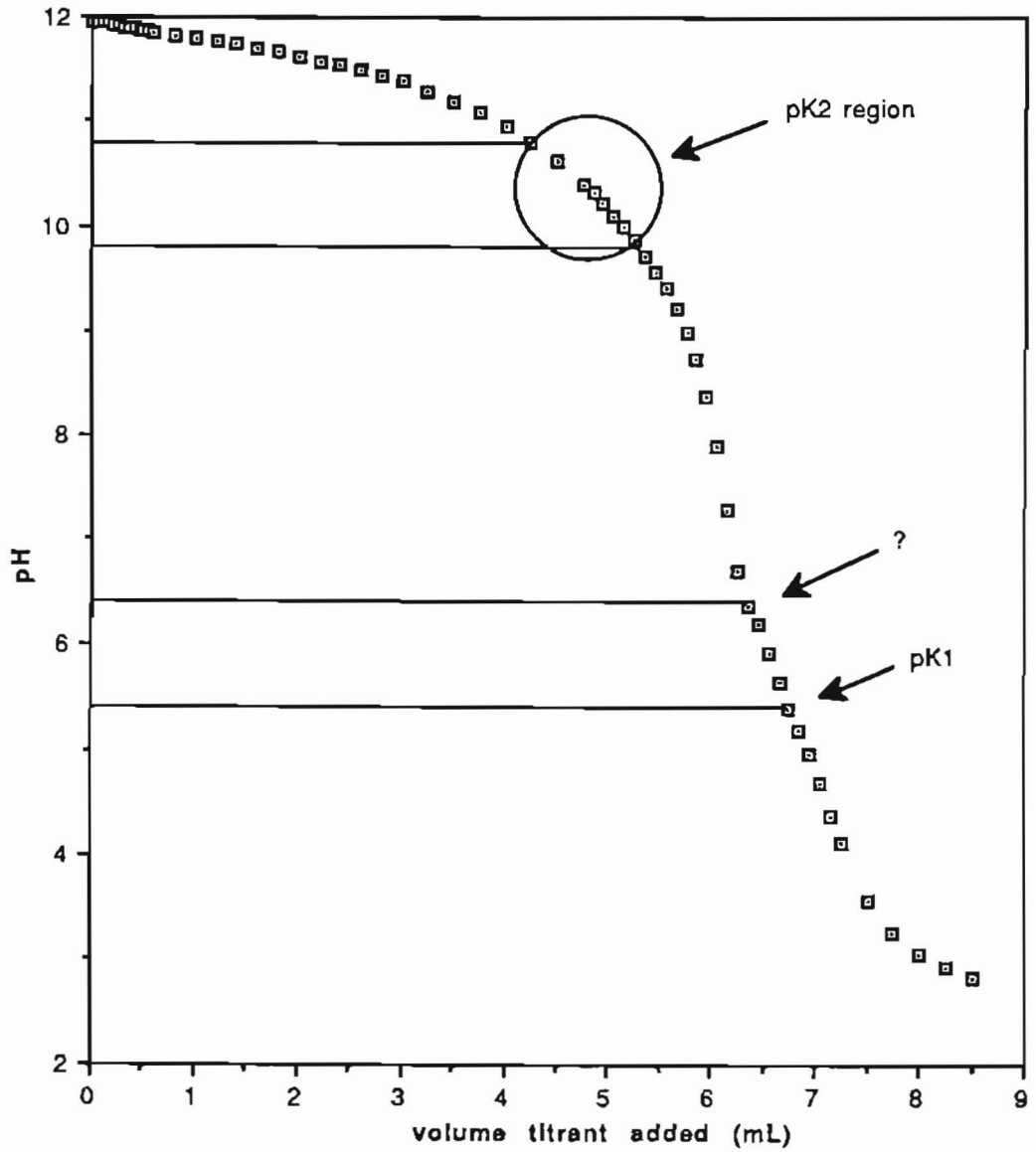

0.0025
0.0015
0.0005
-0.0005
-0.0015
-0.0025
ß of F8HQ
noise
5.40
6.39
10.11
10.41
3
4
5
6
7
8
9
10
11
12
pH

Figure 7.
pH vs. volume of 0.05 M
HCl titrant added (ml).
27 ml slurry of F8HQ.


12
10
8
6
4
2
pH
0
1
2
3
4
5
6
7
8
9
volume titrant added (mL)
pK2 region
?
pK1

CHAPTER 2

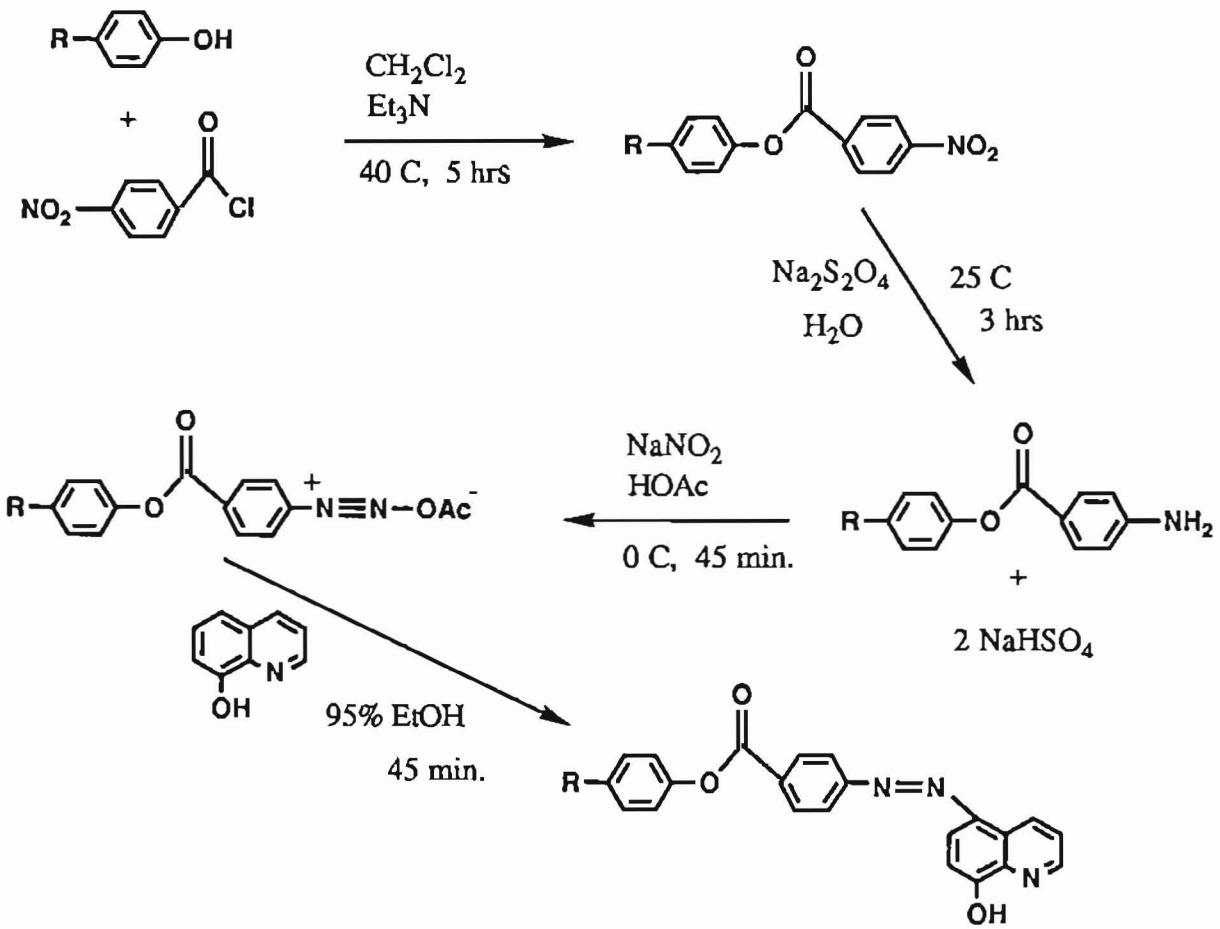
Now that the speciation modelling of Cu(II) and Pb(II) complexes in TRIS/HOAc buffer is complete, and a reasonable conclusion has been reached as to the types of metal species that can be chelated by F8HQ, the column can be characterized using experimental data. However, the reasons for using a TRIS/HOAc buffer system and a fractogel/8-hydroxyquinoline column must first be discussed.

There has been considerable work done using 8-hydroxyquinoline as a chelating ligand for transition and heavy metal cations, and there have been many attempts to immobilize this molecule on different substrates such that the product will be chemically stable, useful at extremes of pH, and have properties of high porosity, high mechanical strength, and fast reaction kinetics. Landing, et. al., [3] have devised a substrate-8HQ complex that exhibits all of these characteristics, and that also does not retain humic or fulvic acids - an important characteristic for analysis of natural water samples. They used Toyopearl Fractogel-TSK HW75F, which consists of vinyl polymer agglomerates supporting easily modified secondary hydroxyl groups. Landing's 8HQ immobilization procedure was carried out by Michael Mackey and Dave Anderson, and the resulting F8HQ was used in this research. The synthesis is presented in figure 8. The fractogel chromatographic material itself did not show any significant retention of Cu(II) or Pb(II) compared to F8HQ. Thus, the vinyl polymer backbone matrix does not introduce a background retention of these metals.

There were several options of buffers that could be used in the Cu(II) and Pb(II) solutions. A polyprotic buffer or a mixture of monoprotic buffers with acid pK's ranging from 3 to 10 was desired to

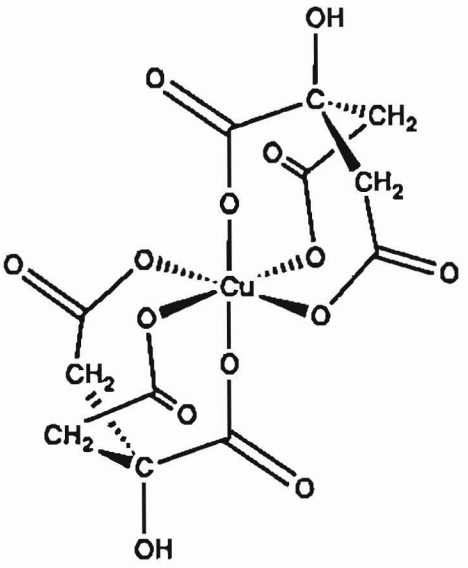
Figure 8.
Synthesis of fractogel immobilized 8-hydroxyquinoline chelating ligand by ester, phenyl, and azo linkages.

fractogel


R
OH
+
O
NO2
Cl
CH2Cl2
Et3N
40 C, 5 hrs
O
R
O
NO2
Na2S2O4
H2O
25 C
3 hrs
NaNO2
HOAc
0 C, 45 min.
R
O
O
NH2
+
2 NaHSO4
R
O
O
+
N≡N−OAc-
N
OH
95% EtOH
45 min.
R
O
O
N=N
N
OH

minimize pH drift during the course of the pH sensitive experiments. Ideally, maximum buffer capacities, β, should occur at about pH 5 and pH 8 since above pH 10, hydroxide ion becomes a good buffer. Carbonic acid, H_2CO_3, has pK's at 10.329 and 6.352 at μ=0 and 25° C. This would be a good candidate except that CO_3^{-2} forms insoluble metal complexes with many metals including Cu(II) and Pb(II), especially at the metal concentrations used here ($1e^{-4}$ M).

Citric acid (2-hydroxypropane-1,2,3-tricarboxcylic acid) was also a good choice with pK's of 6.396, 4.761, and 3.128 at μ=0 and 25° C. However, experiments with this buffer in Cu(II) solution showed that the Cu-citrate complexes formed were kinetically very stable, thus rendering chelation by the F8HQ material a very slow process. This stability is analogous to that of ethylenediaminetetraacetic acid (EDTA) when bound to a metal. Using entropy arguments, the reaction of EDTA with metals is very favorable since it is a hexadentate ligand. Likewise, citric acid could act as a tridentate ligand with 2 molecules wrapping around a metal in octahedral configuration with favorable entropy relative to coordination by monodentate ligands.

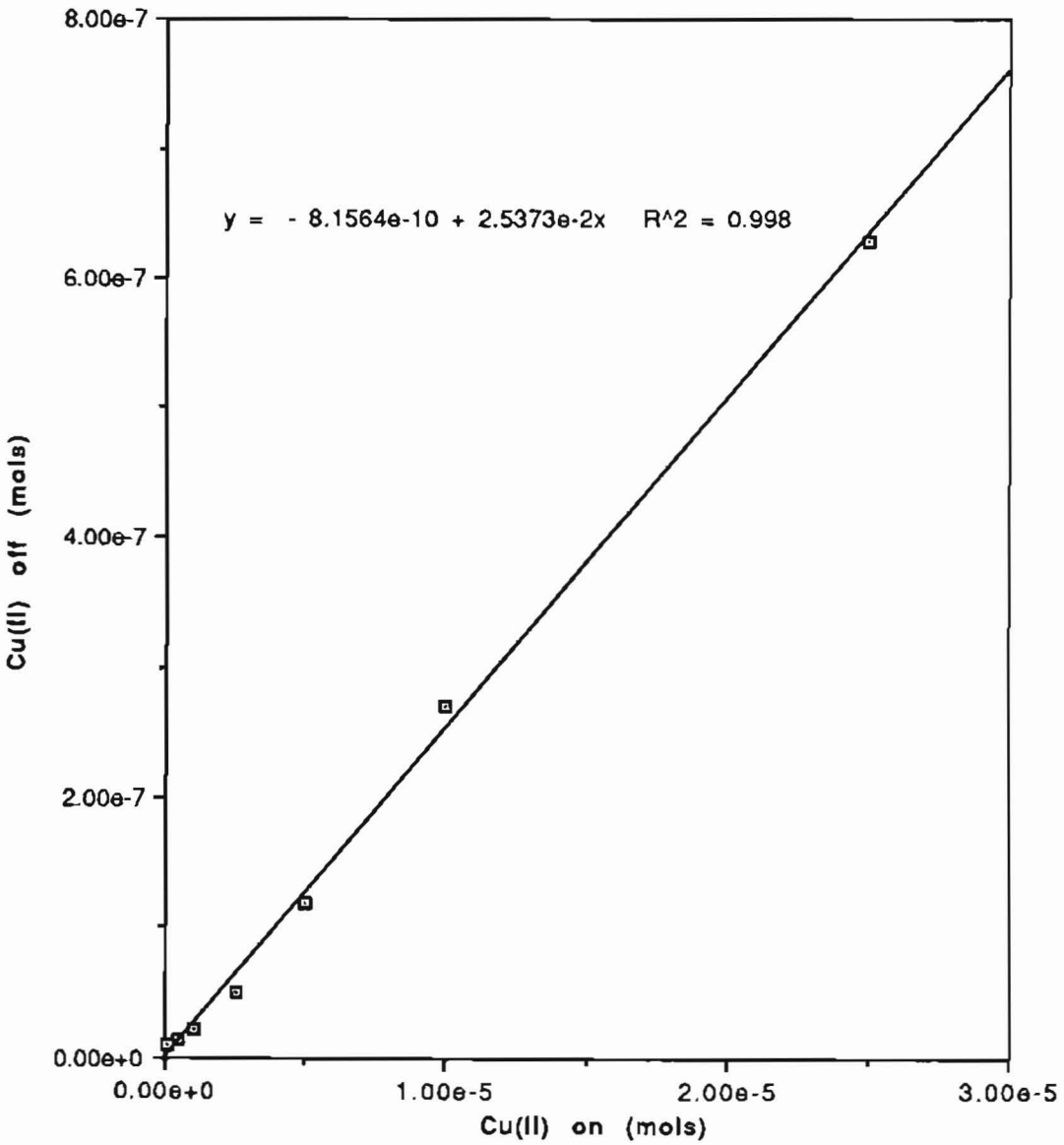


This wrapping of the metal center prevents it from coming in close contact with the chelation sites of F8HQ. As seen in figure 9, only 2.5% of the Cu was complexed by the column. Due to this low recovery, other buffers were investigated.

From these results it is easy to see that a monodentate ligand would be most suited to the situation. Landing, et. al., used a mixture of acetic acid and ammonia with success. In this research the metal solutions were buffered with a 1:1 mixture of HOAc and TRIS (pK = 4.757 and 8.075 respectively and both at μ=0 and 25° C). These buffers were selected for several reasons: ammonia is unpleasant to work with, TRIS is nontoxic and well characterized, and TRIS has a lower pK than that of NH_3 (9.244 at μ=0 and 25° C). This lower pK provides a better β distribution for the solutions being studied.

A breakthrough curve of Cu(II) in $5e^{-3}$ M TRIS solution at pH 8.2 indicated that the exchange kinetics of this buffer system were adequately fast. Acetate was assumed to be a small enough ligand to not have a

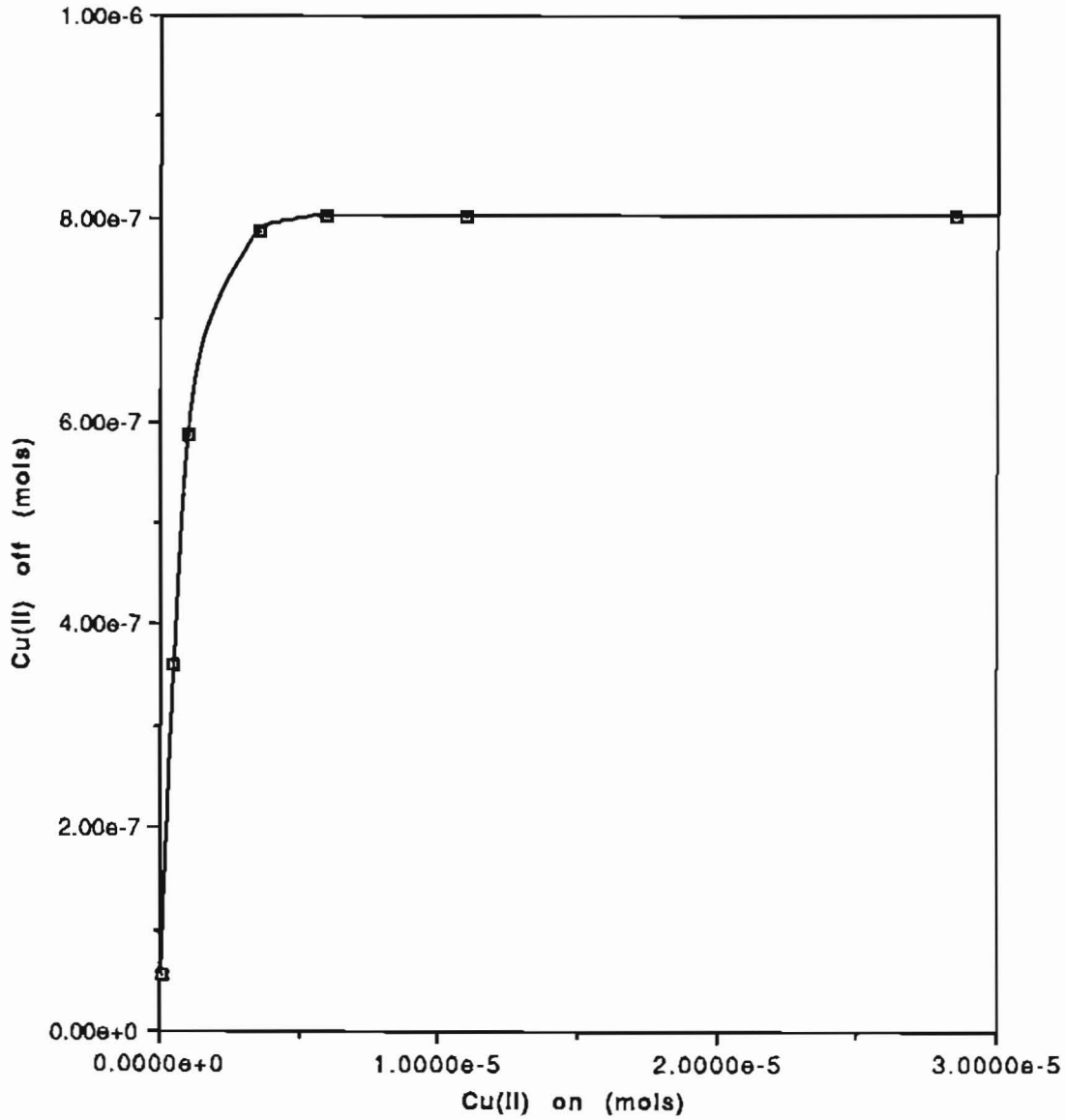
Figure 9.
Cu(II) eluted off the column (mols) vs. Cu(II) loaded on the column (mols). Breakthrough curve of $1e^{-4}$ M Cu(II) in $5e^{-3}$ M citrate buffer at pH = 7.7 using a 70 µL F8HQ solid phase column.


8.00e-7
6.00e-7
4.00e-7
2.00e-7
0.00e+0
y = - 8.1564e-10 + 2.5373e-2x R^2 = 0.998
Cu(II) off (mols)
0.00e+0
1.00e-5
2.00e-5
3.00e-5
Cu(II) on (mols)

deleterious effect on the rate of chelation. The breakthrough curve (figure 10) also indicates the approximate amount of metal that has to be put through a 70 μL F8HQ column to saturate all the active sites.

The ideal conditions for acid elution of Cu(II) and Pb(II) loaded columns were also determined by eluting with HCl solutions of different pH. HNO_3 was not used as either a column eluent or a column wash because of its oxidizing properties, which could lead to cleavage of the ester linkage holding the F8HQ to the resin. Since the efficiency of the nebulizer system on the ICP-AES is a function of the matrix of the solution, the best acid matrix was also determined by varying the pH's of metal samples analyzed. An HCl solution of pH 1 was found to be sufficient for both efficient elution of Cu(II) and Pb(II) and as a matrix for efficient metal detection by ICP-AES.

Figure 10.
Cu(II) eluted off the F8HQ column (mols) vs. Cu(II) loaded on (mols). Breakthrough curve for Cu(II) at pH 8.2 using a 70 µL F8HQ solid phase column and a $1e^{-4}$ M Cu(II) solution in $5e^{-3}$ M TRIS buffer.


1.00e-6
8.00e-7
6.00e-7
4.00e-7
2.00e-7
0.00e+0
Cu(II) off (mols)
0.0000e+0
1.0000e-5
2.0000e-5
3.0000e-5
Cu(II) on (mols)

CHAPTER 3

Now that the chemistry of the aqueous metal species and 8-hydroxyquinoline has been characterized by their α plots, the determination of the thermodynamic stability constants, $K_{Cu(II)}$ and $K_{Pb(II)}$, is a matter of experimentally elucidating the pH, temperature, and ionic strength dependence of Cu(II) and Pb(II) complexation by the F8HQ column. Two methods to do this were investigated: the log(K) vs. pH method, and the Scatchard method. The former method yielded questionable results that were not quantifiable due to some unknown chemical parameters that were overlooked, but the Scatchard method yielded reproducible and credible results. The theory and experimental results of the log(K) vs. pH method are presented in this chapter, and those of the Scatchard method are found in the following chapter.

The general reaction being studied here is,

$$M + Q \leftrightarrow MQ \tag{15}$$

where M represents all metal cations chelating to the column, Q represents the available F8HQ on the column. The equilibrium constant, K, at constant μ, pH, and temperature is defined by:

$$K = \frac{[MQ]}{[M][Q]} \tag{16}$$

Equation 16 can be defined in terms of total metal and F8HQ concentrations:

$$K = \frac{[MQ]}{(TM\alpha_M)(TQ\alpha_Q)} \qquad (17)$$

where TM is the total metal present, α_M is the fraction of metal available to chelate, TQ is the total F8HQ uncomplexed by metal, and α_Q is the fraction of uncomplexed F8HQ available to chelate the metal. If γ is defined as the total amount of F8HQ present on the column, complexed and uncomplexed, then,

$$K = \frac{[MQ]}{(TM\alpha_M)((\gamma\text{-}MQ)\alpha_Q)} \qquad (18)$$

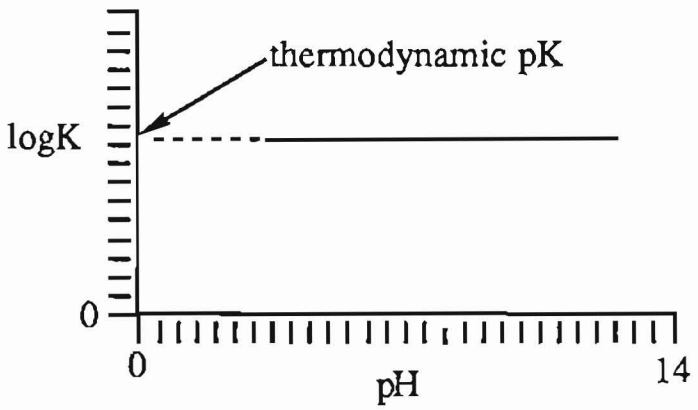
and rearranging equation (18) gives,

$$K\alpha_M\alpha_Q = \frac{[MQ]}{TM(\gamma\text{-}MQ)} = K' \qquad (19)$$

where K' is the conditional stability constant between the metal and F8HQ. K' is also what is measured experimentally. The two equilibrium values are related by equation 20:

$$K = \frac{K'}{\alpha_M\alpha_Q} \qquad (20)$$

Using ICP-AES to determine MQ, the metal and 8-hydroxyquinoline speciation models to determine α_M and α_Q respectively, and estimating a value for γ, a plot of logK vs. pH should yield a curve with the general shape below. This linear plot should have a slope of zero and all y values should average out to be the thermodynamic pK.



The only problem with this approach is that the plot was derived using an estimated value of TQ. If plots for two or more concentrations of TM are used, the true TQ can be calculated by minimizing the sum of residuals of common points in the plots. This best fit for TQ can then be used to calculate K.

The above method was applied to the determination of K between Cu(II) and F8HQ under known conditions of temperature and ionic strength. The experimental procedure below details the steps used, and the plots generated are an application of the theory above.

Experimental

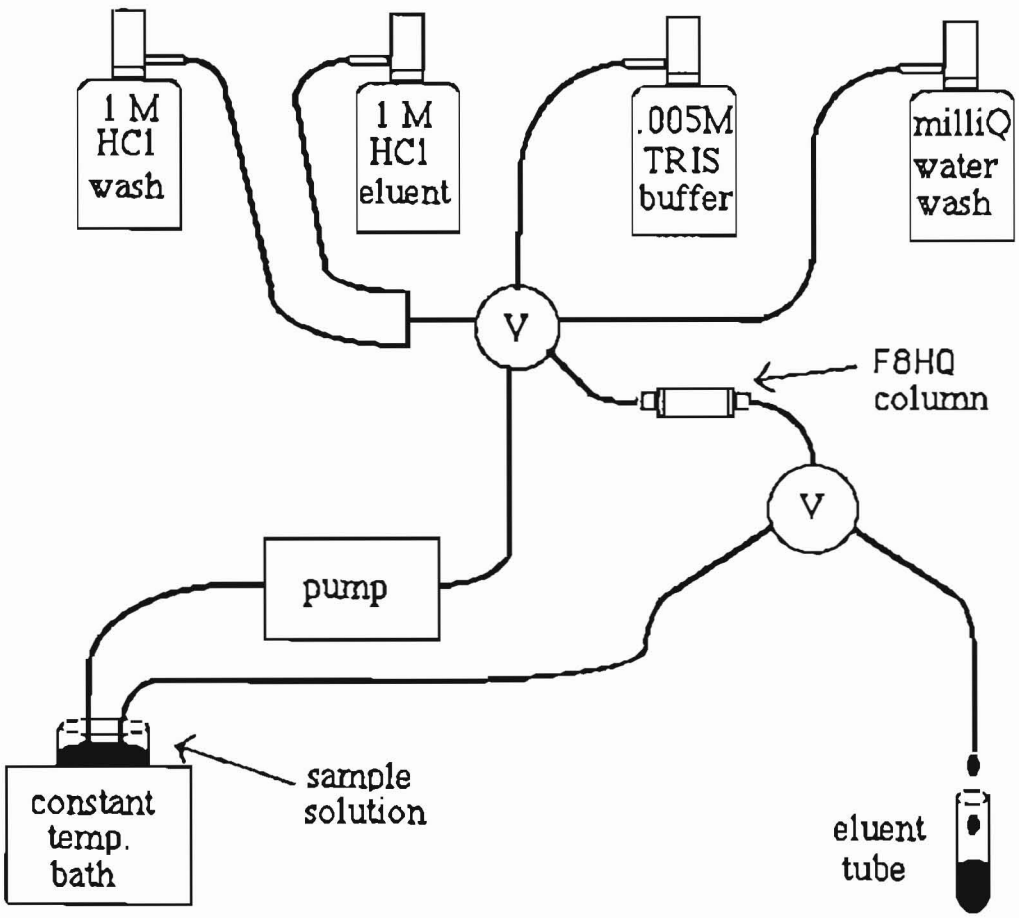
Reagents: All reagents were of highest purity and were used as received. A complete list is provided in appendix 3.

Apparatus: All instruments used in this and all other experiments are listed in appendix 4.

Procedure,part 1: The chelating column for this experiment was constructed by pouring a 200 μL slurry of F8HQ into an emptied Millipore "sep-pak", which was stoppered with glass wool. All solutions were prepared in a clean hood with acid washed glassware. The system diagram (figure 11) shows the flow setup of this experiment. 1.0 M HCl was used as the column wash solution and column eluent for pre-concentrated Cu(II) species. TRIS buffer (0.005 M) titrated to pH 8.2 with HCl was the column equilibration solution for use after elution, and milli-Q water (resistance of approximately 18.3 MΩ) was the clean column wash. A 3 liter solution of $1.00e^{-3}$ M $Cu(NO_3)_2$ in $2.50e^{-3}$ M TRIS and $2.50e^{-3}$ M HOAc buffer was prepared and placed in a polycarbonate vessel in a constant temperature bath. This solution and a 0.05 M standard potassium acid phthalate buffer solution for pH measurements were allowed to thermally equilibrate for at least one hour at 25.0° C. All pH measurements were obtained at 25.0° C, and were calculated relative to the observed potential of the standard KHP buffer. Enough NaOH (50% wt/wt) was added to the Cu(II) solution to bring it to pH 12.700 and μ = 0.06. HCL (37.8%) and 1.0 M HCl were both used to titrate the Cu(II) solution to pH 2.267 and μ = 0.12 over the course of this experiment at approximately half pH unit increments. The experimental steps are summarized below.

1) The bulk Cu(II) solution was titrated to a specific pH (at approximately half pH unit increments) with HCl (37.8%) and 1.0 M HCl.

Figure 11.
System diagram for determination of $K_{Cu(II)}$ and $K_{Pb(II)}$ by both the log(K) vs. pH method and the Scatchard method.


1 M
HCl
wash
1 M
HCl
eluent
.005M
TRIS
buffer
milliQ
water
wash
V
F8HQ
column
V
pump
sample
solution
constant
temp.
bath
eluent
tube

2) The flow system without the column was flushed with the $1.00e^{-3}$ M Cu(II) solution at 25.0° C for 10 minutes to equilibrate temperatures.
3) The column was saturated at a rate of 5 ml/min. for 16 minutes with the column effluent directed back to the Cu(II) vessel.
4) A 5.00 ml sample of the bulk Cu(II) solution was collected and acidified with 25-50 μL HCl (37.8%) for analysis.
5) The column was washed with 10 ml milli-Q water at a rate of 1 ml/sec. to get rid of excess Cu(II) solution on the column.
6) The column was eluted with 5.0 ml 1.0 M HCl at a rate of 5 ml/min., and the eluent was collected for analysis.
7) The column was washed with 5 ml 1.0 M HCl at a rate of 5 ml/min. to get any excess metal or contamination off the column.
8) The column was equilibrated by passing 5 ml 0.005 M pH 8.2 TRIS buffer through it at a rate of 10 ml/min.
9) The column was washed with 5 ml milli-Q water to remove all chemical species from the column and to eliminate any extra β of the TRIS.
10) Start at step one again.

ICP-AES was used to analyze the following samples: the 5.0 ml eluent samples for all pH pre-concentration runs from step 6; the acidified 5.0 ml bulk Cu(II) solution samples from step 4; a 5.0 ml blank of the milli-Q wash acidified by 10 μL HCl (37.8%); a 5.0 ml blank of the 0.005 M TRIS buffer wash acidified with 25 μL of HCl (37.8%); and 5.0 ml blanks of both the 1.0 M HCl column wash and 1.0 M HCl column eluent. The

elemental standards used to obtain the ICP-AES standard curve ranged from 0 to 56 ppm Cu.

Procedure,part 2: The same procedure as above was carried out two more times for solutions of $5.00e^{-4}$ M and $2.50e^{-4}$ M Cu(II) both in the same concentration TRIS and HOAc buffer media as above. The experiments were performed at only four pH's for each of these Cu(II) solutions. These pH's were in the non-precipitate region: approximately pH 12.7, 10.9, 5, and 3. The same samples and blanks as above were analyzed by ICP-AES using the same Cu standards for the standard curve, and the results were used in an attempt to determine the total amount of F8HQ (γ in equation 19).

Calculations and Results

Data: Raw data from both parts of this experiment are listed in appendix 5.

Part 1: A total of 80 ml of the Cu(II) solution ($8e^{-5}$ mols Cu(II)) was passed through the F8HQ column for each pH run. This was enough to saturate the column completely. During the course of the experiment, a pale blue precipitate was noticeably collecting on the glass wool of the column between pH's 6.3 and 8, and it is possible that precipitate was also present in unnoticeable quantity up to pH 10. Consequently, no data points were taken between pH's 6.3 and 8.

The relative moles of Cu(II) bound by the column as a function of pH are shown in figure 12. The plot shows the approximate pH region of good chelation, but this is obscured by the area of precipitate formation in which no data were taken. The shape of the log(K) vs. pH plot (figure 13)

Figure 12.
Relative Cu(II) (mols) complexed by the F8HQ column vs. pH for $1.00e^{-3}$ M Cu(II) solution in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc. No data was taken from pH 6.3 to 8 due to precipitate formation.

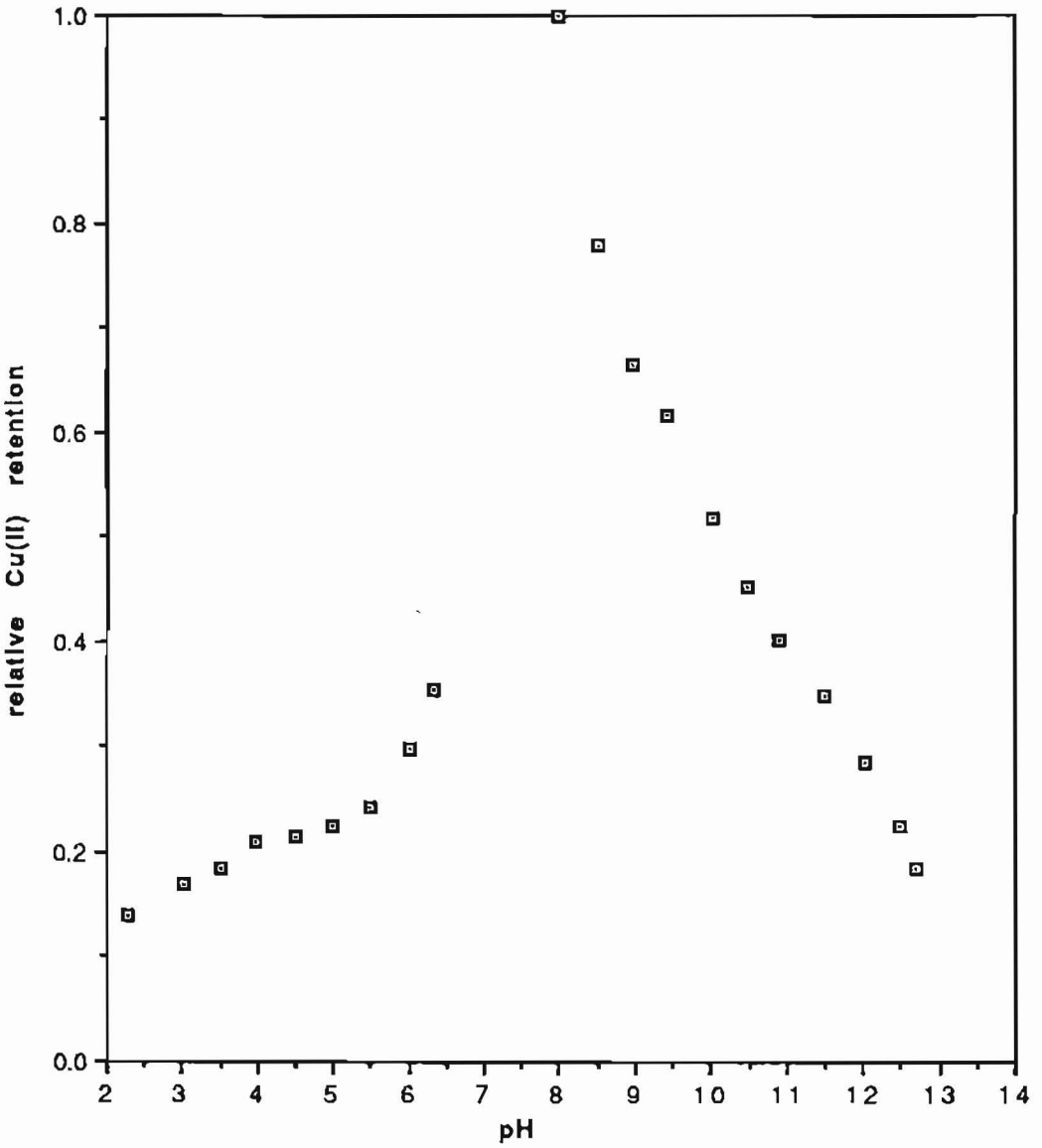
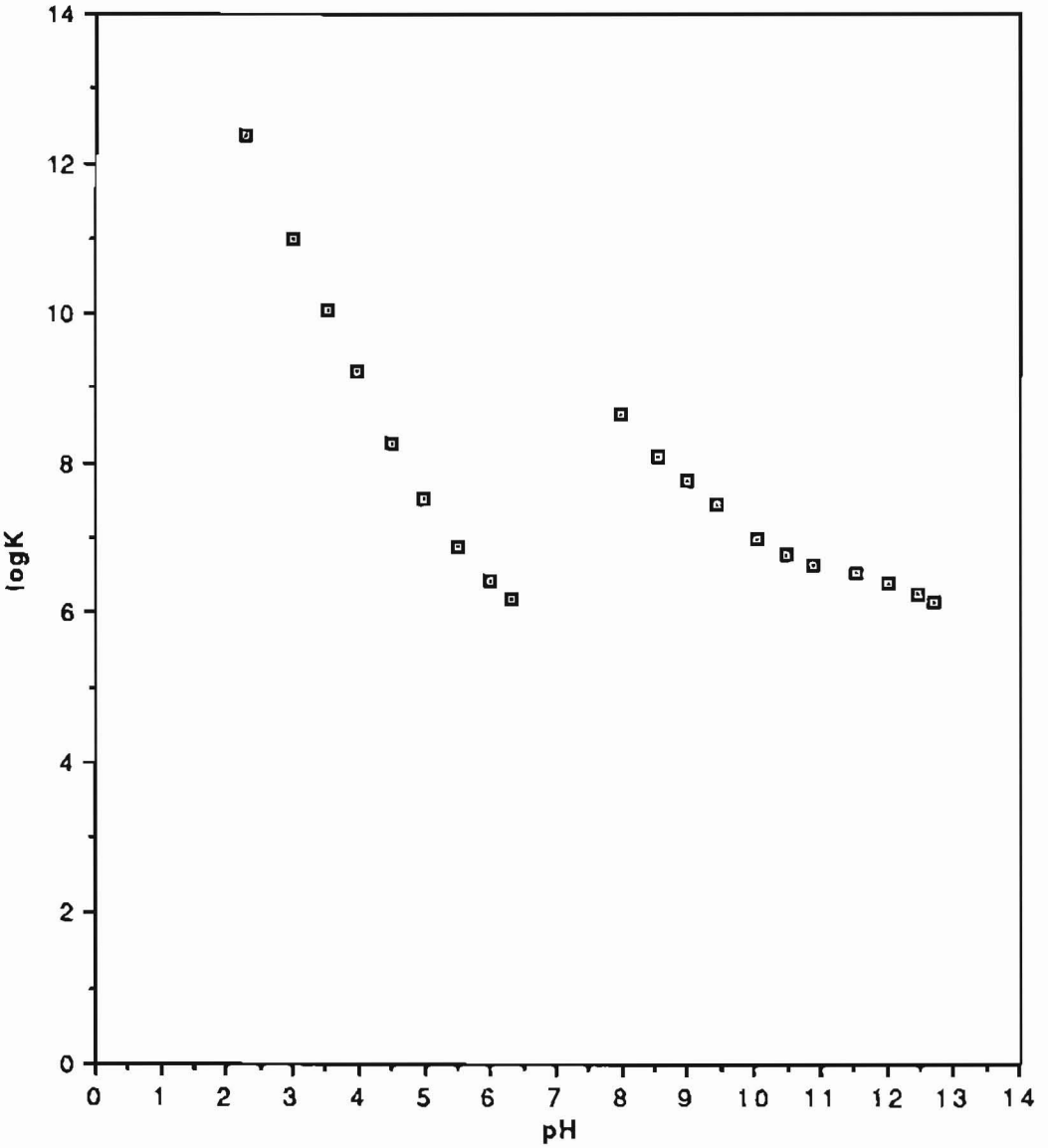

1.0
0.8
0.6
0.4
0.2
0.0
relative Cu(II) retention
2
3
4
5
6
7
8
9
10
11
12
13
14
pH

Figure 13.
Log(K) vs. pH plot for complexation of $1.00e^{-3}$ M Cu(II) to F8HQ in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc. This plot should theoretically be linear with slope = 0 and y-intercept = log(K).


14
12
10
8
6
4
2
0
logK
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH

is not linear as predicted by theory. The region below pH 6.3 has a negative slope. The region approximately after pH 8 is the most linear part of the plot, but the slope is still negative. Formation of precipitate in the region of about pH 8 to possibly as far as pH 10 might be distorting the log(K) values, making them higher than expected. The variation in this plot makes accurate determination of K impossible. The reason (besides the formation of precipitate) that this widespread variation occurs is unknown, but it renders this method inappropriate for the determination of K. The precipitate was probably the insoluble $Cu(OH)_2$ and/or $CuCO_3$ species. High Cu(II) concentrations ($1e^{-3}$ M) were necessary in this experiment to prevent significant changes in the bulk solution concentration as the experiment progressed. If lower concentrations were used to prevent precipitation, the method would take an inordinate amount of time to complete.

Part 2: The attempt to determine the total F8HQ on the column also failed. There wasn't enough difference in the K's derived for each pH value for each of the three Cu(II) solutions to get the value of γ. As the total F8HQ was varied, there was no clear best fit for all the points. Thus, no single γ value could be determined. Ignoring the complications in part 1, this uncertainty in the total F8HQ makes determination of K by this method inappropriate.

Conclusion

It would be wise to choose another method for the determination of K. The new method should take less time, have fewer complications, and give more accurate results.

CHAPTER 4

The next method chosen for the determination of the K's between Cu(II) and Pb(II) species and F8HQ was the Scatchard Plot method. This is a well known technique [7] used mostly for the determination of equilibrium constants in biochemistry, but it has had very little or no application in the characterization of systems like this one. Most often the absorbance of an analyte-substrate solution is measured as a function of the concentration of analyte, which is added incrementally. In this case, concentrations were measured directly by ICP-AES. As in the last method, we start with the basic reaction. M denotes species of either Cu(II) or Pb(II) and Q denotes F8HQ. For the reaction,

$$M + Q = MQ \tag{21}$$

there is a constant, K, describing the equilibrium such that,

$$K = \frac{[MQ]}{[M][Q]} \tag{22}$$

The same substitutions for M and Q can be performed as in the previous chapter:

$$K = \frac{[MQ]}{(TM\alpha_M)(TQ\alpha_Q)} \tag{23}$$

and,

$$K = \frac{[MQ]}{(TM\alpha_M)((\gamma\text{-}MQ)\alpha_Q)} \qquad (24)$$

where the total uncomplexed and complexed F8HQ is γ. Rearranging equation 4 into a linear form gives:

$$\frac{[MQ]}{TM} = K\gamma\alpha_Q\alpha_M - K\alpha_Q\alpha_M[MQ] \qquad (25)$$

Plotting [MQ]/TM vs. [MQ] gives a slope, m, equal to $-(K\alpha_Q\alpha_M)$ and a y-intercept, b, equal to $K\gamma\alpha_Q\alpha_M$ or γm. Thus, the total overall F8HQ is,

$$\gamma = \frac{\gamma K\alpha_Q\alpha_M}{K\alpha_Q\alpha_M} = \frac{\text{y-intercept}}{\text{slope}} \qquad (26)$$

at all pH's. The apparent equilibrium constant, K', is equal to the slope m. Since $K' = m = K\alpha_Q\alpha_M$, then,

$$K = \frac{m}{\alpha_Q\alpha_M} \qquad (27)$$

Again, the importance of the speciations of 8-hydroxyquinoline and Cu(II) and Pb(II) species is understood. Without them, the thermodynamic K for each metal with F8HQ could not be calculated from equation 27.

This method is relatively rapid compared to the log(K) vs. pH method. One only has to run a metal solution through the F8HQ column a number of times, each time determining the concentration of the bulk

metal solution, [TM], and the number of mols being extracted from the solution by the column, [MQ]. This is done by ICP-AES. This treatment turns out to be the same as if the metal were being added incrementally to a bulk solution of aqueous F8HQ, which is the way the experiment would normally be done. Only one series of measurements like this can be done for a given pH. Thus, in order to confirm that the thermodynamic K is independent of pH, two or more experiments at significantly different pH's must be performed. If the experimental method works, calculated K's should be the same at different pH's.

Since protons in high concentration outcompete any metal for F8HQ active sites, doing Scatchard determinations at low pH would be impractical. The experiments should be done at a pH of greater than approximately 5.

Experimental

Reagents: All reagents were of highest purity and were used as received. A complete list is provided in appendix 3.

Apparatus: All instruments used in this and all other experiments are listed in appendix 4.

Procedure,part 1: The chelating column for this experiment was constructed by pouring a 100 µL slurry of F8HQ into an emptied Millipore "sep-pak", which was stoppered with glass wool. All solution preparations were done in a clean hood with acid washed glassware. The system used before (figure 11) was also used for this experiment. HCl (1.0 M) was used as the column wash solution and column eluent for pre-concentrated metal species. TRIS buffer (0.005 M) titrated to pH 8.2 with HCl was the

column equilibration solution for use after elution, and milli-Q water (resistance of approximately 18.3 MΩ) was the clean column wash. Solutions of both $1.00e^{-4}$ M $Cu(NO_3)_2$ and $1.00e^{-4}$ M $Pb(NO_3)_2$ in $2.50e^{-3}$ M TRIS and $2.50e^{-3}$ M HOAc buffer were prepared and placed in the constant temperature bath in small vessels. The volumes and pH's (adjusted by 1 M and 50% wt/wt NaOH) of these solutions are listed in the table below. The higher pH solution had a μ of approximately 0.01 M and the lower pH solutions had a μ of about 0.001 M. These solutions and a 0.05 M standard potassium acid phthalate buffer solution for pH measurements were allowed to thermally equilibrate for at least one hour at 25.0° C before any experimental runs. All pH measurements were obtained at 25.0° C and were calculated relative to the observed potential of the standard KHP buffer. The experimental steps are summarized below.

1) The flow system without the column was flushed with the $1.00e^{-4}$ M analyte solution at 25.0° C for 10 minutes to equilibrate temperatures.
2) The column was saturated at a rate of 20-30 ml/min. so that the solution volume turned over at least 6 times. The column effluent was directed back into the analyte vessel.
3) A 1.00 ml sample of the bulk analyte solution was collected then diluted and acidified with 4.00 ml of 1 M HCl eluent for analysis.
4) The column was washed with 10 ml milli-Q water at a rate of 1 ml/sec. to get rid of excess analyte solution on the column.
5) The column was eluted with 5.0 ml 1.0 M HCl at a rate of 5 ml/min., and the eluent was collected for analysis.

6) The column was washed with 5 ml 1.0 M HCl at a rate of 5 ml/min. to get any excess metal or contamination off the column.

7) The column was equilibrated by passing 5 ml 0.005 M pH 8.2 TRIS buffer through it at a rate of 10 ml/min.

8) The column was washed with 5 ml milli-Q water to remove all chemical species from the column and eliminate any extra β of the TRIS wash.

10) Start at step one again.

The analyte solution pH's and volumes used are below.

metal	pH	volume (ml)
Cu(II)	7.532	40
Cu(II)	11.963	30
Pb(II)	6.209	50
Pb(II)	12.333	25

ICP-AES was used to analyze the following samples: the 5.0 ml eluent samples for all pre-concentration runs from step 5; the acidified and diluted 5.0 ml analyte solution samples from step 3; a 5.0 ml blank of the milli-Q wash acidified by 10 μL HCl (37.8%); a 5.0 ml blank of the 0.005 M TRIS buffer wash acidified with 25 μL of HCl (37.8%); and 5.0 ml blanks of both the 1.0 M HCl column wash and 1.0 M HCl column eluent. The Cu and Pb elemental standards used to obtain a standard curve ranged from 0 to 2 ppm of each metal. A Leeman Labs Certified check standard of approximately 2 ppm Cu and Pb was periodically analyzed during the

analysis to monitor the consistency of the ICP-AES and the accuracy of the standard curve being used.

Calculations and Results

Scatchard plots of Cu(II) and Pb(II) were not obtained for pH's of approximately 9 to 10 since the chemistry of the system is not well understood in this region (pK2 of F8HQ is somewhere between 9 and 10). Exactly defined α's are required to get accurate speciations, and as seen in chapter 1, the acid pK's for F8HQ vary from those of 8-hydroxyquinoline. The raw data, other data, and calculated values for the Scatchard plots are listed in appendices 6-9. The methods presented in the beginning of the chapter and the α values determined previously were used to calculate the K's of Cu(II) and Pb(II) with F8HQ, and the total ligand concentration, γ, on the 100 μL column.

The values for $K_{Cu(II)}$ determined using the Scatchard method at the two pH's agreed to within 0.3%. All stability constants of Cu(II) species, including that of $[Cu(TRIS)_4]^{+2}$, were known for this calculation. It would be safe to assume that the K's for Pb(II) and F8HQ at each of the two pH's would also be in reasonable agreement. This is the case if the overall pK for Pb(II) binding to four TRIS ligands is estimated at 14.2. So not only is the Pb(II)-F8HQ pK calculated, but so is the $[Pb(TRIS)_4]^{+2}$ pK.

Figures 14, 16, 18, and 20 show the Cu and Pb recoveries as a function of run number. The only part of these curves that give information about the metal-F8HQ complexation is where the slope is changing significantly. The points used in the Scatchard plots (figures 15, 17, 19, and 21) are indicated by arrows in the previous plots. All data

that do and do not contribute to the determination of K can be found in appendices 6-9.

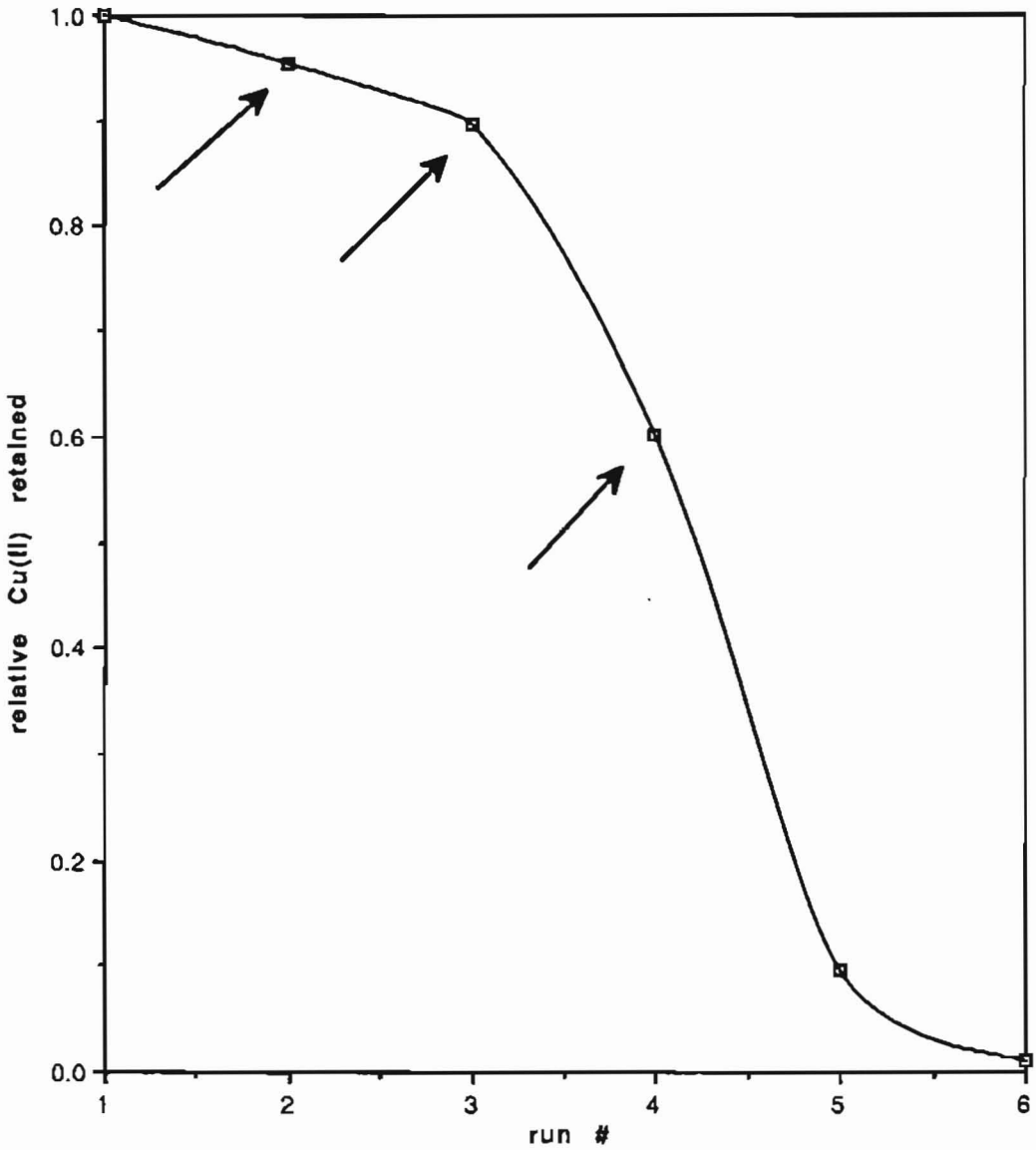
The pK values determined by this method are 9.17 for Cu(II)-F8HQ, and 9.68 for Pb(II)-F8HQ (at 25.0° C degrees and μ between 0.001 and 0.01 M). The literature values of pK's for these metals and 8-hydroxyquinoline, 12.10 and 9.02 respectively, differ significantly from these. The Cu pK has dropped by three orders of magnitude and the Pb pK has increased by about half a pK unit. The pK drop was predicted and is understandable. Since the fractogel linkage to 8-hydroxyquinoline is electron withdrawing by resonance, the hydroxyl group stabilizes a negative charge and F8HQ will bind a metal at lower pK. The slight rise in pK for Pb from 9.02 to 9.68, however, was not expected and remains unexplained.

Conclusion

The bottom line is that the Scatchard method quickly and accurately determined the metal-F8HQ K's compared to the method presented in chapter 3. Consequently, the constants derived by this method will be used in the speciation analysis of Penobscot Bay seawater in the next section.

Figure 14.
Plot of Cu(II) species retained by the column vs. run number at pH 7.532
in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.

Figure 15.
Scatchard plot of Cu(II) species binding to F8HQ at pH 7.532
in 2.5e-3 M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.


1.0
0.8
0.6
0.4
0.2
0.0
relative Cu(II) retained
1
2
3
4
5
6
run #

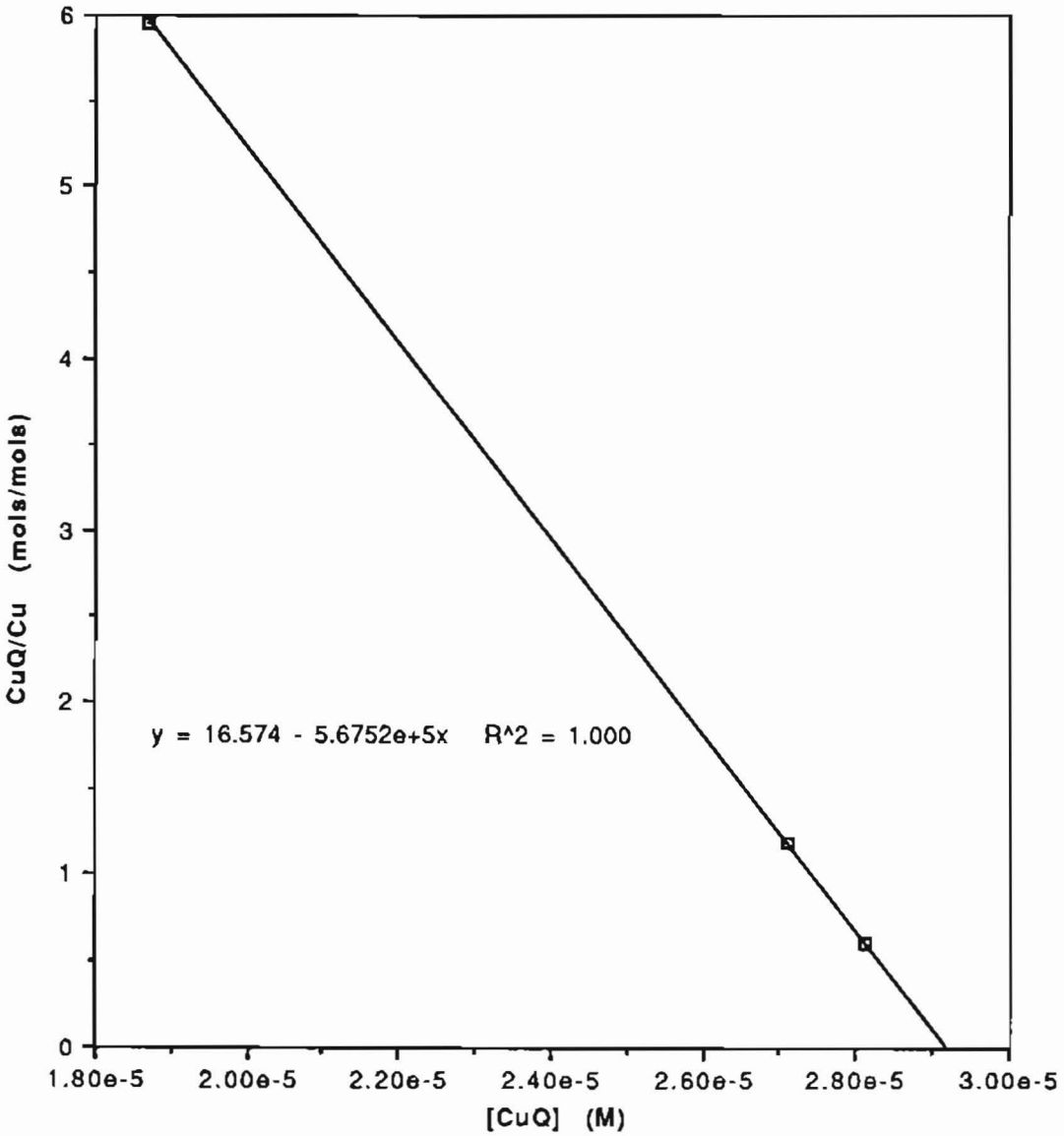
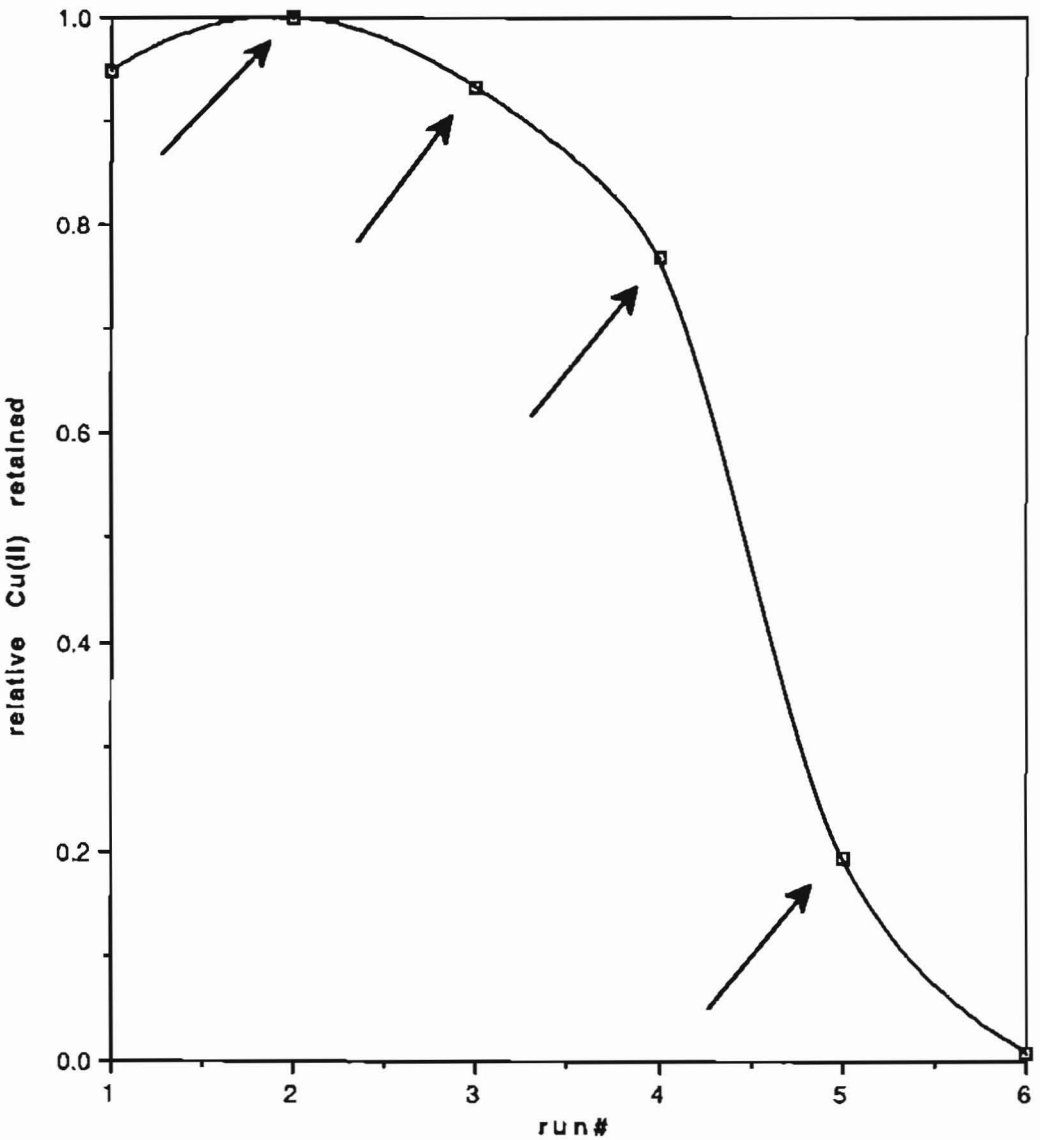

6
5
4
3
2
1
0
CuQ/Cu (mols/mols)
y = 16.574 - 5.6752e+5x R^2 = 1.000
1.80e-5
2.00e-5
2.20e-5
2.40e-5
2.60e-5
2.80e-5
3.00e-5
[CuQ] (M)

Figure 16.
Plot of Cu(II) species retained by the column vs. run number
at pH 11.963 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.

Figure 17.
Scatchard plot of Cu(II) species binding to F8HQ at
pH 11.963 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.


1.0
0.8
0.6
0.4
0.2
0.0
relative Cu(II) retained
1
2
3
4
5
6
run#

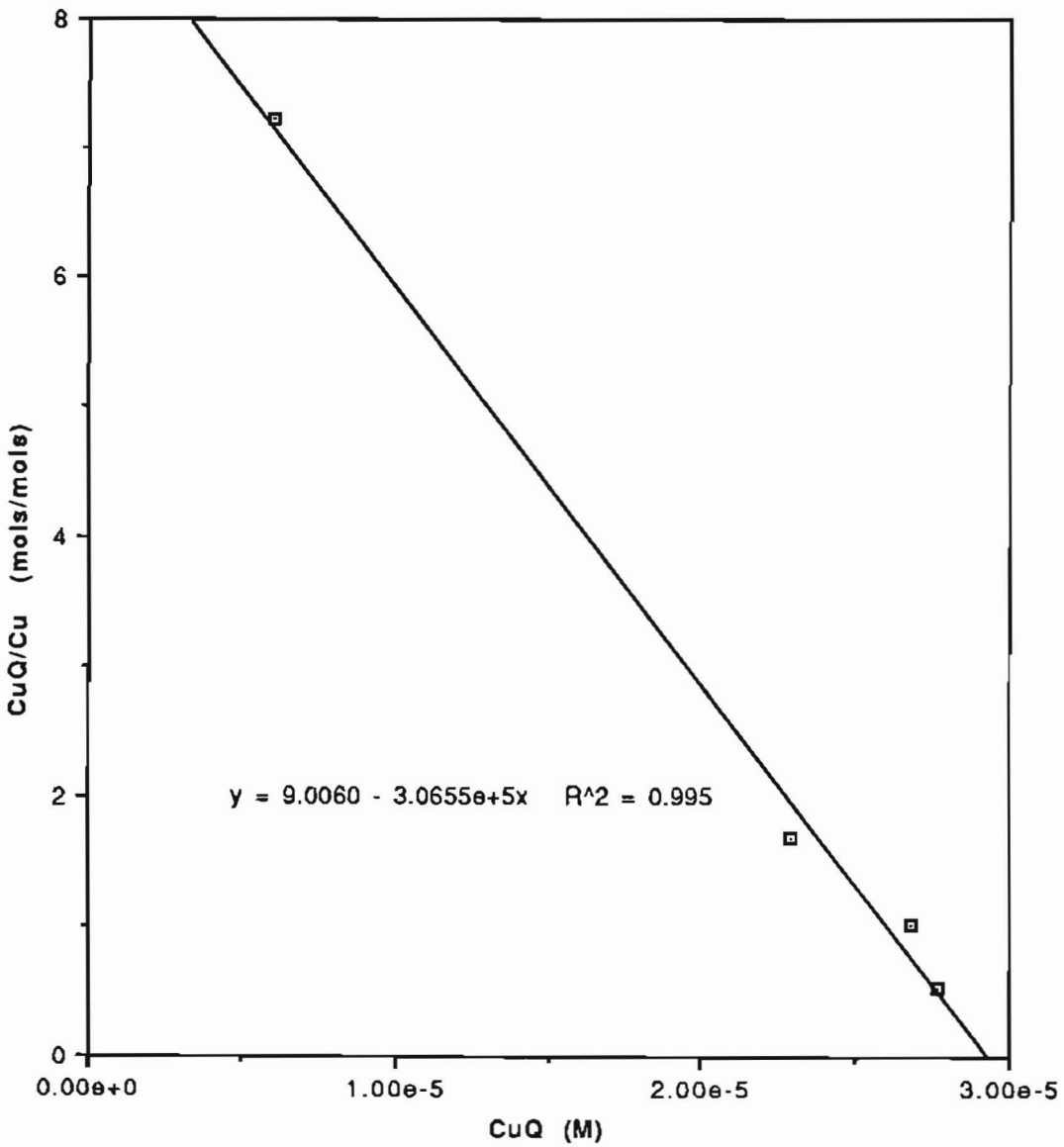
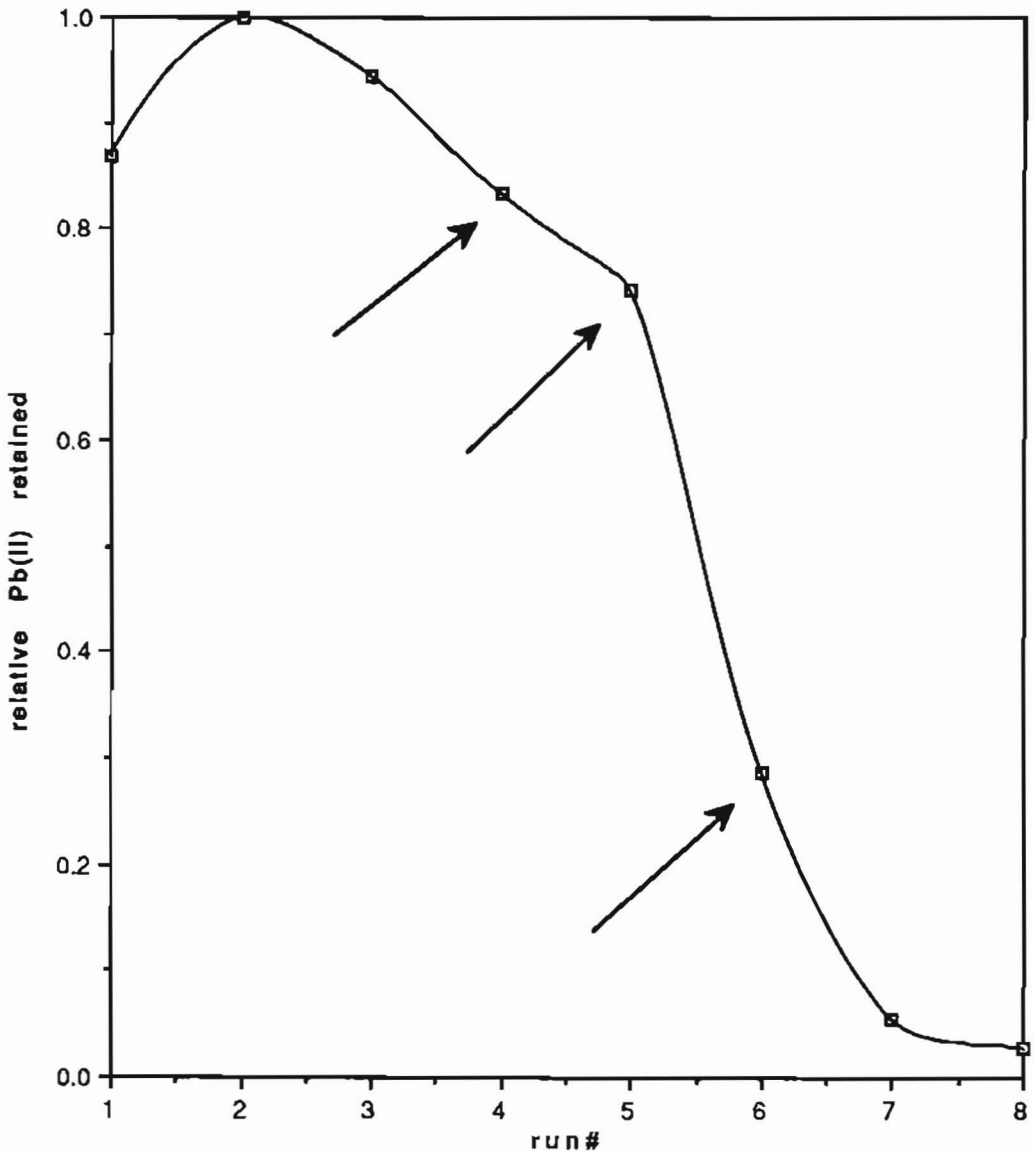

8
6
4
2
0
CuQ/Cu (mols/mols)
y = 9.0060 - 3.0655e+5x R^2 = 0.995
0.00e+0
1.00e-5
2.00e-5
3.00e-5
CuQ (M)

Figure 18.
Plot of Pb(II) species retained by the column vs. run number at pH 6.209 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.

Figure 19.
Scatchard plot of Pb(II) species binding to F8HQ at pH 6.209 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.


1.0
0.8
0.6
0.4
0.2
0.0
relative Pb(II) retained
1
2
3
4
5
6
7
8
run#

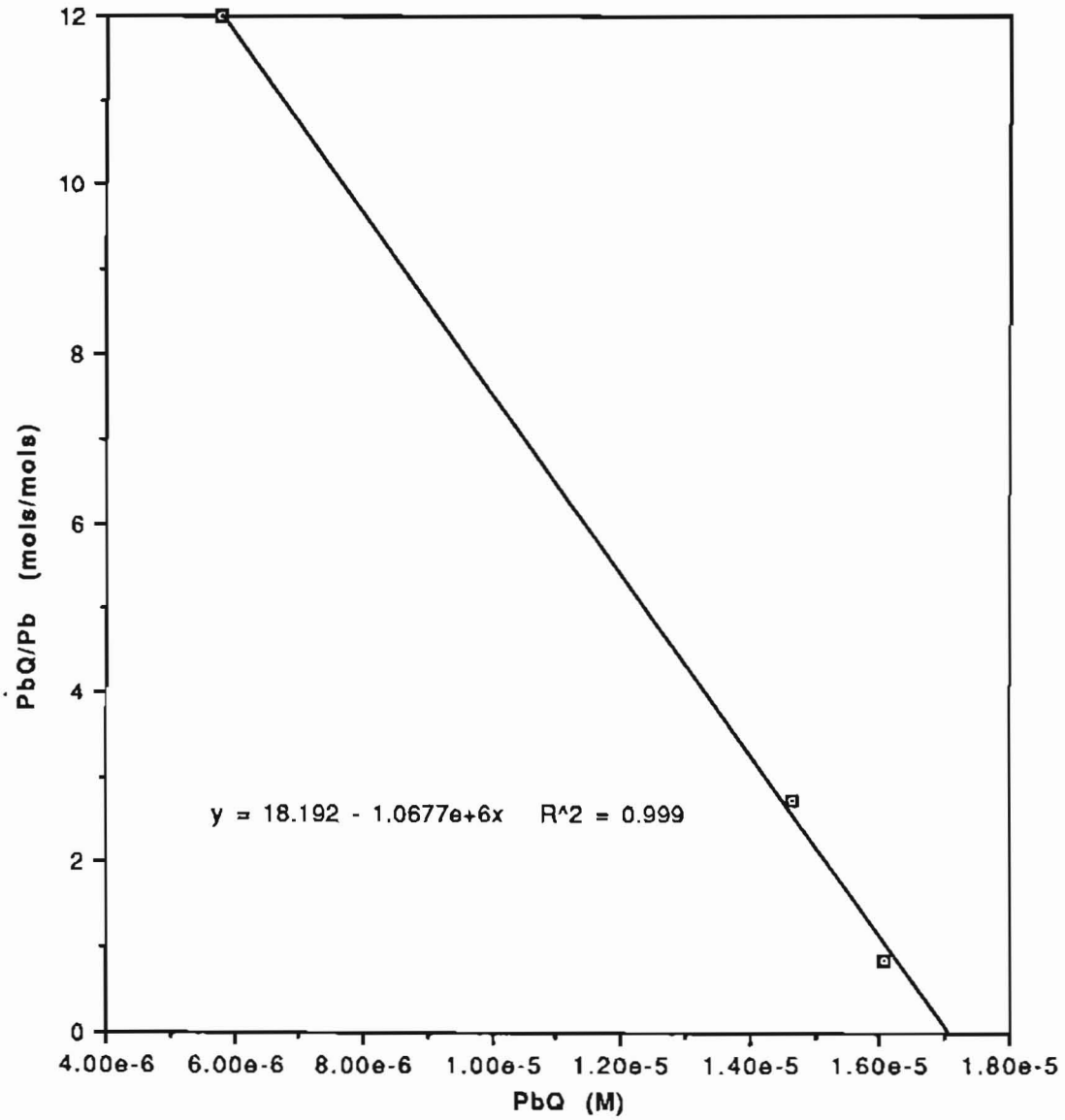
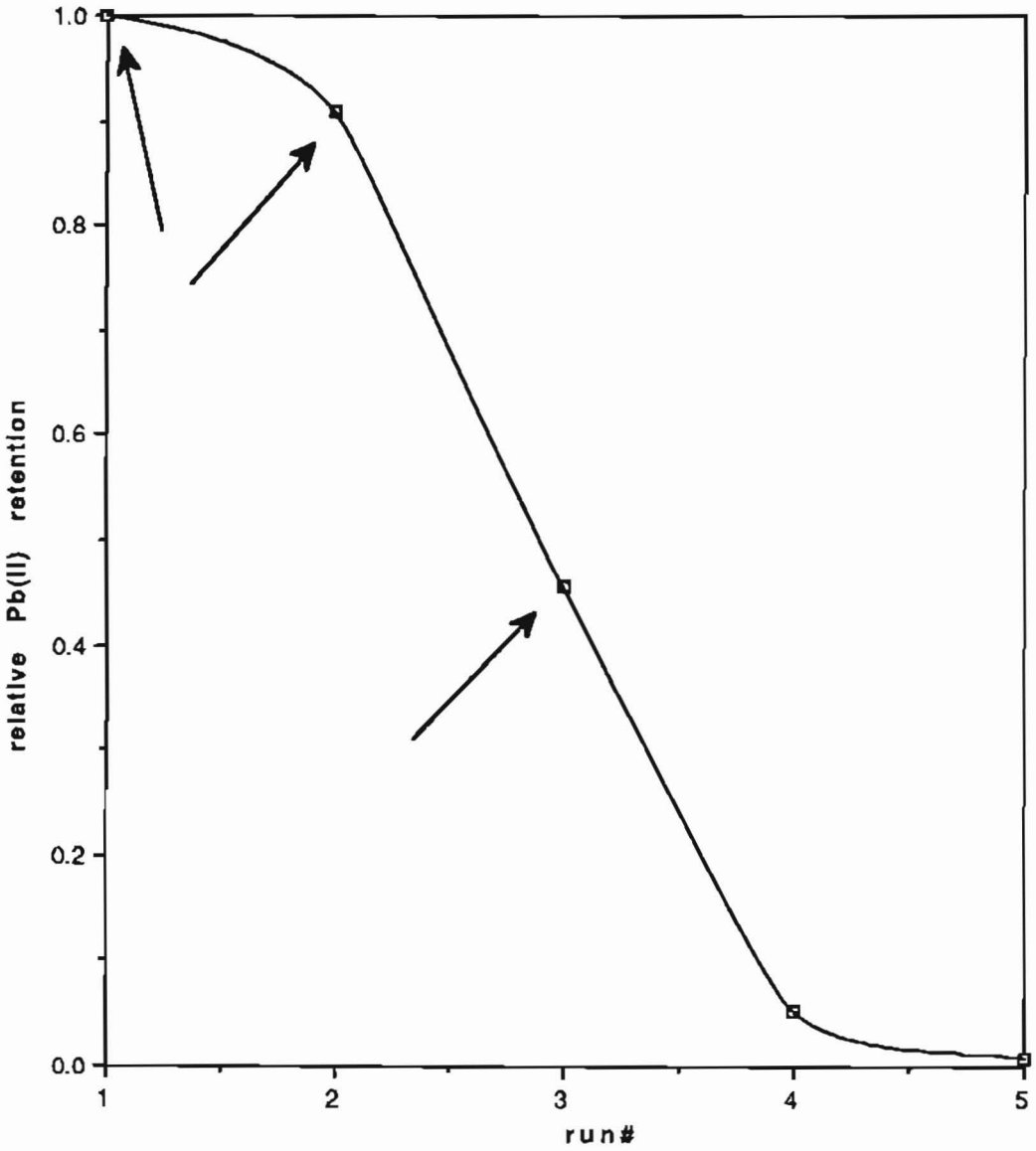

12
10
8
6
4
2
0
PbQ/Pb (mols/mols)
y = 18.192 - 1.0677e+6x R^2 = 0.999
4.00e-6
6.00e-6
8.00e-6
1.00e-5
1.20e-5
1.40e-5
1.60e-5
1.80e-5
PbQ (M)

Figure 20.
Plot of Pb(II) species retained by the column vs. run number
at pH 12.333 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.

Figure 21.
Scatchard plot of Pb(II) species binding to F8HQ at
pH 12.333 in $2.5e^{-3}$ M TRIS and $2.5e^{-3}$ M HOAc buffer at 25° C.


1.0
0.8
0.6
0.4
0.2
0.0
relative Pb(II) retention
1
2
3
4
5
run#

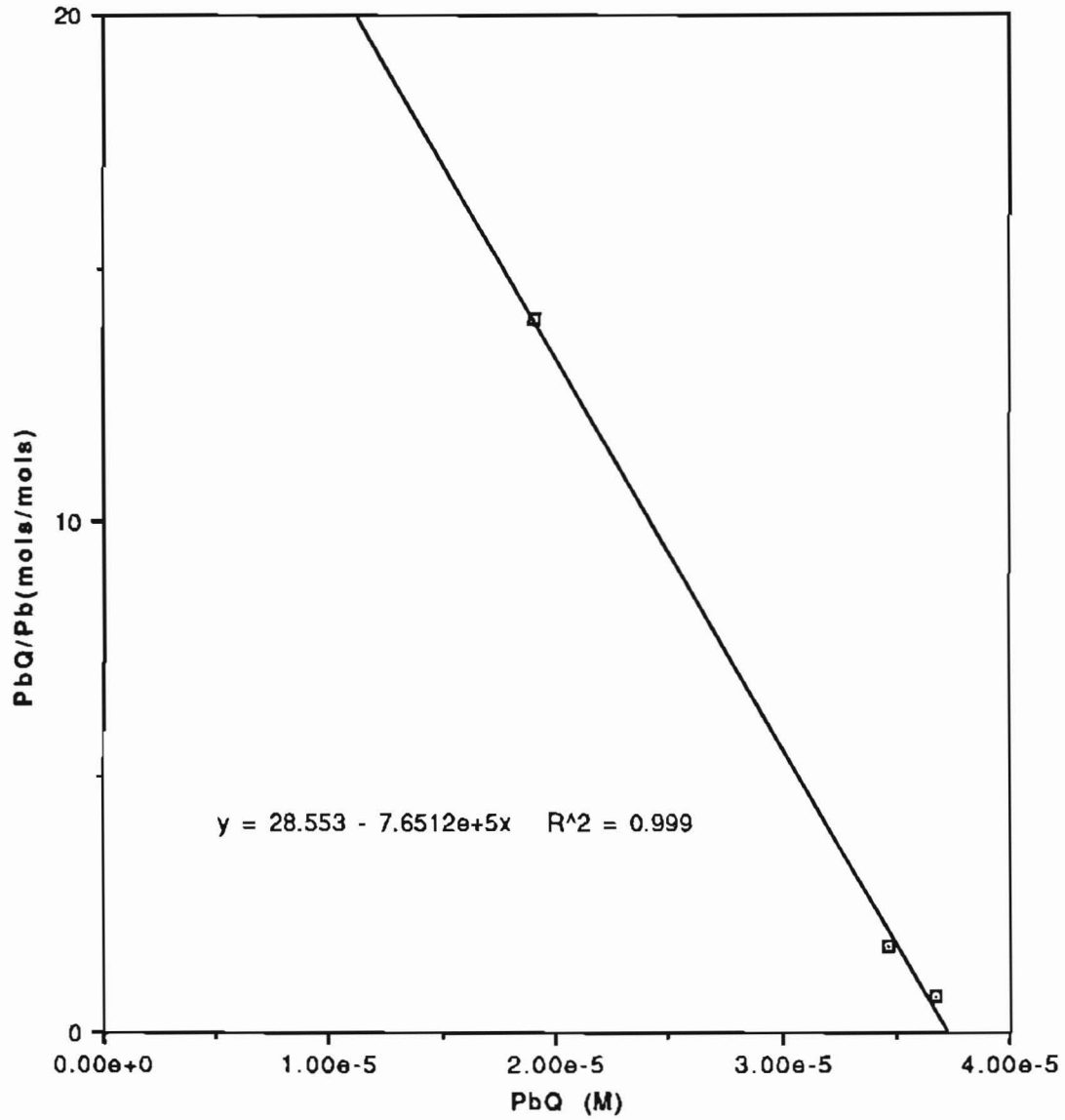

20
10
0
PbQ/Pb(mols/mols)
y = 28.553 - 7.6512e+5x R^2 = 0.999
0.00e+0
1.00e-5
2.00e-5
3.00e-5
4.00e-5
PbQ (M)

Table II.
Summary of results for the Scatchard determinations of K_{Cu} and K_{Pb} for F8HQ.

	Cu(II) pH 7.532	Cu(II) pH 11.963	Pb(II) pH 6.209	Pb(II) pH 12.333
Scatchard Slope (L/mol)	$5.675e^5$	$3.066e^5$	$1.068e^6$	$7.651e^5$
Scatchard y-intercept	16.57	9.006	18.19	28.55
K (L/mols)	$1.42e^9$	$1.52e^9$	$4.78e^9$	$3.00e^9$
pK (L/mol)	9.15	9.18	9.68	9.68*
average pK (L/mol)	9.17		9.68	
% difference in pK's	0.3%		0%	
γ (mols/L)	$2.92e^{-5}$	$2.94e^{-5}$	$1.70e^{-5}$	$3.73e^{-5}$
solution volume (L)	0.04	0.03	0.05	0.025
γ (mols)	$1.17e^{-6}$	$8.82e^{-7}$	$8.52e^{-7}$	$9.33e^{-7}$
average γ (mols)	$9.60e^{-7}$			
γ standard deviation (mols)	$1.44e^{-7}$			

* at an estimated $[Pb(TRIS)_4]^{+2}$ pK of 14.2

CHAPTER 5

Part 1: Before proceeding to analyze natural samples, the applicability of the constants derived in the last chapter needed to be verified. This was done experimentally by determining the metal recovery from the column as a function of pH, and comparing these recoveries to what is predicted by the model. Samples were pre-concentrated using a computer interfaced continuous flow pre-concentration system built in 1991. Figure 22 shows this automated system in detail.

The pre-concentration system was operated as follows: 100 ml volumes of a solution of $1e^{-5}$ M $Cu(NO_3)_2$ and $1e^{-5}$ M $Pb(NO_3)_2$ in $2.5e^{-3}$ M TRIS and HOAc were run through a 100 µL F8HQ column at 10 ml/min. The same column eluent, column wash solutions, blanks, and ICP-AES standard concentrations used in the previous experiments were used for this verification experiment.

As can be seen in figure 23, the predicted recoveries are much higher than the actual recoveries. The observed Cu(II) curve is very broad with a maximum recovery of about 69%, and the observed Pb(II) curve is sharp with a maximum recovery of about 64%. The difference between observed and predicted recoveries could very likely be due to kinetics; the various TRIS-metal cations are bulky, and they might not have had enough time to chelate to the column in the time that they passed through. The fact that the Cu(II) curve is broad and the Pb(II) curve is sharp is consistent with the relative sizes of the metal atoms. Pb has more surface area with which to spread out TRIS ligands thereby exposing itself to more F8HQ. Cu(II) as a smaller atom, in comparison, would have much slower exchange kinetics as a result of being surrounded by TRIS ligands. The

Figure 22.
Schematic for the continuous flow pre-concentration system. The bold lines are compressed air pipes, the solid thin lines are aqueous solution pipes, and the dotted lines represent electrical leads. The electrical schematic for the black box is in appendix 11, and the computer program controlling the system is in appendix 12.

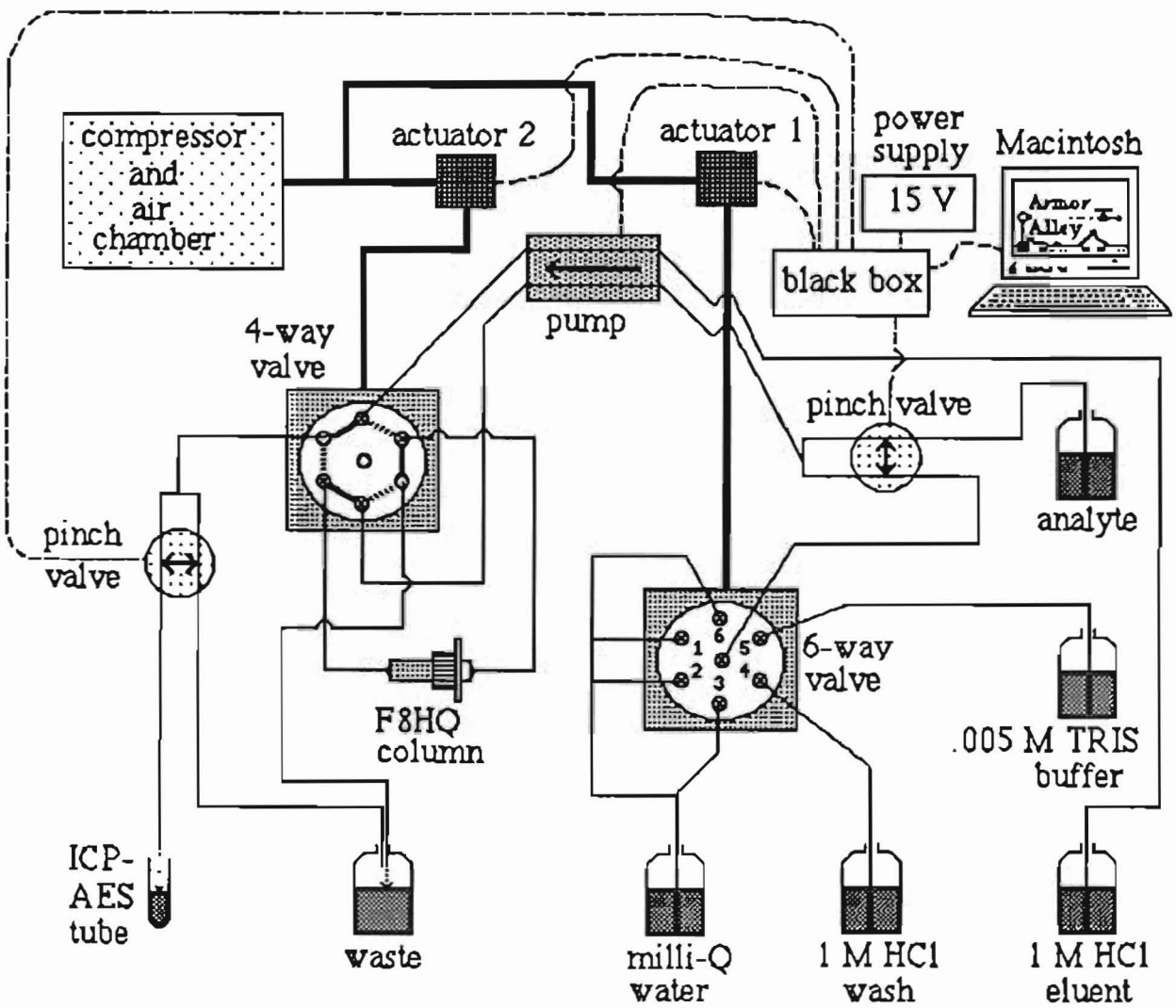
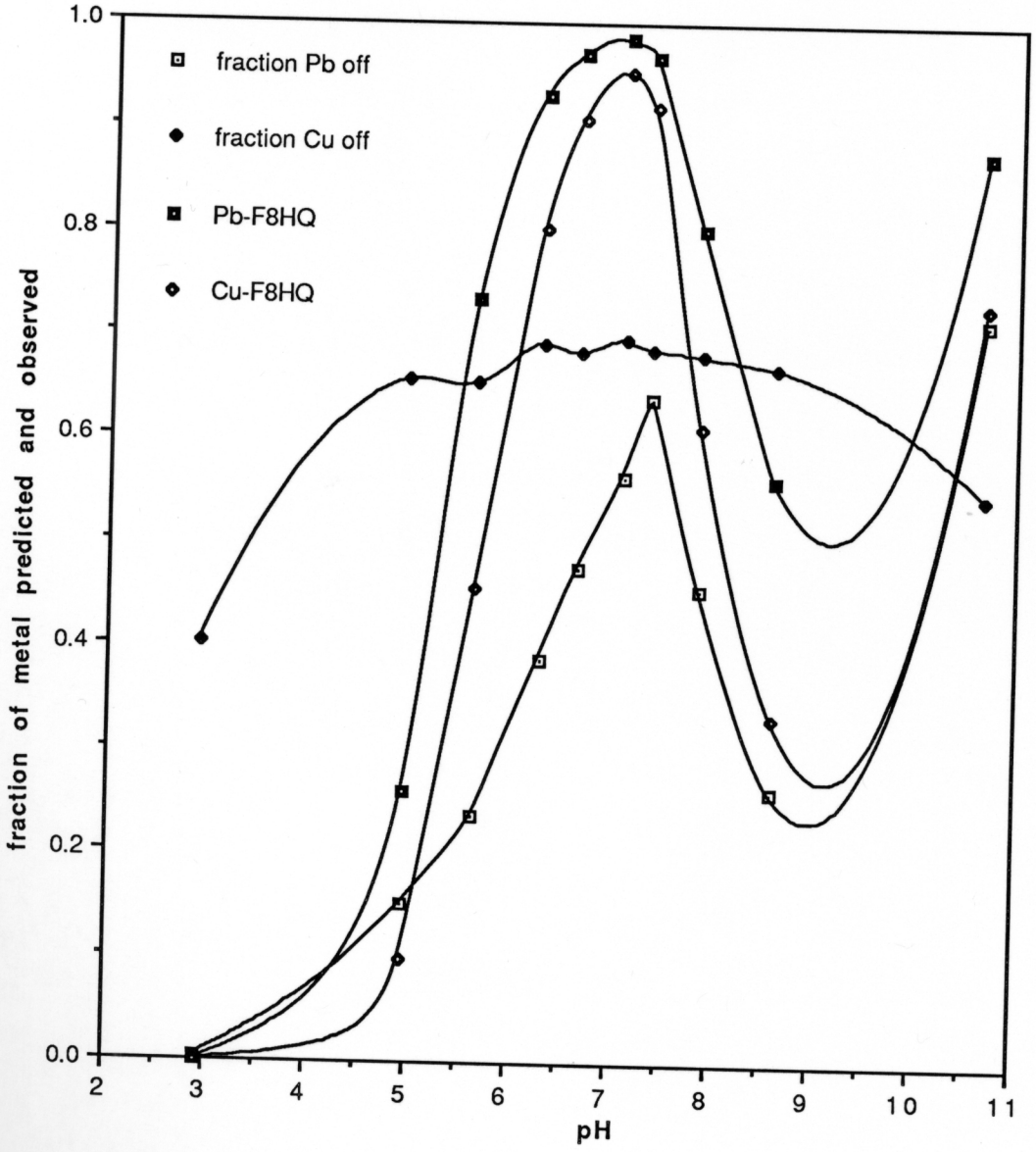

compressor and air chamber
actuator 2
actuator 1
power supply
15 V
Macintosh
black box
pump
4-way valve
pinch valve
analyte
pinch valve
6-way valve
F8HQ column
.005 M TRIS buffer
ICP-AES tube
waste
milli-Q water
1 M HCl wash
1 M HCl eluent

Figure 23.
Theoretical and observed amounts of Cu(II) and Pb(II) coming off a 100 μL F8HQ column vs. pH. Pb-F8HQ and Cu-F8HQ denote the theoretical curves. $1e^{-5}$ M Cu(II) and Pb(II) in $2.5e^{-3}$ M TRIS and HOAc. $3e^{-6}$ M F8HQ.


fraction Pb off
fraction Cu off
Pb-F8HQ
Cu-F8HQ
fraction of metal predicted and observed
pH
1.0
0.8
0.6
0.4
0.2
0.0
2
3
4
5
6
7
8
9
10
11

upward bend of the predicted recovery curves after pH 9 is consistent with F8HQ shifting the equilibrium toward metal chelation at higher pH. For the same reasons as above, Pb(II) complexes would have fast exchange kinetics and therefore follow the shape of the predicted recovery curve. Cu(II) complexes don't have an adequate rate of ligand exchange in the flow system to reach equilibrium with F8HQ, and thus the observed shape of the curve is different from the predicted shape after pH 9.

The reduced recovery of metal is disappointing, but the fact that the most metal came off at the pH predicted for maximum recovery in each case makes the technique useful. It shows that the derived constants might not provide quantitative recoveries, but they do give the optimal pH for pre-concentration of the metals on the F8HQ column. This is extremely important for efficient analysis of natural water samples since the other option to determine optimal pH involves multiple time-consuming standard additions. By generating speciations for natural water systems using the derived pK's, these standard additions can be avoided.

Part 2: The application of the characterized column to natural samples is perhaps the most important part of this research because it shows whether or not the method works. Seawater taken from Penobscot Bay, Maine, was the natural sample chosen for analysis. A knowledge of the chemical species in seawater that could possibly react with Cu(II) and Pb(II), the most common form of these metals in seawater, was necessary to create a speciation model. The derived constants for these metals with the column material are also necessary for the model.

The only ligand included in the Cu(II) model was carbonate (CO_3^{-2}). It is well known that the predominant aqueous Cu(II) species in seawater is

aqueous $CuCO_3$ [8]. Thus, the theoretical model for Cu(II) recovery off the F8HQ column includes only this neutral, non-chelating species. Copper Bicarbonate ($CuHCO_3^+$) is not included in the speciation because it is bound by the column as a cation. As seen in figure 24, the predicted recovery for Cu(II) at the natural pH of the seawater collected is approximately 71%. This recovery is located within a plateau region of pH's, all of which would give the same result. Since Cu(II) would start to precipitate at pH's above 8.5 in seawater, it would not be wise to increase the pH of the solution before pre-concentration in hopes of a higher recovery. Consequently, the pre-concentration of Cu(II) should be done at this seawater's natural pH. The case will be the same with Pb(II) below.

Pb(II) in seawater has a more complicated speciation because it is not clear whether carbonate or chloride is the most important ligand for Pb(II) in seawater. D. R. Turner et. al. [9] used periodic trends to estimate stability constants of many metal complexes. Fitting $PbCO_3$ to $CdSO_4$, they determined the lead-carbonate pK to be 7, thus making this species the dominant form of Pb(II) in seawater. However, work by R. H. Byrne, which accounts for the special chemical conditions of seawater, resulted in a $PbCO_3$ pK of 3 [10]. Furthermore, continued work of Byrne and W. L. Miller showed lead-chloride species to be the most important forms of Pb(II) in seawater - specifically, aqueous $PbCl_2$ is the predominant form and $PbCl_3^-$ and $PbCl^+$ are less prevalent [11]. Only $PbCl_2$ is included in the speciation, however, since it is the only important species which does not bind to the column.

Based on these conflicting constants, two different Pb(II) speciation models are presented in figures 25 and 26. Only speculation as to the truth is possible at this point because so many parameters are involved in

Figure 24.
α of aqueous Cu(II) species vs.pH.
Theoretical speciation of Cu(II) in seawater.
$6e^{-4}$ M CO_3^{-2} in seawater, $1e^{-5}$ M Cu(II),
and $3e^{-6}$ M F8HQ.

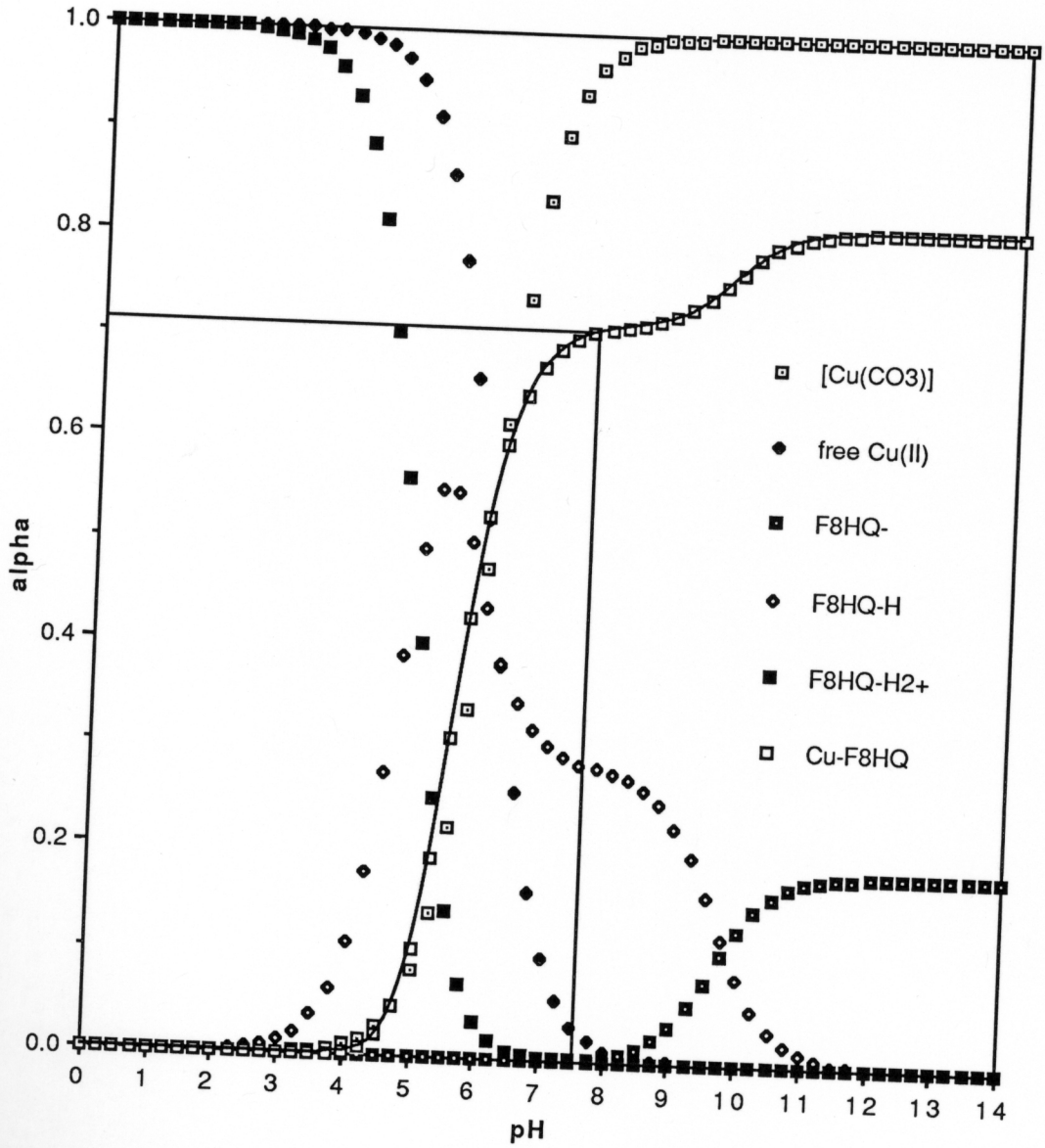

1.0
0.8
0.6
0.4
0.2
0.0
alpha
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH
[Cu(CO3)]
free Cu(II)
F8HQ-
F8HQ-H
F8HQ-H2+
Cu-F8HQ

Figure 25.
α of aqueous Pb(II) species vs pH.
Theoretical speciation of Pb(II) in seawater
based on Turner et. al. (pK $PbCO_3$ = 7).
$6e^{-4}$ M CO_3^{-2}, $1e^{-5}$ M Pb(II), $3e^{-6}$ M F8HQ.

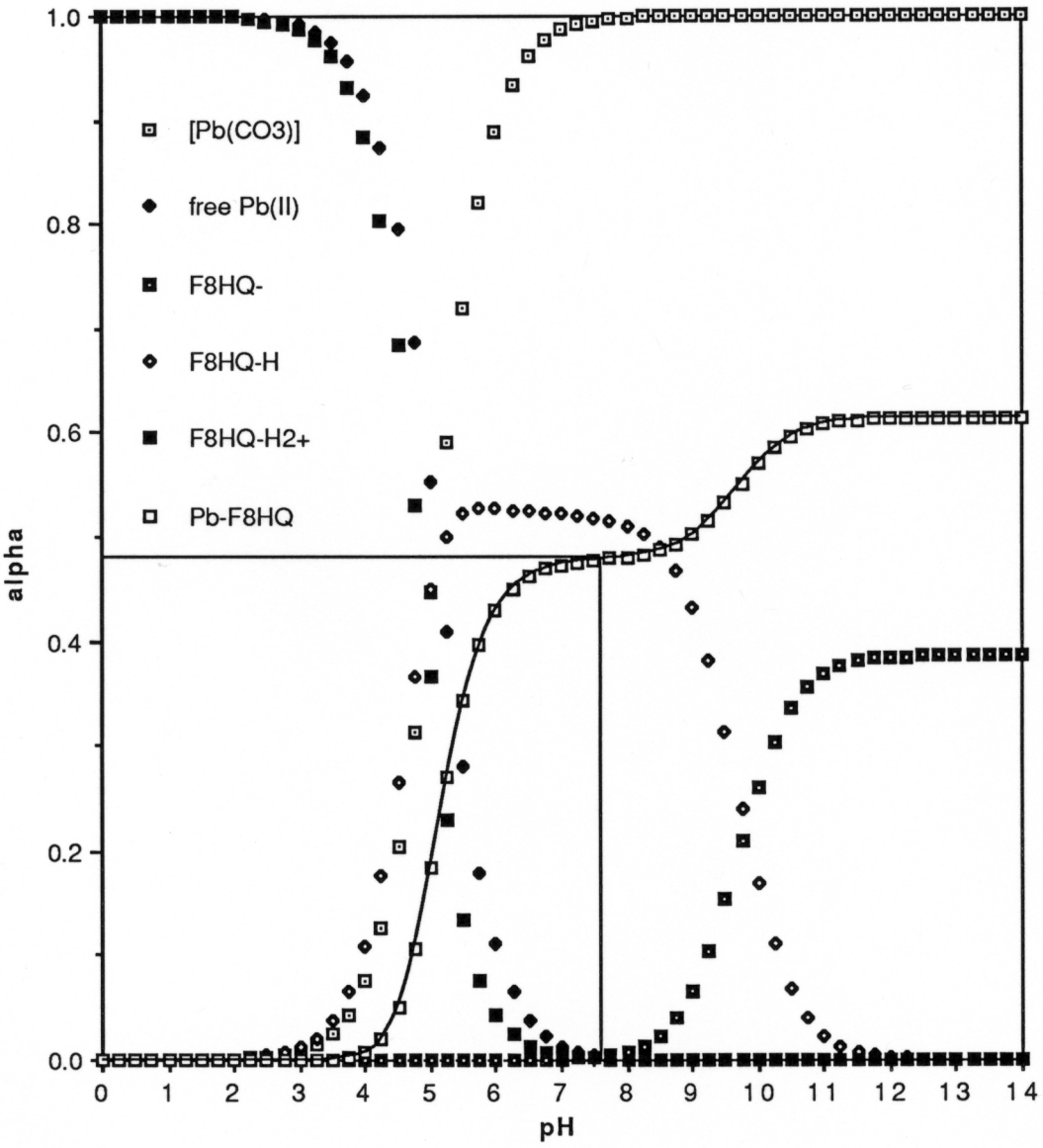
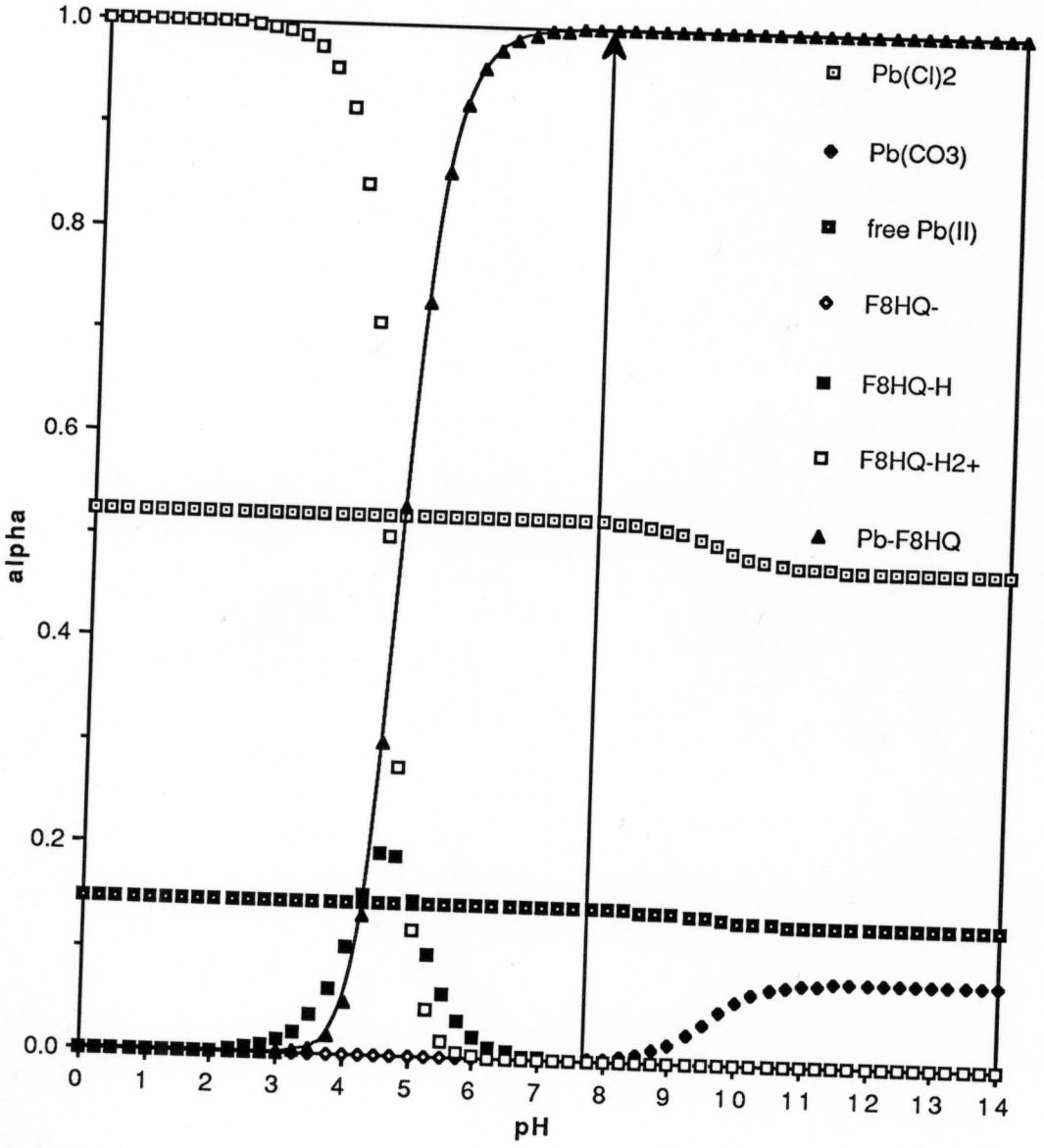

1.0
0.8
0.6
0.4
0.2
0.0
alpha
[Pb(CO3)]
free Pb(II)
F8HQ-
F8HQ-H
F8HQ-H2+
Pb-F8HQ
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pH

Figure 26.
α of aqueous Pb(II) species vs. pH
Theoretical speciation of Pb(II) in seawater
based on Byrne and Miller (pK $PbCO_3$ = 3).
0.52 M Cl^-, $1e^{-5}$ M Pb(II), $3e^{-6}$ M F8HQ.


1.0
0.8
0.6
0.4
0.2
0.0
alpha
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
pH
Pb(Cl)2
Pb(CO3)
free Pb(II)
F8HQ-
F8HQ-H
F8HQ-H2+
Pb-F8HQ

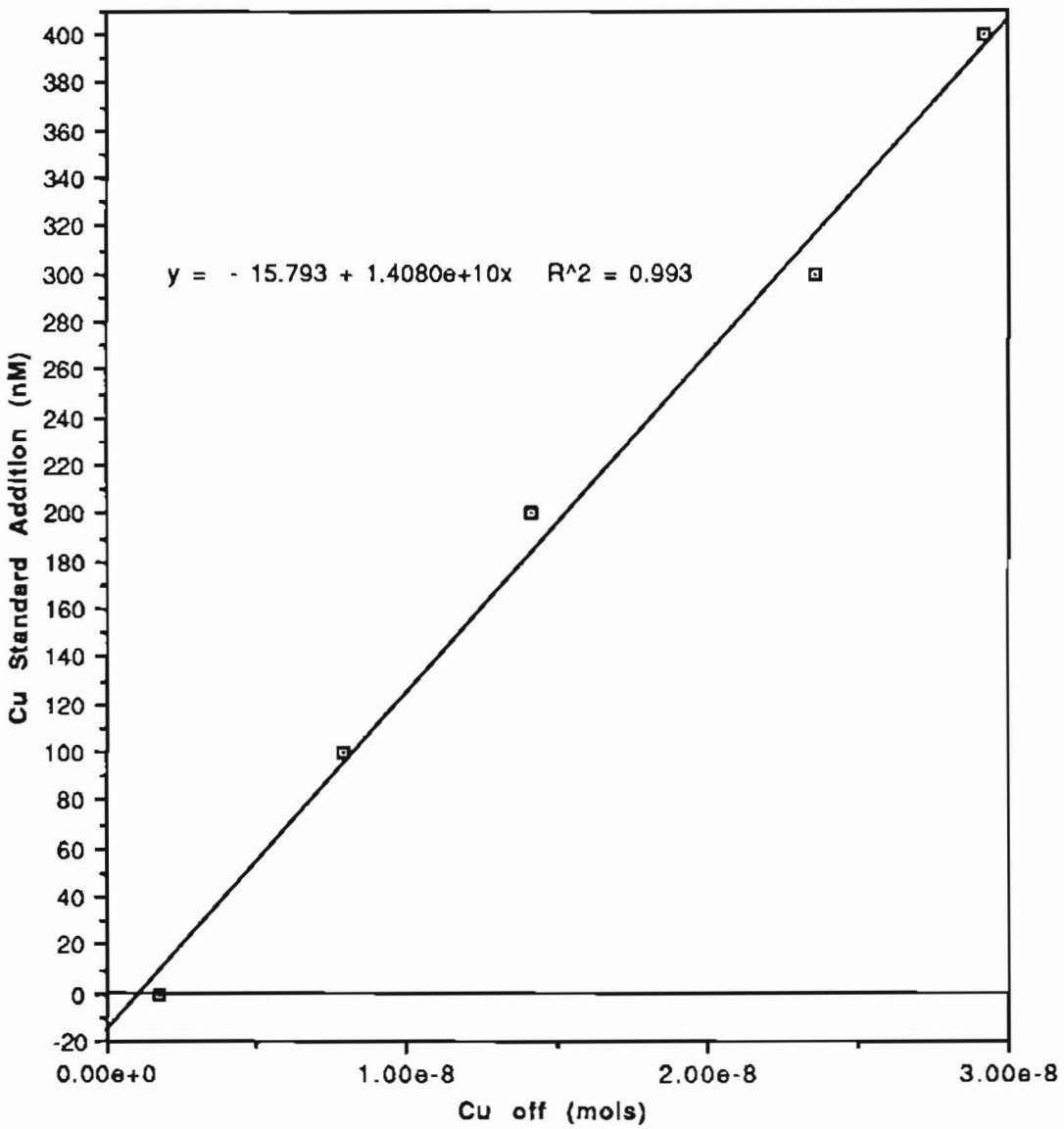
seawater speciation. The model based on Turner's work predicts an optimal pre-concentration pH similar to that for Cu(II), but again to avoid precipitation, it shows that the Pb(II) should be loaded at the natural pH (7.553) of this seawater. Byrne and Miller, however, predict 100% recovery at this pH.

The Penobscot Bay seawater was analyzed by the characterized F8HQ column for Cu(II) and Pb(II). The R/V Friendship of the Maine Maritime Academy was used to collected samples in go-flo bottles at depths of 5, 10, 15, 20, and 25 meters at 44° 21' 55" N latitude 68° 49' 30" W longitude. The samples were stored in acid/milli-Q washed Nalgene bottles. The salinity of the samples was approximately 32% and their pH was 7.553. The computerized continuous flow system was again used to pre-concentrate all standard additions and other samples in this analysis. This system minimized any contamination of the samples since all solutions were enclosed in Teflon bottles or tubes at all times.

In order to determine the recovery of the column at the pH of seawater, standard additions ranging from 20 to 450 nM Cu and 7 to 150 nM Pb were made to 100 ml volumes of seawater. These standard additions were pre-concentrated at pH 7.553, eluted with 1 M HCl from the F8HQ column in known volumes between 5 and 8 ml, and analyzed by ICP-AES. The standard addition curves presented in figures 27 and 29 allow the concentrations of Cu(II) and Pb(II) in the seawater to be determined. By adding these concentrations to each of the original standard addition concentrations, and by knowing the total volume of the sample being loaded, one can calculate the number of moles of metal being run through the column. A plot of moles of metal put on the column vs. moles observed coming off the column was used to determine the recovery

Figure 27.
Standard addition of Cu(II) (nM) vs. Cu(II) coming off the column (nmols).

Figure 28.
Cu(II) put through the column (nmols) vs. Cu(II) coming off the column (nmols). Recovery slope.


y = - 15.793 + 1.4080e+10x R^2 = 0.993
Cu Standard Addition (nM)
Cu off (mols)
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
0.00e+0
1.00e-8
2.00e-8
3.00e-8

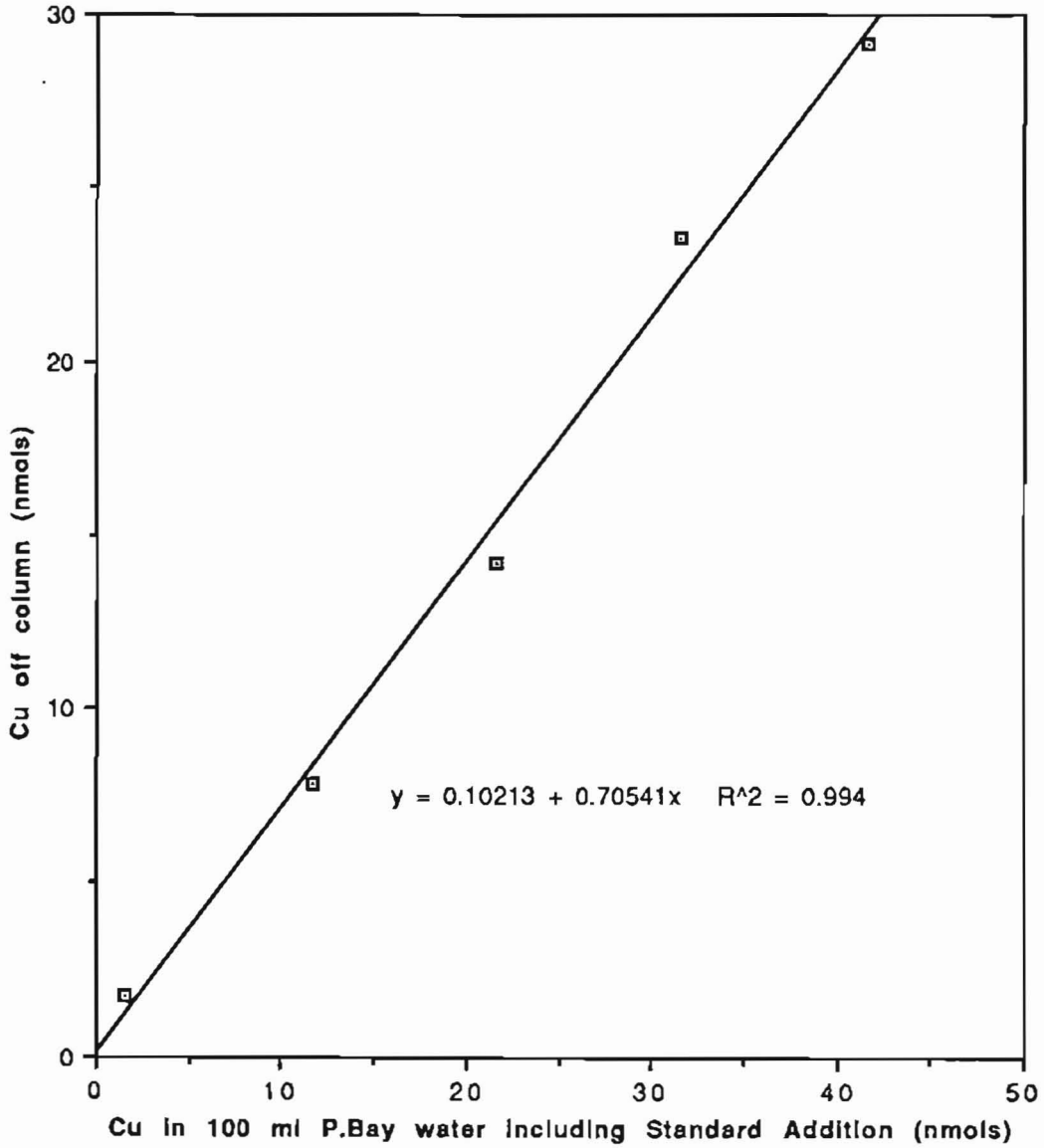
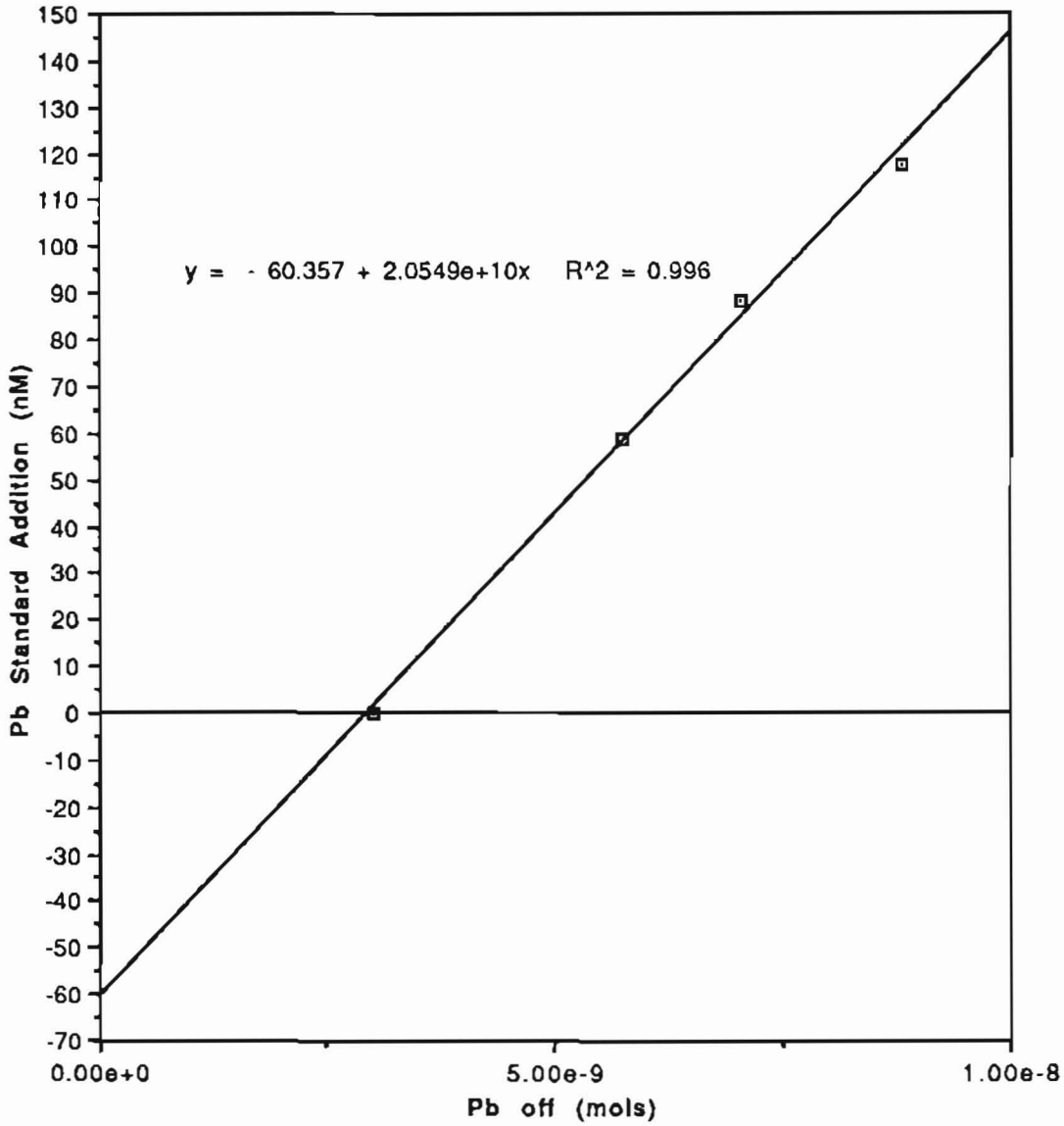
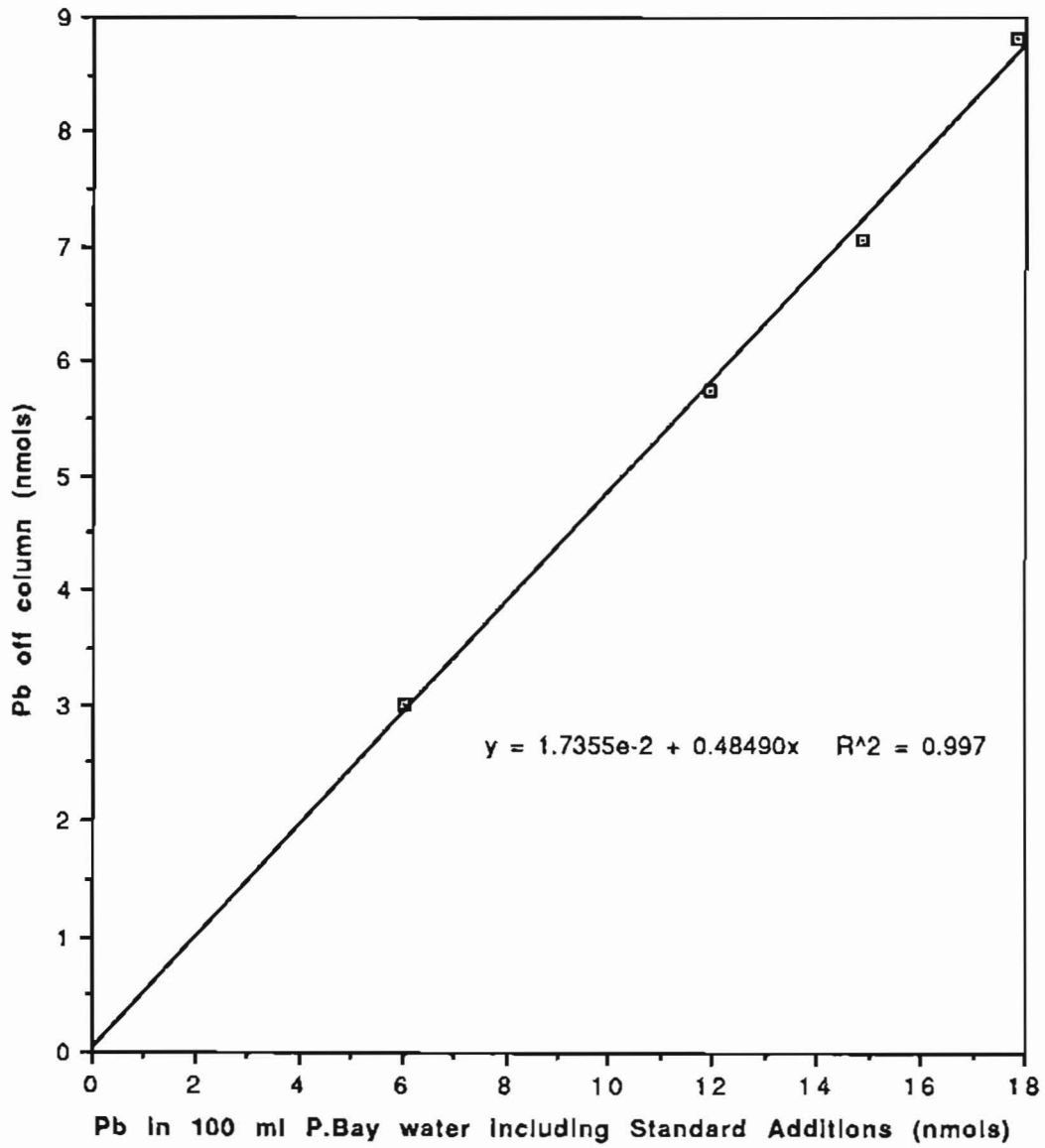

30
20
10
0
Cu off column (nmols)
y = 0.10213 + 0.70541x R^2 = 0.994
0
10
20
30
40
50
Cu in 100 ml P.Bay water including Standard Addition (nmols)

Figure 29.
Standard addition of Pb(II) (nM) vs. Pb(II) coming off the column (nmols).

Figure 30.
Pb(II) put through the column (nmols) vs. Pb(II) coming off the column (nmols). Recovery slope.


y = - 60.357 + 2.0549e+10x R^2 = 0.996
Pb Standard Addition (nM)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
0.00e+0
5.00e-9
1.00e-8
Pb off (mols)


Pb off column (nmols)
Pb in 100 ml P.Bay water including Standard Additions (nmols)
y = 1.7355e-2 + 0.48490x R^2 = 0.997
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
12
14
16
18

of each metal at pH 7.553. The recovery curves are figures 28 and 30.

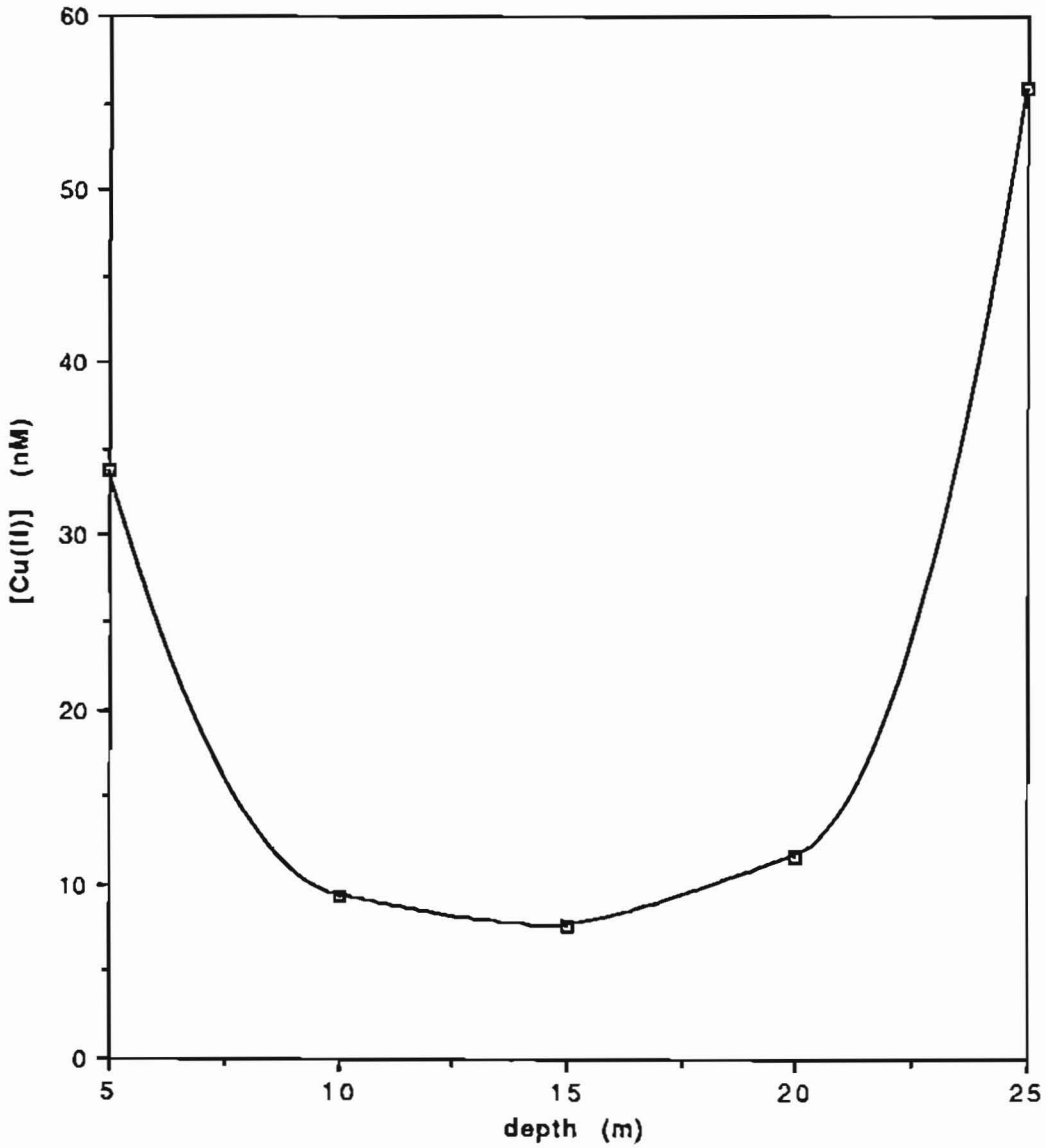
The recovery predicted by the speciation for Cu(II) agrees remarkably well with what is observed: 71% and 70.5% respectively. The observed recovery for Pb(II), 48.5%, is consistent with $PbCO_3$ being the most important Pb(II) species in seawater as Turner would argue. But it is not consistent with the 100% Cu(II) recovery predicted by Byrne and Miller. The conclusion that can be drawn from this work is that the model is a good qualitative tool for prediction of the best metal pre-concentration conditions. Quantitative information, however, cannot be obtained for metal recovery unless the system in question has well understood chemistry for all species present. Conflicting values for $PbCO_3$ pK's from Turner and Byrne is an example of the problem faced in creating an accurate model. Organic ligand speciation of metals is another complication not considered in this work.

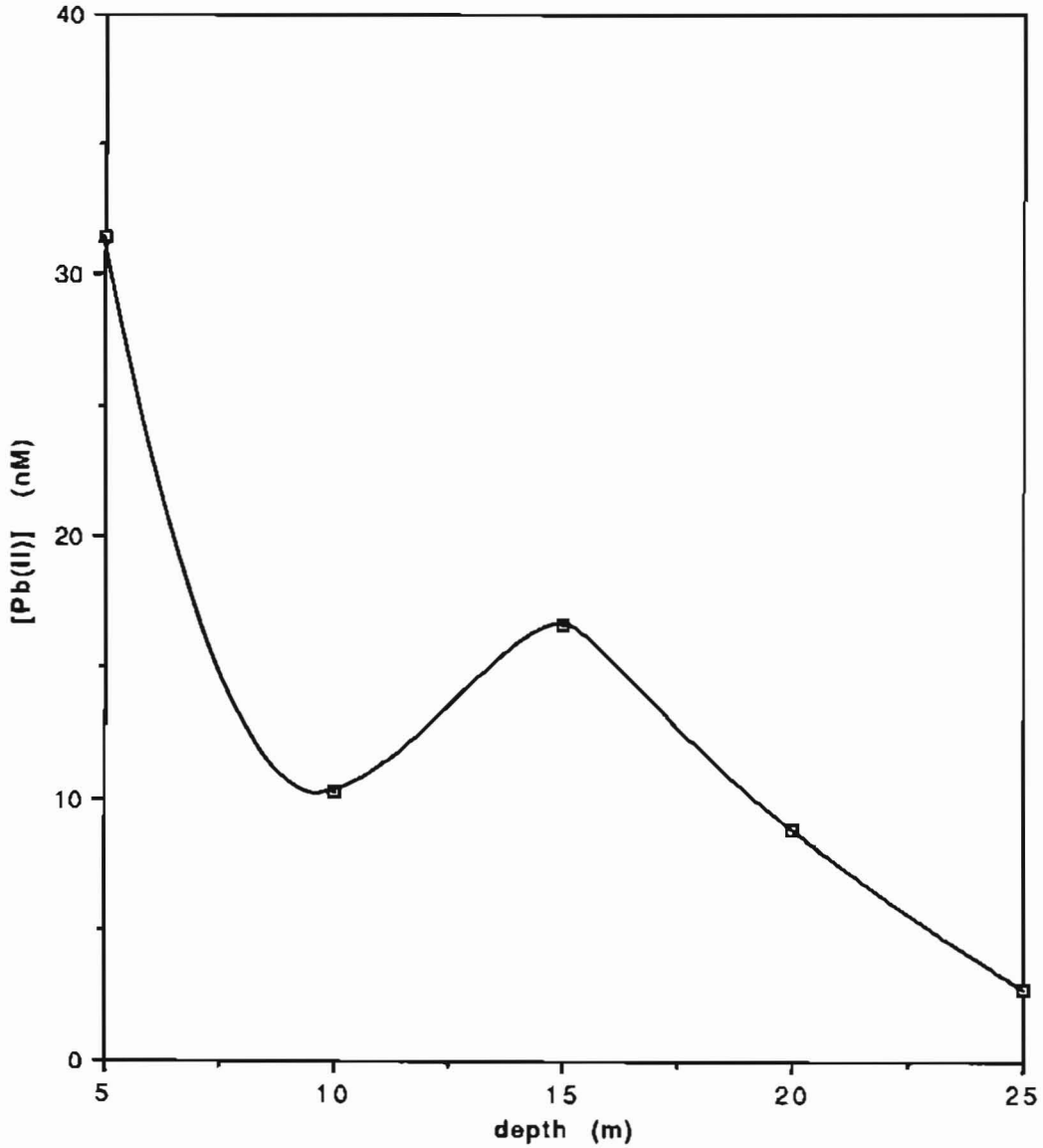
The experimental recoveries, 70.5% for Cu(II) and 48.5% for Pb(II), were used to determine the concentrations of these metals in Penobscot Bay seawater at several depths. The results are graphically depicted in figures 31 and 32 and the data are listed in appendix 10. The Cu(II) concentration is highest near the surface of the water and near the sediment at 25 m depth. Pb(II) concentration, on the other hand, is highest near the surface and drops off erratically as the depth increases. This is not surprising since Pb-containing pollution from the air and land runoff is deposited on the water surface, but is quickly precipitated by organic species, hydroxide, and carbonate.

These results demonstrate that the characterized F8HQ column can readily be used for the determination of metals in the low nanomolar to high picomolar concentration range, provided enough natural sample is

Figure 31.
[Cu(II)] (nM) vs. depth (m) in
Penobscot Bay seawater.

Figure 32.
[Pb(II)] (nM) vs. depth (m) in
Penobscot Bay seawater.


60
50
40
30
20
10
0
[Cu(II)] (nM)
5
10
15
20
25
depth (m)


40
30
20
10
0
[Pb(II)] (nM)
5
10
15
20
25
depth (m)

pre-concentrated. The pre-concentration above was only for a volume of 100 ml of seawater. Now that F8HQ has been shown to work successfully in analysis of seawater, the method can be used to determine trace metal concentrations in other natural samples. The application of this method to Pb detection in tapwater is an example of an important application.

ACKNOWLEDGMENTS

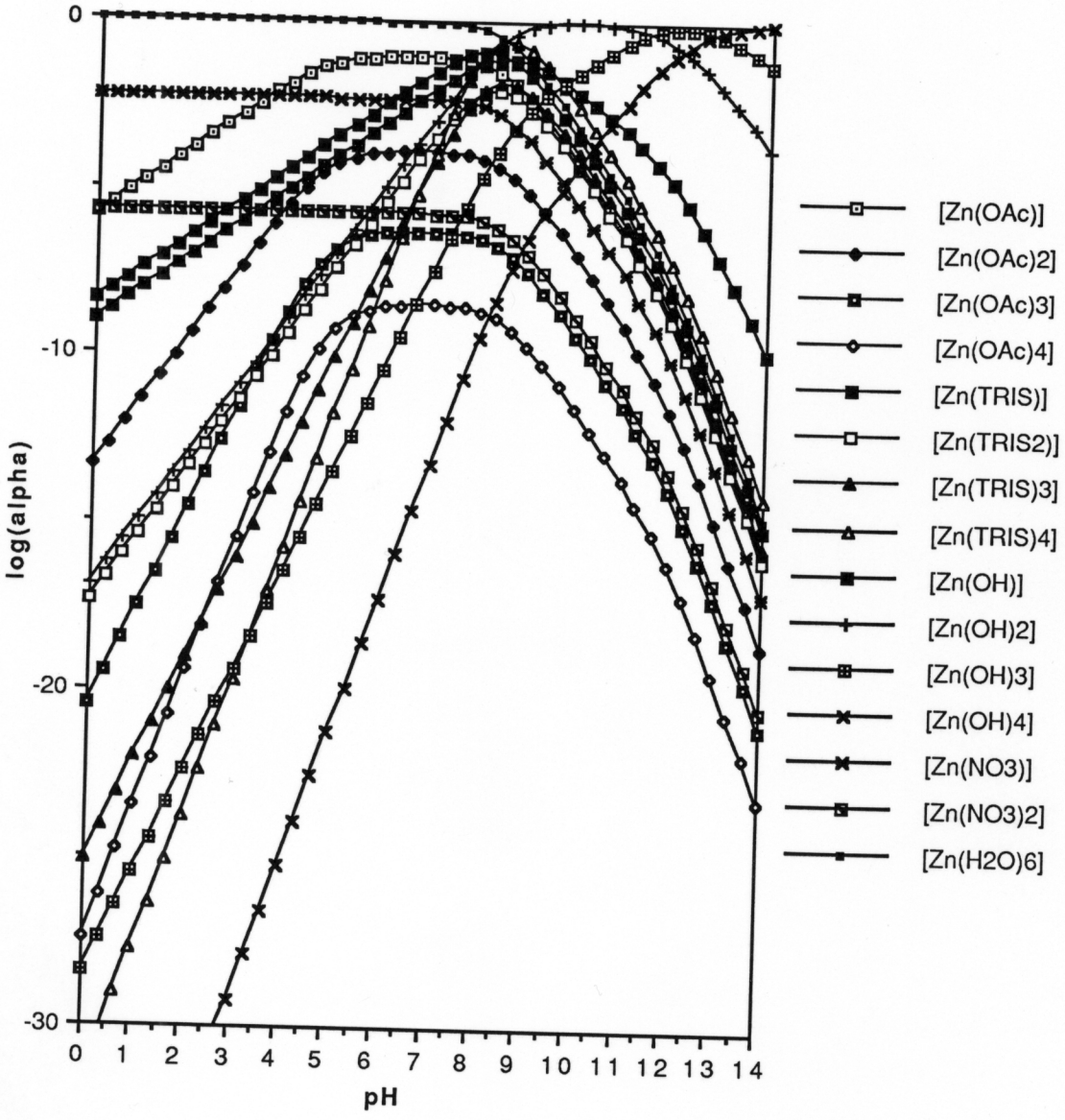
I would like to thank a number of people for their help in making this thesis possible. This project would have been impossible without the encouragement, enthusiasm, and patient support of Whitney King - thank-you. Thanks also to Wayne Smith and Dan Libby for their extra help in difficult problems. Mike Rooney, Matt Mackey, and Dave Anderson all started the project with me in 1991 and helped make the continuous-flow system and chelating ligand which I used in this research. Robb Aldrich helped to expand the electronics of the continuous-flow system in the summer of 1991. And thanks to Jocelyn Boucher and Paul the R/V Friendship captain at Maine Maritime Academy for helping with seawater sample collection.

APPENDIX 1

ligand	association	Cu^{+2} logK	Zn^{+2} logK	Pb^{+2} logK
OH^-	$ML/M.L$	6.3*	5.0*	6.3*
	$ML_2/M.L^2$	12.8***	11.1*	10.9*
	$ML_3/M.L^3$	14.5***	13.6*	13.9*
	$ML_4/M.L^4$	16.4*	14.8*	(15*)
TRIS	$ML/M.L$	3.95**	2.27**	(4**)
	$ML_2/M.L^2$	7.63**	(4**)	(8**)
	$ML_3/M.L^3$	11.10**	(7**)	(11**)
	$ML_4/M.L^4$	14.1**	(11**)	(14.2**)
OAc^-	$ML/M.L$	2.22*	1.57*	2.68*
	$ML_2/M.L^2$	3.63*	1.36****	4.08*
	$ML_3/M.L^3$	3.58****	1.57****	3.6****
	$ML_4/M.L^4$	3.3****	(2*)	2.9****
NO_3^-	$ML/M.L$	0.5*	0.4*	1.17*
	$ML_2/M.L^2$	-0.4*	-0.3*	1.4*
CO_3^{-2}	$ML/M.L$	6.75†		7.00† 3.00††
Cl^-	$ML/M.L$			0.9††
	$ML_2/M.L^2$			1.12††
	$ML_3/M.L^3$			1.0††

Approximations are in parentheses.
All values are at 25° C.
* @ $\mu = 0$
** @ $\mu = 0.1$
*** @ $\mu = 1.0$
**** @ $\mu = 3.0$
Constants for OH^-, TRIS, OAc^-, NO_3^- are from Smith and Martell [2].
Constants for CO_3^{-2} labelled † are from Turner, et. al. [9].
Constants for CO_3^{-2} labelled †† are from Byrne [10].
Constants for Cl^- are from Byrne and Miller [11].

APPENDIX 2


0
-10
-20
-30
log(alpha)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
pH
[Zn(OAc)]
[Zn(OAc)2]
[Zn(OAc)3]
[Zn(OAc)4]
[Zn(TRIS)]
[Zn(TRIS2)]
[Zn(TRIS)3]
[Zn(TRIS)4]
[Zn(OH)]
[Zn(OH)2]
[Zn(OH)3]
[Zn(OH)4]
[Zn(NO3)]
[Zn(NO3)2]
[Zn(H2O)6]

APPENDIX 3

Elemental Standards:

Pb: Lead, granular, ACS reagent, "Baker Analyzed" lot#90194

Cu: Copper, shot, source unknown

Acids and Bases:

HNO_3: Nitric Acid 70%, ICP-AES grade, "Baker Analyzed" lot#C09045

HCl: Hydrochloric Acid 37.8%, ICP-AES grade, "Baker Analyzed" lot#D21059

NaOH: Sodium Hydroxide 50%wt/wt, "Baker Analyzed" lot#E09039

Buffers:

Citric Acid Monohydrate, ACS reagent, "Baker Analyzed" lot#43137

TRIZMA Base: tris(hydroxymethyl)aminomethane, Reagent grade purity, "Sigma" lot#129F5620

CH_3COOH: Glacial Acetic Acid 99.7%, ACS Reagent, "Fisher" lot#754473

Pb(II) and Cu(II):

$Cu(NO_3)_2$: Cupric Nitrate, ACS Reagent, "Baker Analyzed" lot#44244

$Pb(NO_3)_2$: Lead Nitrate, ACS Reagent, "Mallinkrodt", lot#5744

APPENDIX 4

all pH measurements made by:	Orion Sure-flow Ross combination pH electrode, and Orion pH meter model SA 720.
all solution temperatures maintained by:	Neslab thermocirculator model RTE-220.
all flow systems powered by:	Cole-Parmer Masterflex model 7550-90 peristaltic pump and Rainin Rabbit model 55207 peristaltic pump.
all pure water for solutions was from:	SYBRON/Barnstead Nanopure II DI water system (purity to 18 MΩ).
all mass measurements were made on:	Mettler model College150 analytical balances.
all experimental metal concentrations (raw data) were determined by:	Leeman Labs PS-series 1000 inductively coupled plasma atomic emission spectrometer.
all speciation and other complicated calculations were made on:	Macintosh SE/30 computer.

APPENDIX 5

Tot. Cu(II)(M)	0.001		1 M HCl eluent blank (ppm)		0.039
solution pH	Cu(II) conc. after eluent blank correction (ppm)	amount Cu(II) chelated by the column (mols)	relative amount of Cu off the column	alpha(Cu(II)	alpha(F8HQ)
2.267	18.461	1.45E-06	0.13989739	0.99999972	6.5015E-11
3.004	22.261	1.75E-06	0.16869378	0.99999197	1.9171E-09
3.516	24.461	1.92E-06	0.18536537	0.99992141	1.9716E-08
3.971	27.661	2.18E-06	0.20961496	0.99947279	1.4952E-07
4.488	28.261	2.22E-06	0.21416176	0.99674613	1.308E-06
4.983	29.661	2.33E-06	0.22477096	0.98961964	8.0711E-06
5.489	32.161	2.53E-06	0.24371595	0.98137802	3.7789E-05
5.996	39.361	3.10E-06	0.29827752	0.97670011	0.00014181
6.332	46.761	3.68E-06	0.3543547	0.97486278	0.00032043
7.984	131.961	1.04E-05	1	0.00503069	0.01469617
8.518	102.961	8.10E-06	0.7802381	0.00069634	0.04855954
8.966	87.961	6.92E-06	0.66656815	0.00032972	0.12526717
9.429	81.561	6.42E-06	0.61806897	0.00024175	0.29373445
10.026	68.561	5.39E-06	0.51955502	0.00021256	0.62183654
10.49	59.861	4.71E-06	0.45362645	0.00020645	0.82717764
10.903	53.261	4.19E-06	0.40361167	0.00020452	0.92530598
11.513	45.961	3.62E-06	0.3482923	0.0002036	0.98056974
12.02	37.561	2.96E-06	0.28463713	0.0002034	0.99387184
12.48	29.561	2.33E-06	0.22401316	0.00020334	0.9978666
12.7	24.261	1.91E-06	0.18384977	0.00020332	0.99871341
Tot. Cu(II)(M)	0.0005		1 M HCl eluent blank (ppm)		0.116
3.002	22.084	1.74677E-06	0.75510204	0.99999204	1.8996E-09
4.982	29.284	2.31328E-06	1	0.98963727	8.0441E-06
10.905	24.584	1.94347E-06	0.84013605	0.00020451	0.92562365
12.703	23.084	1.82545E-06	0.78911565	0.00020332	0.99872225
Tot. Cu(II)(M)	0.00025		1 M HCl eluent blank (ppm)		0.116
3.004	17.784	1.40843E-06	0.72177419	0.99999197	1.9171E-09
4.978	24.684	1.95134E-06	1	0.98970775	7.9366E-06
10.906	21.284	1.68382E-06	0.86290323	0.00020451	0.92578201
12.702	20.784	1.64448E-06	0.84274194	0.00020332	0.99871931

APPENDIX 6

Experiment 1:		alpha(Cu(II))	0.0763
Cu(II) @ pH 7.532		alpha(F8HQ-)	0.00523

CS was only 94.8% of its real value throughout the experiment.

run#	[CuQ] in 5 ml after CS and blank correction (ppm)	Cu(II) chelated by the column (mols)	relative [Cu(II)] chelated by column	[CuQ] (M)	
6	0.15	1.1802E-08	0.01022495	3.353E-07	
5	1.393	1.0961E-07	0.09495569	3.0278E-06	
4	8.844	6.9587E-07	0.60286299	1.8706E-05	***
3	13.16	1.0355E-06	0.89706885	2.7107E-05	***
2	14.01	1.1024E-06	0.95501022	2.8121E-05	***
1	14.67	1.1543E-06	1	2.8713E-05	

run#	[Cu] in 5 ml after CS and blank correction (ppm)	total Cu(II) in solution after dilution corrections (mols)	[Cu(II)] in solution (M)	CuQ/Cu(II) (mols/mols)	
6	0.0333	9.2229E-08	2.6201E-06	0.12796888	
5	0.0229	6.5227E-08	1.8018E-06	1.6803783	
4	0.0399	1.1679E-07	3.1395E-06	5.9584445	***
3	0.29	8.7165E-07	2.2818E-05	1.18794006	***
2	0.579	1.7859E-06	4.5558E-05	0.61726763	***
1	0.941	2.9764E-06	7.4041E-05	0.38780592	

The data in the asterisked rows were used to generate the Scatchard plot.

APPENDIX 7

Experiment 2:		alpha(Cu(II))	0.000203
Cu(II) @ pH 11.963		alpha(F8HQ-)	0.993

CS was 128.3% of its real value throughout the experiment.

run#	[CuQ] in 5 ml after CS and blank correction (ppm)	Cu(II) chelated by the column (mols)	relative [Cu(II)] chelated by column	[CuQ] (M)	
6	0.09	7.08148E-09	0.008783058	2.8213E-07	
5	1.997	1.5713E-07	0.194886308	6.0203E-06	***
4	7.877	6.19787E-07	0.768712794	2.287E-05	***
3	9.557	7.51975E-07	0.932663219	2.6761E-05	***
2	10.247	8.06266E-07	1	2.7707E-05	***
1	9.717	7.64564E-07	0.948277545	2.5401E-05	

run#	[Cu] in 5 ml after CS and blank correction (ppm)	total Cu(II) in solution after dilution corrections (mols)	[Cu(II)] in solution (M)	CuQ/Cu(II) (mols/mols)	
6	0.0106	2.17685E-08	8.34041E-07	0.77949073	
5	0.1726	3.68037E-07	1.35807E-05	7.21824622	***
4	0.3366	7.44222E-07	2.64848E-05	1.68420446	***
3	0.6536	1.49653E-06	5.14273E-05	1.01041819	***
2	0.9776	2.31531E-06	7.69207E-05	0.53875548	***
1	1.3676	3.23897E-06	0.000107607	0.33022087	

The data in the asterisked rows were used to generate the Scatchard plot.

APPENDIX 8

Experiment 3:		alpha(Pb(II))	0.934
Pb(II) @ pH 6.209		alpha(F8HQ-)	0.000239

CS was 140.0% of its real value throughout the experiment.

run#	[PbQ] in 5 ml after CS and blank correction (ppm)	Pb(II) chelated by the column (mols)	relative [Pb(II)] chelated by column	[PbQ] (M)	
8	1.067	2.5748E-08	0.02827464	5.9602E-07	
7	2.047	4.9397E-08	0.05424385	1.1176E-06	
6	10.837	2.6151E-07	0.28717174	5.7856E-06	***
5	28.037	6.7657E-07	0.74295784	1.4644E-05	***
4	31.437	7.5861E-07	0.83305509	1.6072E-05	***
3	35.637	8.5997E-07	0.9443517	1.7842E-05	
2	37.737	9.1064E-07	1	1.8509E-05	
1	32.837	7.924E-07	0.87015396	1.5785E-05	

run#	[Pb] in 5 ml after CS and blank correction (ppm)	total Pb(II) in solution after dilution corrections (mols)	[Pb(II)] in solution (M)	PbQ/Pb(II) (mols/mols)	
8	0.008	8.3398E-09	1.9305E-07	3.08738426	
7	0.005	5.333E-09	1.2066E-07	9.26244344	
6	0.02	2.1815E-08	4.8263E-07	11.9878319	***
5	0.221	2.4639E-07	5.333E-06	2.74597951	***
4	0.768	8.7475E-07	1.8533E-05	0.86723716	***
3	1.478	1.7191E-06	3.5666E-05	0.50024144	
2	2.198	2.6096E-06	5.3041E-05	0.34895914	
1	3.138	3.8013E-06	7.5724E-05	0.20845236	

The data in the asterisked rows were used to generate the Scatchard plot.

APPENDIX 9

Experiment 4:		alpha(Pb(II))	0.000256
Pb(II) @ pH 12.333		alpha(F8HQ-)	0.997

CS was 112.4% of its real value throughout the experiment.

run#	[PbQ] in 5 ml after CS and blank correction (ppm)	Pb(II) chelated by the column (mols)	relative [Pb(II)] chelated by column	[PbQ] (M)	
5	0.313	7.5531E-09	0.00822732	3.8341E-07	
4	2.014	4.86E-08	0.0529387	2.3478E-06	
3	17.344	4.1853E-07	0.45589318	1.9024E-05	***
2	34.544	8.3359E-07	0.90800126	3.4733E-05	***
1	38.044	9.1805E-07	1	3.6722E-05	***

run#	[Pb] in 5 ml after CS and blank correction (ppm)	total Pb(II) in solution after dilution corrections (mols)	[Pb(II)] in solution (M)	PbQ/Pb(II) (mols/mols)	
5	0.0192	9.1274E-09	4.6332E-07	0.82751692	
4	0	0	0	0	
3	0.0562	2.9836E-08	1.3562E-06	14.0278227	***
2	0.8452	4.895E-07	2.0396E-05	1.70294999	***
1	2.1752	1.3123E-06	5.249E-05	0.69959544	***

The data in the asterisked rows were used to generate the Scatchard plot.

APPENDIX 10

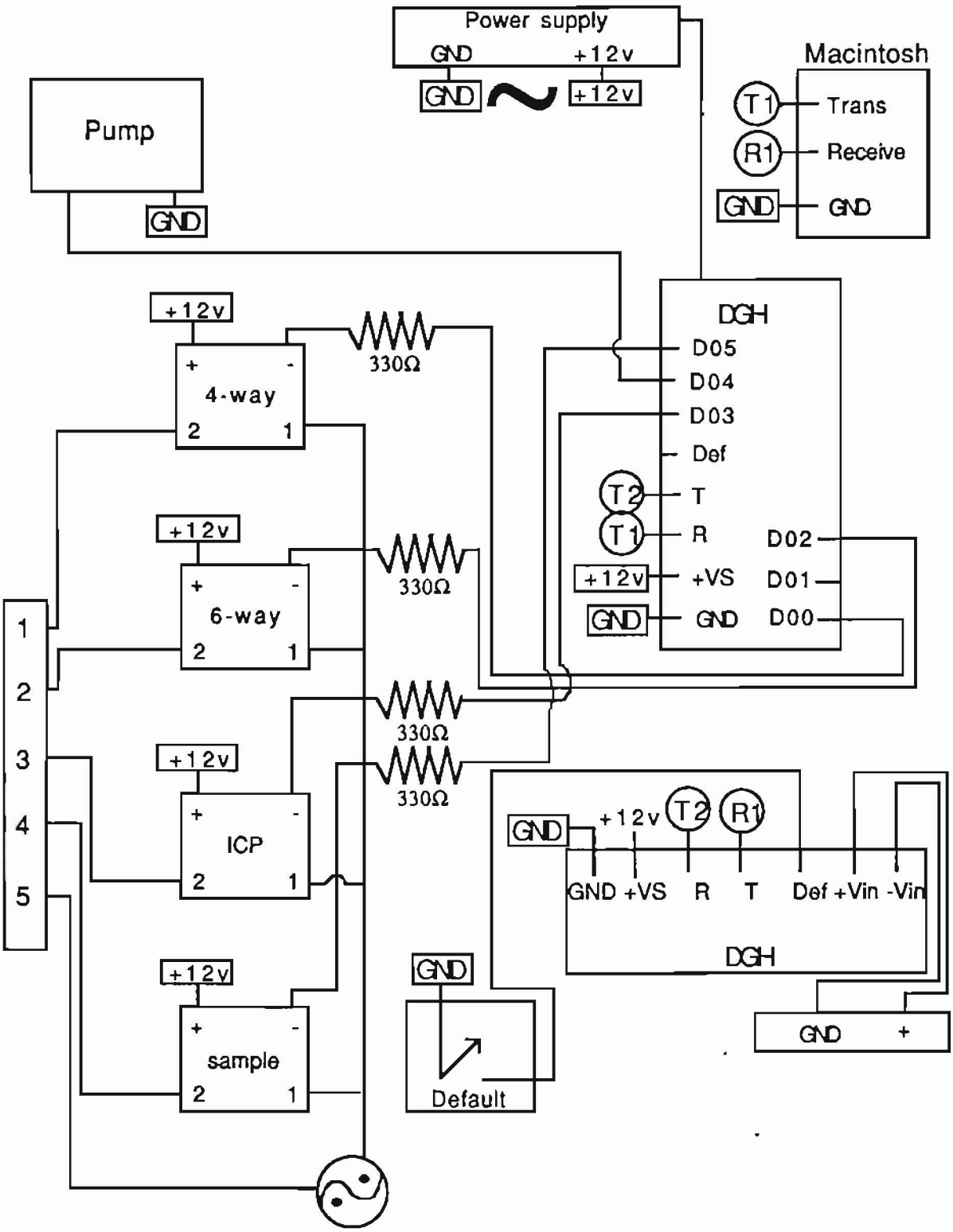
Cu(II) eluent blank (ppb)	19.6
Pb(II) eluent blank (ppb)	19

recovery of Cu by the column at pH 7.553	70.50%
recovery of Pb by the column at pH 7.553	48.50%

depth (m)	observed Cu(II) (ppb)	observed Pb(II) (ppb)	Cu coming off the column (mols)	Pb coming off the column (mols)
5	42.3	66.5	4.4266E-09	2.1343E-09
10	26.1	34.9	2.6697E-09	1.0948E-09
15	25.1	45.6	2.4884E-09	1.3865E-09
20	27.5	32.3	2.8778E-09	1.0367E-09
25	58.1	23.2	5.9429E-09	7.278E-10

true Cu coming off the column (mols)	true Pb coming off the column (mols)	[Cu] in P Bay seawater (nM)	[Pb] in P Bay seawater
6.2789E-09	4.4006E-09	62.7891606	44.0059905
3.7868E-09	2.2574E-09	37.8683627	22.5739362
3.5297E-09	2.8587E-09	35.2969302	28.5873502
4.082E-09	2.1374E-09	40.8203763	21.3743383
8.4297E-09	1.5006E-09	84.2970067	15.0061696

APPENDIX 11


Power supply
GND
+12v
GND
+12v
Macintosh
T1
Trans
R1
Receive
GND
GND
Pump
GND
+12v
330Ω
4-way
2
1
DGH
D05
D04
D03
Def
T2
T
T1
R
D02
+12v
+VS
D01
GND
GND
D00
+12v
330Ω
6-way
2
1
1
2
3
4
5
330Ω
+12v
330Ω
ICP
2
1
GND
+12v
T2
R1
GND +VS R T Def +Vin -Vin
DGH
+12v
sample
2
1
GND
Default
GND
+

APPENDIX 12

```
REM Trace Metal Control
REM Whitney King, Winter 1991

DIM sequence(100,7), byte$(4,2)
Snum = 0
xx$ = "9600": REM Communication Baud Rate
xx$ = "com1:"+xx$+", N, 8, 1"
OPEN "I",#1,xx$
OPEN "O", #2,xx$

dataacqr = 2

functionlist:
update$ = "T"
CLS: PRINT"Option List": PRINT
PRINT "1. DGH Module Setup"
PRINT "2. Set Digital Out"
PRINT "3. Read Command Sequence"
PRINT "4. Execute Command Sequence"
PRINT "5. Initialize Pump System"
PRINT "6. Read Single Data Point"
PRINT "7. TBDef"
PRINT "8. TBDef"
PRINT "9. Quit"
PRINT:PRINT
INPUT "Select Option Number"; function
IF (function>0) AND (function<10) THEN GOTO s2
GOTO functionlist
s2:
ON function  GOSUB setup, SetOut, ReadCom, ExCom, InitSys, readone,
TBdef, TBdef, stopit
GOTO functionlist
end.

REM default mode
setup:
CLS:PRINT:PRINT
PRINT"                          DGH Communications Setup Procedure"
PRINT"                             Please ground the DEFAULT PIN "
INPUT "                                (hit RETURN when ready)",x$
CLOSE #1
```

```
CLOSE #2
OPEN "I",#1,"com1:300, N, 8, 1"
OPEN "O", #2,"com1:300, N, 8, 1"
changesetup:
PRINT #2,"$1RS"
INPUT #1, a$
FOR I = 1 TO 4
byte$(I,1) = MID$(a$,I*2,2)
NEXT I
CLS:PRINT:PRINT:PRINT
PRINT "                              Current values for SetUp Bytes (HEX)"
PRINT
FOR I = 1 TO 4
PRINT "                                   ", I,byte$(I,1)
NEXT I
PRINT
INPUT "Select the byte you want to change or 9 to exit", xx
IF xx = 9 THEN GOTO endsetup
PRINT "Enter a new value for byte ";xx;
INPUT byte$(xx,1)
PRINT #2, "$1WE"
INPUT #1, a$
PRINT #2, "$1SU";byte$(1,1);byte$(2,1);byte$(3,1);byte$(4,1)
INPUT #1,a$
GOTO changesetup
endsetup:
CLS:PRINT:PRINT:PRINT "                         SetUp complete remove
default ground"
INPUT "Hit any key to continue", a$
IF byte$(2,1) = "01" THEN xx$ = "19200"
IF byte$(2,1) = "02" THEN xx$ = "9600"
IF byte$(2,1) = "03" THEN xx$ = "4800"
IF byte$(2,1) = "04" THEN xx$ = "2400"
IF byte$(2,1) = "05" THEN xx$ = "1200"
IF byte$(2,1) = "06" THEN xx$ = "600"
IF byte$(2,1) = "07" THEN xx$ = "300"
xx$ = "com1:"+xx$+", N, 8, 1"
CLOSE #1
CLOSE #2
OPEN "I",#1,xx$
OPEN "O", #2,xx$
RETURN
```

```
REM Set Digital Out
SetOut:
CLS:PRINT:PRINT:
INPUT "Enter Hex String to output to DGH",x$
PRINT #2,"$1DO"+x$
INPUT #1, a$
INPUT #1, a$
PRINT a$
PRINT
INPUT "Hit 'r' to return, any other key to change output";x$
IF x$ = "r" THEN RETURN ELSE SetOut
RETURN

REM Read Command Sequence
ReadCom:
filename$ = FILES$(1)
OPEN "I", #3, filename$
INPUT #3, Snum
PRINT Snum
FOR I = 1 TO Snum
INPUT #3, sequence(I,1), sequence(I,2), sequence(I,3),
sequence(I,4),sequence(I,5),sequence(I,6),sequence(I,7)
NEXT I
CLS:PRINT:PRINT
PRINT "Number of Steps  ";Snum
PRINT "Time    ";"Valve 1      ";"Valve 2      ";"Valve 3      "; "Pump
";"Acquire Data  ";"Sample Valve"
FOR I = 1 TO Snum
PRINT sequence(I,1);"    ";sequence(I,2);"      ";sequence(I,3);"
";sequence(I,4);"      ";sequence(I,5);"      ";sequence(I,6);"
";sequence(I,7)
NEXT I
INPUT "Hit any key to continue", b$
CLOSE #3
RETURN

REM Execute Command Sequence
ExCom:
GOSUB openfile
TIME$ = "00:00:00"
CLS
sequence(0,6) = 0
PRINT "Execute Command Sequence"
```

```
PRINT
PRINT "Time    ";"Valve 1 ";"Valve 2 ";"Valve 3 ";"Pump   ";"Acquire
Data  ";"Sample Valve"
FOR I = 1 TO Snum
PRINT sequence(I,1);"    ";sequence(I,2);"    ";sequence(I,3);"
";sequence(I,4);"    ";sequence(I,5);"    ";sequence(I,6)
loopa:
IF sequence(I-1,6) = 1 THEN GOSUB readdata
Tt$ = TIME$
timesec=
VAL(MID$(Tt$,1,2))*3600+VAL(MID$(Tt$,4,2))*60+VAL(MID$(Tt$,7,
2))
IF timesec > sequence(I,1) THEN GOTO continue ELSE GOTO loopa
continue:
hc =  0 OR sequence(I,2)*1 OR sequence(I,3)*4 OR sequence(I,4)*8 OR
sequence(I,5)*16 OR sequence(I,7)*32
hc$ = HEX$(hc)
IF LEN(hc$) = 1 THEN PRINT #2, "$1DO0" + hc$ ELSE PRINT #2,
"$1DO" + hc$
INPUT #1,a$
INPUT #1,a$
NEXT I
CLOSE #3
RETURN

REM initialize Valves and Pumps
InitSys:
delay = 5000
PRINT #2, "$1DO04"
INPUT #1,a$
INPUT #1,a$
INPUT "What is the position of the six position valve (1-6) "; position
IF position = 1 THEN steps = 0
IF position = 2 THEN steps = 5
IF position = 3 THEN steps = 4
IF position = 4 THEN steps = 3
IF position = 5 THEN steps = 2
IF position = 6 THEN steps = 1
FOR j = 1 TO steps
PRINT #2, "$1DO00"
INPUT #1,a$
INPUT #1,a$
FOR l = 1 TO delay: NEXT l
```

```
PRINT #2, "$1DO04"
INPUT #1, a$
INPUT #1,a$
FOR r = 1 TO delay: NEXT r
NEXT j
CLS:PRINT:PRINT
PRINT "Turn on the power to the Pump. CAUTION the pump is about to
start"
INPUT "(hit any key TO continue)", m$
PRINT:PRINT
PRINT #2,"$1DO14"
INPUT #1, a$
INPUT #1,a$
PRINT "Flushing #1, Hit any key when finished"
loop3:
IF INKEY$ = "" THEN GOTO loop3
PRINT "Flushing #2, Hit any key when finished"
PRINT #2, "$1DO10"
INPUT #1,a$
INPUT #1,a$
FOR m = 1 TO delay: NEXT m
PRINT #2, "$1DO14"
INPUT #1, a$
INPUT #1,a$
loop4:
IF INKEY$ = "" THEN GOTO loop4
PRINT "Flushing #3, Hit any key when finished"
PRINT #2, "$1DO10"
INPUT #1,a$
INPUT #1,a$
FOR n = 1 TO delay: NEXT n
PRINT #2, "$1DO14"
INPUT #1, a$
INPUT #1,a$
loop5:
IF INKEY$ = "" THEN GOTO loop5
PRINT "Flushing #4, Hit any key when finished"
PRINT #2, "$1DO10"
INPUT #1,a$
INPUT #1,a$
FOR n = 1 TO delay: NEXT n
PRINT #2, "$1DO14"
INPUT #1, a$
```

```
INPUT #1,a$
loop6:
IF INKEY$ = "" THEN GOTO loop6
PRINT "Flushing #5, Hit any key when finished"
PRINT #2, "$1DO10"
INPUT #1,a$
INPUT #1,a$
FOR n = 1 TO delay: NEXT n
PRINT #2, "$1DO14"
INPUT #1, a$
INPUT #1,a$
loop7:
IF INKEY$ = "" THEN GOTO loop7
PRINT "Flushing #6, Hit any key when finished"
PRINT #2, "$1DO10"
INPUT #1,a$
INPUT #1,a$
FOR n = 1 TO delay: NEXT n
PRINT #2, "$1DO14"
INPUT #1, a$
INPUT #1,a$
loop8:
IF INKEY$ = "" THEN GOTO loop8
FOR I = 1 TO 1
PRINT #2, "$1DO00"
INPUT #1, a$
INPUT #1,a$
FOR o = 1 TO delay: NEXT o
PRINT #2, "$1DO04"
INPUT #1, a$
INPUT #1,a$
FOR o = 1 TO delay: NEXT o
NEXT I
CLS:PRINT:PRINT
INPUT "Initialization Complete (Hit any key and RETURN to continue)",
m$
RETURN

REM quit
stopit:
END
RETURN
```

```
REM sub read data
readdata:
y=0
FOR n = 1 TO 4
PRINT #2,"$2ND"
INPUT#1,Q$
INPUT #1,b$
b$=MID$(b$,2,9)
b=VAL(b$)
y=y+b
NEXT n
y=y/4
PRINT #3, timesec,",",y
RETURN

REM sub read Single data point
readone:
y=0
FOR n = 1 TO 8
PRINT #2,"$2ND"
INPUT#1,Q$
INPUT #1,b$
b$=MID$(b$,2,9)
b=VAL(b$)
y=y+b
NEXT n
y=y/8
PRINT , timesec,y
INPUT "Hit 'r' to return, any other key for more data";x$
IF x$ = "r" THEN RETURN ELSE readone
RETURN

REM open file for Fluorometer data
openfile:
filename$ = FILES$(0)
OPEN "O", #3, filename$
RETURN
```

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