

Portland State University

PDXScholar

Dissertations and Theses

Dissertations and Theses

1-1-1982

The copper complexation properties of dissolved organic matter from the Williamson River, Oregon

Charles Russell Lytle
Portland State University

Follow this and additional works at: https://pdxscholar.library.pdx.edu/open_access_etds

Let us know how access to this document benefits you.

Recommended Citation

Lytle, Charles Russell, "The copper complexation properties of dissolved organic matter from the Williamson River, Oregon" (1982). *Dissertations and Theses*. Paper 815.
<https://doi.org/10.15760/etd.815>

This Dissertation is brought to you for free and open access. It has been accepted for inclusion in Dissertations and Theses by an authorized administrator of PDXScholar. Please contact us if we can make this document more accessible: pdxscholar@pdx.edu.

THE COPPER COMPLEXATION PROPERTIES OF DISSOLVED
ORGANIC MATTER FROM THE WILLIAMSON RIVER, OREGON

by

CHARLES RUSSELL LYTLE

A dissertation submitted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY
in
ENVIRONMENTAL SCIENCES AND RESOURCES: CHEMISTRY

Portland State University


1982


TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the dissertation of
Charles Russell Lytle presented August 10, 1982.


Edward M. Perdue, Chairperson


Dennis W. Barnum


Kwan Hsu


Joann S. Loehr


Richard R. Petersen


David K. Roe

APPROVED:


David W. McClure, Head, Department of Chemistry


Robert O. Tinnin, Coordinator, Environmental
Sciences and Resources Ph.D. Program


Stanley E. Rauch, Dean of Graduate Studies and Research

ACKNOWLEDGEMENTS

I would like to thank all the members of my committee for their time, criticisms, and concern over the past five years. Special thanks are due to Professor David Roe, who suggested the metal-ion buffer experiments and who was an invaluable resource person on all matters analytical, and Professor Mike Perdue, who thought up the whole project and directed the research.

Appreciation is extended to Karen Schwartzkoph, who typed the manuscript from a set of barely legible notes; to Dolores Oberson and Peg Pankratz, who smoothed over a plethora of administrative hurdles; to Dr. Rudy Parrish, who wrote the original versions of the LSTSQR and GAUSSM3 programs; and to all my graduate student friends.

Finally, the most important thank you is to Lynn C. Fox - fiance, friend, life companion - who shared all the joy and frustration, endured all the complaints and anxieties, understood the long hours and weekend work days, and provided the spiritual and physical support without which this research would have been a much more arduous task.

AN ABSTRACT OF THE DISSERTATION OF Charles Russell Lytle for the
Doctor of Philosophy in Environmental Sciences and Resources - Chemistry
presented August 10, 1982.

Title: The Copper Complexation Properties of Dissolved
Organic Matter from the Williamson River, Oregon

APPROVED BY MEMBERS OF THE DISSERTATION COMMITTEE:



Edward M. Perdue, Chairperson



Dennis W. Barnum



Kwan Hsu



Joann S. Loehr



Richard R. Petersen



David K. Roe

Recent research has indicated that dissolved organic matter (DOM) may play an important role in the ability of natural waters to complex metals. This research was conducted because the quantitative nature of this role is uncertain.

Gas-liquid chromatography was used to study the hydrolyzable amino acids at twelve sampling sites on the Williamson River at monthly intervals for two years. The relative abundances showed little spatial or temporal variation. The two-year averages for total amino acids ranged from about $0.5\text{ }\mu\text{M}$ to about $8\text{ }\mu\text{M}$. A separation technique was used to show that $\geq 96\%$ of the dissolved amino acids were associated with aquatic humus. Since it was found that amino acids contributed less than 1% to humic carbon and since a published report found that carbohydrates contributed less than 2% to humic carbon, this research provided the necessary data to conclude that DOM in the Williamson River is essentially aquatic humus.

Humus complexation capacity is often operationally defined as amount of metal bound per unit weight of humus. This research has shown that the titrimetric methods commonly used to obtain this parameter underestimate its magnitude. However, it was shown that these methods can be combined with acidic functional group analyses to determine upper and lower limit for this parameter. For Williamson River humus, the range was $7.2 - 15.4\text{ }\mu\text{moles}$ copper per mg humic carbon.

Titration of humus into a copper-oxalate metal-ion buffer enabled the determination of the copper-humus binding "constant" at humus: copper ratios found in the Williamson River, ≤ 4300 . The binding "constant" was a variable and a function of pH. At a humus: copper

ratio of 4300, the values of the function at pH 5.0, 5.5, 6.0, and 6.5 were: 3.0×10^6 , 8.9×10^6 , 3.0×10^7 , and 1.7×10^8 .

Current models of metal-humus complexation, were shown to be inappropriate via rigorous mathematical examination and via application to computer-simulated titrations. A model, in which it is assumed that the concentrations of binding sites in humus are normally distributed with respect to the log of the metal binding constant for each site, is proposed. Application of this model to simulated titrations and to experimental data proved it to be superior to other current models.

DEDICATION

This work is dedicated to the memory of my father, the late
Peter Edward Lytle.

We ask, "What is the answer? How can a dream be realized, any dream?" Perhaps the answer is if a dream is seen as a perfectly created material state, it is inevitably doomed to failure. Only when it is a striving toward an attainable goal can it be achieved. In short, what is attainable is spritual fulfillment and growth . . .

When I sit in my cabin watching the flames in my fireplace, it reminds me of countless fires I have built all over the North -- but even more, I remember that mankind has gazed into fires and dreamed his dreams for centuries. The longing for Hudson Bay is behind me, and that for all other explorations I have been on, but the great dream, that of finally growing into the vast world of comprehension and knowing, is still very much alive. This is the grandest dream of all.

At last I am beginning to believe I am part of all this life and to know how I evolved from the primal dust to a creature capable of seeing beauty. This is compensation enough. No one can ever take this dream away; it will be with me until the day I have seen my last sunset, and listened for a final time to the wind whispering through the pines."

-- Sigurd F. Olson

TABLE OF CONTENTS

| | <u>Page</u> |
|--|-------------|
| Acknowledgements | iii |
| List of Tables | viii |
| List of Figures | x |
| CHAPTER | |
| I INTRODUCTION | 1 |
| Trace Metal Speciation | 1 |
| Dissolved Organic Matter in Natural Waters | 6 |
| OVERALL PLAN OF THE RESEARCH | 9 |
| II CHOICE OF METHODS | 12 |
| Amino Acid Analysis | 12 |
| Copper-Aquatic Humus Interactions | 13 |
| EXPERIMENTAL | 18 |
| Reagents | 18 |
| Apparatus | 21 |
| Methods for Amino Acid Analysis | 25 |
| Methods for Complexation Capacity Study | 34 |
| Methods for Copper-Humus Stability Function Study | 37 |
| III CHEMICAL EQUILIBRIA IN MULTILIGAND MIXTURES | 40 |
| DISCRETE MULTILIGAND MODELS | 43 |
| CONTINUOUS MULTILIGAND MODELS | 49 |

| | |
|--|-----|
| | vii |
| IV RESULTS AND DISCUSSION | 53 |
| Amino Acid Study | 53 |
| The Application of the Two-Component Scatchard | |
| Equation to Defined Ligand Mixtures | 61 |
| The Application of the Continuous Distribution | |
| Model to Proton and Copper Binding by | |
| Aquatic Humus | 77 |
| The Complexation Capacity of Williamson | |
| River Humus | 88 |
| Copper-Aquatic Humus Stability Function | 97 |
| SUMMARY AND CONCLUSIONS | 119 |
| REFERENCES | 124 |
| APPENDICES | 138 |
| Appendix A: HATIT Program Listing | 138 |
| Appendix B: SMPLX Program Listing | 144 |
| Appendix C: LSTSQR and GAUSSM3 Program | |
| Listings | 149 |
| Appendix D: Amino Acid Raw Data | 169 |

LIST OF TABLES

| TABLE | | PAGE |
|-------|---|------|
| I | Desalting Scheme for 0.5 mL Dowex 50W-X4 Columns | 27 |
| II | Conditions for Gas-Liquid Chromatography | 28 |
| III | Standard Curves: ng Amino Acid versus Area Amino Acid/Area 30 ng GABA | 31 |
| IV | Raccoon Alpha-Hemoglobin, First 31 Residues | 34 |
| V | Mol Percent of Amino Acids Found in the Williamson River, Averaged Over Two Years | 53 |
| VI | Sensitivity of Scatchard Equation Parameters to Titration Range for the Gaussian Ligand Set | 65 |
| VII | Ligand Set for the MINEQL Simulated Copper Titration | 68 |
| VIII | Gaussian and Scatchard Fits for the Copper-Twenty Three Ligand MINEQL Simulated Titration | 69 |
| IX | Variation in Bimodal Gaussian Fitting Parameters with Changes in C_L | 81 |
| X | Comparison of Methods for Analyzing Complexation Capacity Data | 89 |
| XI | SMPLX Determinations of the Copper-oxalate Binding Constant | 97 |
| XII | Calculated and Found Free Copper Concentrations for a Copper-oxalate Metal Ion Buffer | 100 |
| XIII | Formation Reactions and Constants for the Copper- oxalate Metal Ion Buffer | 101 |
| XIV | Fitting Parameters for the Single and Bimodal Gaussian Modeling of the Copper Metal Ion Buffer Titrations | 108 |

| TABLE | PAGE |
|--|------|
| XV Comparison of Fitting Parameters from this Research and from "Calculated" Raw Data from the Literature | 115 |

LIST OF FIGURES

| FIGURE | PAGE |
|--|------|
| 1. Constant-Temperature Titration System | 24 |
| 2. Location of Sampling Sites | 26 |
| 3. Fractionation Scheme | 33 |
| 4. Typical Chromatogram: Total Amino Acids at Sampling Site KL-10, September, 1978 | 54 |
| 5. Seasonal Amino Acid Distribution at Selected Sampling Sites | 56 |
| 6. Two-Year Averages for Total Amino Acids and Humic Carbon | 57 |
| 7. Total Amino Acids Versus Humic Carbon for the December, 1978 Field Trip | 59 |
| 8. Discharge, Humic Carbon, and Total Amino Acids at Sprague River Over Two Years | 60 |
| 9. Amino Acid Concentrations (μM) in River Water Fractions | 62 |
| 10. Synthetically-Generated \bar{v} and Calculated Fits for the Gaussian Ligand Set Using the Two- Component Scatchard Equation | 64 |
| 11. Synthetically-Generated $\log \bar{K}'$ Values for the Gaussian Ligand Set and $\log \bar{K}'$ Values Calculated from the Two-Component Scatchard Equation | 66 |
| 12. Synthetically-Generated \bar{v} and Calculated Fits for the MINEQL Ligand Set Using the Scatchard and Gaussian Models | 70 |
| 13. Simulated $\log \bar{K}'$ Values and Calculated Fits for the MINEQL Ligand Set Using the Scatchard and Gaussian Models | 71 |

| FIGURE | PAGE |
|---|------|
| 14. Comparison of Discrete and Continuous Ligand Mixtures | 74 |
| 15. Variation of Scatchard Parameters with the Standard Deviation of a Continuous Distribution of Ligands | 75 |
| 16. Computed Values of \bar{K}' and K'_I and K'_{II} for a Simulated Metal Titration into a Continuous, Bimodal Gaussian Distribution | 78 |
| 17. Base-Titratable Functional Groups Versus pH for a 4 g/L Solution of Williamson River Humus in 0.1 M NaClO ₄ | 80 |
| 18. Experimental \bar{v} Values and Calculated Fit for the Bimodal Modeling of a Proton Titration of Williamson River Humus | 82 |
| 19. Bimodal Gaussian Distribution that Yielded the "Best" Fit for the Proton-Humus Titration | 84 |
| 20. Experimental \bar{v} Values and Calculated Fit Using the Gaussian Distribution Model for a Copper Titration into Williamson River Humus | 85 |
| 21. Data Constructed from the Scatchard Parameters of Sposito <i>et al.</i> (1979) and Calculated Fit Using the Gaussian Distribution Model | 86 |
| 22. Complexation Capacity Plots for pH 5 Titration #2 | 91 |
| 23. \bar{v} Versus C_M/C_L at Various C_L for Copper Binding to Oxalic Acid | 92 |
| 24. \bar{v} Versus pM for Copper Binding to Williamson River Humus at pH 5.0, 5.5, 6.0, and 6.5 | 94 |
| 25. Variability in Gaussian Fitting Parameters With Changes in C_L | 96 |
| 26. Copper ISE Response For $10^{-3} \text{ M} \geq [\text{Cu}] \geq 10^{-8} \text{ M}$ | 99 |
| 27. \bar{K}' Versus $\log C_L/C_M$ at pH 5.0 | 103 |
| 28. \bar{K}' Versus $\log C_L/C_M$ at pH 5.5 | 104 |

| FIGURE | PAGE |
|--|------|
| 29. \bar{K}' Versus $\log C_L/C_M$ at pH 6.0 | 105 |
| 30. \bar{K}' Versus $\log C_L/C_M$ at pH 6.5 | 106 |
| 31. \bar{K}' Versus $\log C_L/C_M$. One Titration Each at pH 5.0, 5.5, 6.0, and 6.5 | 107 |
| 32. Experimental Data and Calculated Fit for the Single Mode Gaussian Modeling of the pH 5.0 Metal Ion Buffer Titration #1 | 110 |
| 33. Experimental Data and Calculated Fit for the Single Mode Gaussian Modeling of the pH 5.5 Metal Ion Buffer Titration #1 | 111 |
| 34. Experimental Data and Calculated Fit for the Single Mode Gaussian Modeling of the pH 6.0 Metal Ion Buffer Titration #1 | 112 |
| 35. Experimental Data and Calculated Fit for the Single Mode Gaussian Modeling of the pH 6.5 Metal Ion Buffer Titration #2 | 113 |
| 36. $\log \bar{K}'$ versus $\log C_L/C_M$ for Gaussian Fits of Data from this Research and Data Calculated from the Literature | 116 |
| 37. Experimental Conditions of Total Metal and Total Ligand for this Research and for Literature Reports | 118 |

CHAPTER I

INTRODUCTION

Trace Metal Speciation in Natural Waters

The natural and anthropogenic occurrences of trace metals in the aquatic environment and their potentially toxic effects on biota have stimulated much current research on the chemistry of dissolved metals in natural waters. Of particular interest have been the interactions between metals and the various naturally-occurring ligands, because recent work indicates it is the chemical activity of a species of a given metal, not its stoichiometric concentration, that determines its biological effect. While aqueous metal complexation with common inorganic and simple organic ligands is well understood, the reactions between metals and dissolved organic matter (DOM) in natural waters are not well-characterized. This lack of knowledge is significant because dissolved organic carbon is second only to bicarbonate in abundance in the "world average river" (Livingstone, 1963; Schlesinger and Melack, 1981).

Johnston (1964) found that the growth of phytoplankton in sea water could be enhanced by the addition of a synthetic chelator. Barber and Ryther (1969) postulated that the enhanced growth of phytoplankton in certain upwelling sea water was due to an increase in nutrient trace metal solubility caused by the presence of undefined natural organic chelators released by the phytoplankton themselves. Spencer (1957) and

Erickson et al. (1970) showed that the presence of strong synthetic chelators such as EDTA and NTA reversed copper growth inhibition of selected test marine algae. Further, Stiff (1971) showed that copper toxicity was reduced by carbonate complexation as bicarbonate alkalinity was increased. Steemann-Nielsen and Wium-Anderson (1970) found that free copper ion, at the concentration found typically for total copper in natural waters, is toxic and concluded that copper is primarily complexed to organic matter, in which form its toxicity is lost. Their implication that the free metal ion activity is the critical parameter in toxicity studies was postulated by Gachter et al. (1973) and confirmed by the important work of Sunda and Guillard (1976) and Kaiser (1980).

The general term "complexation capacity" has evolved to describe the ability of natural waters to bind trace metals and thus decrease their toxic effects upon biota. Chau et al. (1973) used differential pulse anodic stripping voltammetry to measure the apparent complexing capacity of lake waters. Davey et al. (1973) used the sensitivity of the growth of Thalassiosira pseudonana to free copper ion activity to quantify the complexation capacity of sea water. Hanck and Dillard (1973) determined the complexation capacity of fresh waters by a novel cobalt complexation technique. Excess cobalt(II) was added to the sample, and the cobalt(II) complexes were oxidized to chemically inert cobalt(III) complexes. The excess cobalt(II) was then analyzed by differential pulse polarography. Kunkel and Manahan (1973) used a copper(II) solubilization technique followed by filtration and atomic absorption analysis to determine the complexation capacity of natural water and of waste

water. No attempt was made in the above studies to determine the identities of the complexing ligands.

The complex problem of identifying all the bound species of a given trace metal in the aquatic environment was soon found to be a function not only of properties of the water system such as pH, pE, types and concentrations of adsorbing surfaces and types and concentrations of organic and inorganic ligands (Stumm and Belinski, 1972; Vuceta and Morgan, 1978; Andrew et al., 1976) but also of the measurement techniques themselves (Gachter et al., 1973; Ernst et al., 1975; Campbell et al., 1977). McDuffie et al. (1976) showed that suspended solids adsorbed a large fraction of the trace metal load in a test river and that bottom sediments scavenged trace metals as their concentrations increased during low flow. This general result was also found by Pagenkopf and Cameron (1979). The importance of the sediment in the overall scheme of trace metal speciation was emphasized by Boyle et al. (1977) who postulated that adsorption onto oxide surfaces may control some trace metal concentrations in the world's oceans. The mechanisms of adsorption and current models have been recently discussed by Balistrieri and Murray (1979), Davis and Leckie (1979), and Westall and Hohl (1980).

Studies devoted exclusively to speciation by inorganic ligands include Pagenkopf et al. (1974) who found that the copper species toxic to fishes were Cu^{+2} and $\text{Cu}(\text{OH})^+$. Shaw and Brown (1974) concluded that CuCO_3 was as toxic as Cu^{+2} to rainbow trout. Andrew et al. (1977) found Cu^{+2} , $\text{Cu}(\text{OH})^+$, and $\text{Cu}_2(\text{OH})_2^{+2}$ were toxic to Daphnia magna. The literature dealing with the toxicities of the hydroxy and carbonate complexes of copper has been recently reviewed by Magnuson et al.

(1979), who conclude that the carbonate complexes are not toxic, that Cu^{+2} and the neutral and cationic copper-hydroxide complexes contribute 60-70% of the toxic effect and that anionic copper-hydroxide complexes are responsible for the remainder.

However, in a series of carefully controlled experiments on filtered river water, Sunda and Lewis (1978) found that inorganic complexes accounted for only 1.0% of total copper, that natural dissolved organic matter complexes were the dominant copper species, and that the organic complexes were not toxic to a test alga, Monochrysis lutheri. The former important finding was substantiated by Giesy et al. (1978), who found for lake waters in Maine that the observed binding capacity of the water for copper and lead was almost entirely due to organic constituents, and by Van den Berg and Kramer (1979). Baccini and Suter (1979), in a study of selected Swiss lakes, found that $\geq 95\%$ of the dissolved copper was complexed with organic ligands and that the presence of other cations, such as Ca^{+2} , Zn^{+2} , Cd^{+2} , in excess of copper, did not reduce the copper binding properties of the organic ligands. Even though the importance of dissolved organic matter in the trace metal chemistry of natural waters had been recognized before the above-mentioned studies (Hood, 1970; Reuter and Perdue, 1977), some researchers acknowledge and then dismiss the role of organic matter (Stumm and Belinski, 1972; Stumm and Morgan, 1970) while others employ simple model compounds to approximate DOM in the natural environment (Vucetta and Morgan, 1978).

Even when the cation exchange capacity (CEC) of suspended solids is considered, DOM is still an important part of the overall complexation

capacity of natural waters. For example, Livingstone (1963) reports a value of 120 mg total dissolved inorganic solids (TDS) per liter for the world average river. Garrells and Mackenzie (1971) have shown that total suspended inorganic solids (TSS) are about four times TDS and that particulate iron is about 30 mg Fe per liter. Subtracting these two values gives an estimate for suspended clays of 450 mg/L. An estimate for the CEC due to clays can be obtained from data given by Stumm and Morgan (1970). They report an average CEC for a 1:1 kaolinite:montmorillonite mixture of about 0.7 meq/g. Thus the CEC for clays in the world average river is about 0.32 meq/L. Similarly, Benjamin and Leckie (1981) report a CEC for iron of 1.0 meq/g. Thus the particulate iron contribution towards the CEC is about 0.03 meq/L, and the CEC for total suspended solids is about 0.35 meq/L. Schlesinger and Melack (1981) report total organic matter in the world average river as 20 mg/L. Wetzel (1975) has shown that DOM is approximately 90% total organic matter. Thus DOM is about 18 mg/L, and by subtraction, suspended organic matter (SOM) is about 2 mg/L. Beck et al. (1974) have found a CEC value of 10.0 meq/g for both DOM and suspended organic matter. Thus, in the world average river, the CEC for DOM is 0.20 meq/L and for suspended organics is 0.02 meq/L. The sum of all four fractions, clays, iron, DOM, and SOM is 0.57 meq/L., and DOM contributes 35% of the CEC of the world average river. Thus, for such an important constituent in the overall trace-metal speciation scheme for natural waters, detailed knowledge about the identity, concentration, and trace metal chemistry of dissolved organic matter is clearly warranted.

Dissolved Organic Matter in Natural Waters

One of the earliest studies on the nature of DOM in natural waters was conducted by Shapiro (1957). The extractable organic matter was in two main fractions, one yellow colored and one colorless. Both fractions gave infrared spectra indicative of a mixture of hydroxycarboxylates, both gave a positive test for phenol, and both exhibited resistance to oxidation. Their similarity to the organic acids found in soils prompted the label "humolimnic acids." Wilson (1959) stated that, for the colored organic fraction, this similarity was more than coincidental and that the source was soil fulvic acid carried into water through leaching by surface water. Lamar (1968) showed that there was no relation between organic color and the amount of iron present in surface waters. Further work by Christman and co-workers (Black and Christman, 1963a, 1963b; Christman and Ghassemi, 1966; Christman, 1970; Christman and Minear, 1971) using soil chemistry techniques confirmed the polyphenolic, aromatic, acidic nature of DOM and the close structural resemblance to soil humic substances. Soil humic substances are a complex mixture of stable, acidic polyelectrolytes possessing phenolic and carboxyl functionalities that are thought to be formed as byproducts of microbial degradation of plants (Stevenson and Butler, 1969; Schnitzer and Khan, 1972). The fulvic acid fraction is soluble in acid and base, the humic acid fraction in base only, and the humin fraction is insoluble. Reuter and Perdue (1977) used the available literature to conclude that 60-80% of DOM is humic substances that closely resemble soil fulvic acid and that the remainder of DOM is predominately carbohydrates and proteinaceous matter. In an assessment

of the current data, they report concentrations of 10 -13 mg humic substances/L for the lower Mississippi River, 3 mg/L for the Columbia River, and 45 mg/L for coastal plain rivers in the southeastern United States.

Leenheer and Huffman (1976) found that 60% of the DOM in a Wyoming ground water was in the hydrophobic fraction by using separations on macroreticular resins. Leenheer (1980) found that 50% of the soluble organic matter in the Amazon River was humic substances. He postulated that the humic substances originated from shallow soils where biomass input exceeds decay rates and the accumulated biomass is converted to humic substances. Langford et al. (1979) found that almost all the complexation capacity of DOM was in the hydrophobic, acid fraction and stressed that aquatic humic substances are polyelectrolytes in which no two carboxyl groups are inherently chemically identical.

Leenheer and Malcolm (1973) used a free-flow electrophoretic technique to fractionate DOM. They found that polysaccharides can constitute up to 10% of DOM. The literature on the carbohydrate fraction of DOM has been reviewed by Sweet (1979) who reported that values for free sugars in sea, lake, and river water have been found to be in the range of $0.1\mu\text{M}$ - $1.1\mu\text{M}$. Semenov et al. (1967) found that proteinaceous matter constituted less than 10% of DOM in their study of selected Russian rivers. Studies on sea water (Pocklington, 1972; Lee and Bada, 1977; Macko and Green, 1979) report values for total amino acids in the range of $0.1\mu\text{M}$ - $2.0\mu\text{M}$. Peake et al. (1972) found in their study of the Mackenzie River system that 76% of the amino acids were associated with suspended particulate matter at $1,200\mu\text{g/g}$ solids. Gardner and

Lee (1973) used gas-liquid chromatography to analyze lake water for ten dissolved amino acids and found concentrations of $2\mu\text{M} - 3\mu\text{M}$.

Hullett and Eisenreich (1979) used high-performance liquid chromatography of phenacyl ester derivatives to analyze Mississippi River water for free and bound fatty acids and found they constituted 3.3% of the dissolved organic carbon.

It can be seen then that the quantification of the role of dissolved organic matter is an important part in the overall study of trace metal speciation in natural waters and that this quantification will primarily involve the elucidation of trace metal-humic substances interactions. This realization has important consequences in the modeling of natural aquatic systems. In recent years, a host of sophisticated computer programs have been developed that, given the analytical concentrations of all metal ions, inorganic and simple organic ligands, well-defined surfaces, and dissolved gases and gross parameters such as pH, pE, and temperature, will calculate the equilibrium concentration of all possible species. (For a review of many of the currently available programs, see Nordstrom et al., 1979). While thermodynamic data are readily available for the binding of metals to most common inorganic and simple organic ligands, such data for aquatic humic substances are uncertain and currently the subject of much debate in the literature (Reuter and Perdue, 1977). Because of this uncertainty, humic substances are omitted from such computer models. In light of the above discussion, it can be seen that this omission may cause significant errors when these models are applied to natural waters in which humic substances occur. What is called for is a more precise

understanding of trace metal-humic substances interactions in the aquatic environment.

OVERALL PLAN OF THE RESEARCH

The overall goal of this research was to arrive at a better understanding of the role of dissolved organic matter in trace metal transport in a particular river system. It was hoped that the results of this work would find a broader application to natural fresh waters in general.

The first problem was the selection of an appropriate river to study. Highly colored streams draining marshes or swamps contain unusually high concentrations of DOM and are thus often the most advantageous natural systems for studying the role of DOM in natural processes. The Williamson River in Klamath County, Oregon not only possesses high concentrations of DOM but also provides a unique "before-and-after" situation. The river begins as a clear spring, flows about 25 miles through basaltic terrain, and then drains into Klamath Marsh. After passing through the marsh, the river is dark brown in color and contains high concentrations of DOM. After joining Spring Creek and the Sprague River, the Williamson drains into Upper Klamath Lake, about 35 miles from the Marsh (Peterson and McIntyre, 1970; Leonard and Harris, 1974). Since the Williamson provides about 46% of the water and nutrients flowing into Upper Klamath Lake, the river is also a logical focus for one of the causes of the lake's intense, seasonal bloom of the cyanobacterium Aphanizomenon flos-aquae (Miller and Tash, 1967; Gahler, 1969). As part of the larger study of

the river system, this researcher and co-workers completed a two-year, in-depth survey of the Williamson and its major tributaries (Perdue et al., 1981).

The second problem was the characterization of the three main DOM fractions in the Williamson River. While humic substances can be readily estimated via their color at 420 nm (Blunk, 1982), analysis for the carbohydrate and proteinaceous fractions is non-routine. The focus of this research was on the proteinaceous fraction. The abundance and fractionation of the carbohydrate portion was a thesis project of a co-worker (Sweet, 1979).

The third problem was the determination of the complexation capacity of Williamson River DOM towards the test trace metal, copper. Copper was chosen because of the many analytical techniques available for its measurement and because of its ubiquity in natural waters (Hutchinson, 1957). Complexation capacity data allows the calculation of an operational molar concentration unit for DOM in terms of equivalents of metal bound per unit weight of DOM. This number then can be used in equilibrium calculations involving metal-DOM interactions.

The fourth and final problem was the estimation of the extent of copper-DOM binding in the Williamson River. This can be accomplished by calculating the copper-DOM binding constant at the DOM:copper ratio found in the Williamson River and/or by using modeling techniques to extrapolate data found at higher laboratory concentrations of metal and DOM down to levels that are environmentally relevant.

In summary, the results presented in this thesis are intended to:

(1) quantitate the proteinaceous portion of dissolved organic matter

occurring in the Williamson River, Oregon; (2) quantitate the complexation capacity of this DOM for the test metal copper; (3) quantitate the copper-DOM binding constant at DOM:copper ratios found in the Williamson River; (4) model the variable nature of this binding constant at these same, environmentally relevant ratios.

CHAPTER II

CHOICE OF METHODS

Amino Acid Analysis

Common techniques for the quantification of amino acids from protein hydrolysates include partition chromatography on silica columns, two-dimensional paper chromatography, high-voltage paper electrophoresis, thin-layer chromatography, ion-exchange chromatography, gas-liquid chromatography, and high performance liquid chromatography (Blackburn, 1968; Husek and Macek, 1975; Bayer et al., 1976). Because of experimental simplicity, analysis using the automated, ion-exchange analyzer has become the standard method since the instruments first introduction in the late 1950's (Spackman et al., 1958). Two disadvantages are the relative high cost of the instrument and its single-purpose design. High performance liquid chromatography offers high speed (typical analysis time is 30 - 45 minutes) and a lower instrument cost. New specific fluorescent derivative techniques (Lindroth and Mopper, 1979) have made possible direct analysis of natural water samples.

At the time this research was initiated, neither of these two instruments were available. Of the remaining techniques, only gas-liquid chromatography offered both the sensitivity and the operational ease desired. The latter facet was critical in the choice due to the large number (almost 300) of analyses to be done. The large array of derivatization methods and their relative merits have been reviewed

(Husek and Macket, 1975). Most of these methods involve the formation of an ester at the carboxylate group and an amide at the alpha-amine group. The choice of alcohol and anhydride used is determined by amino acid solubility in the reagents, ease of derivative formation, derivative volatility and stability, availability of suitable chromatographic liquid phase, chromatographic elution profile, detector sensitivity to the derivatives, etc. Detection limits are typically in the nanogram range, and use of fluorinated anhydrides and electron capture detection can lower these limits into the picogram range (Zumwalt et al., 1971). The agreement of results found by gas-liquid chromatography and by the automated, ion-exchange analyzer has been demonstrated (Tajima, 1978; Burleson et al., 1980).

The derivatization procedure of Zanetta and Vincendon (1973) involves esterification with isoamyl alcohol and acylation with heptafluorobutyric anhydride. It was chosen because a readily-available, stable liquid phase is used and because the derivatives are not subject to volatility loss during drying steps. The modification of using acetyl chloride/alcohol instead of HCl/alcohol (Felker and Bandurski, 1975) was used because of its experimental simplicity.

Copper-Aquatic Humic Substances Interactions

The methods used to investigate the complexation capacity of natural waters and the binding of trace metals to aquatic humic substances largely center on the measurement of the metal of interest, as free metal ion activity, total metal concentration, or both. Often, metal determination is combined with a separation technique to determine spe-

ciation in a natural water system. Thus, while the actual determination of metal is generally confined to a few instrumental techniques (for example, voltammetry, potentiometry, atomic absorption spectroscopy), the experimental methods employing these techniques are many and varied. They can be conveniently grouped as direct and indirect electrochemical titrations, chromatographic separations, and miscellaneous.

In the miscellaneous category, the cobalt(III) complexation method of Hanck and Dillard (1973) and the copper(II) solubilization technique of Kunkel and Manahan (1973) have already been discussed. Van den Berg and Kramer (1979) used a dispersion of manganese dioxide as a weak ion exchanger to estimate the complexing capacity of natural water for copper. Truit and Weber (1981) used membrane dialysis to separate free metal from complexes with fulvic acid and measured total and free metal concentrations by atomic absorption spectrophotometry (AAS). Ryan and Weber (1982) used the loss of fluorescence of fulvic acid upon binding with metals as a measure of the amount of metal bound. Ultrafiltration and ion selective electrodes were used by Ramamoorthy and Kushner (1975) to determine the complexing capacity of molecular weight fractions of DOM in estuarine waters. A similar methodology was employed by Smith (1976), who used anodic stripping voltammetry (ASV) to measure metal. Ultrafiltration was combined with dialysis by Guy et al. (1975) and Guy and Chakrabarti (1976) in a similar size fractionation scheme. Both AAS and ASV were used to measure metal. Tessler et al. (1979) used a sequential extraction technique to speciate eight metals into five groups: exchangeable, bound to carbonates, bound to iron/manganese oxides, bound to organic matter, and residual.

Mantoura and Riley (1975), Mantoura et al. (1978), and Hirata (1981) used Sephadex gels to chromatographically separate humic-bound species from free metal and used AAS to analyze for total metal before and after separation. Bowen et al. (1979) combined gel filtration with gamma counting of radio-isotopes to investigate Sb, Hg, and Zn complexation with humic substances. In the ion-exchange technique, the competitive equilibria of an exchange resin and of humic substances for binding to a test metal are used to provide data for complexation capacity calculations. This method has been in use for many years by soil scientists (for example, Gamble et al., 1970), who generally follow the experimental procedures of Schubert (1948). An important modification of the basic method, allowing its use for metal-polyelectrolyte complexes, was developed by Ardakani and Stevenson (1972). The mathematical expressions derived from the technique were rigorously examined by MacCarthy and Mark (1977) and MacCarthy (1977a) and applied to mono- and polynuclear complexes by MacCarthy (1977b). Crosser and Allen (1977, 1978) applied the technique to soluble test ligands in water and to industrial wastewater, using AAS to measure metal concentration. Chelating resin has been used by Batley and Florence (1976) and Florence (1977) along with UV irradiation to determine seven species of copper, lead, cadmium, and zinc in natural waters. Metal was determined in the various fractions by ASV. Sturgeon et al. (1980) compared chelating resins and solvent extraction as techniques for metal preconcentration in speciation studies and found both methods gave comparable results.

Indirect electrochemical titrations make use of the fact that metal-humic substances complexation proceeds with release of protons.

The extent of metal binding is quantified by titrating solutions of humic substances with base before and after equilibration with metal (Gamble, 1973). The technique was used by Stevenson et al. (1973) and Stevenson (1976) to measure stability constants of humic substances binding to copper, lead, and cadmium.

Direct electrochemical titrations make use of ion selective electrodes or anodic stripping voltammetry to measure free metal ion and/or "labile" metal complexes. The ion selective electrode (ISE) has received much attention because it measures free metal ion activity and thus does not require a separation step to remove bound metal. Its main drawback is a loss of sensitivity at concentrations less than $10^{-7}M$. The early work of Stiff (1971) has already been discussed. Gardiner (1974) used the cadmium ISE to study complex formation in a variety of waters. He extended the sensitivity limit of the electrode to environmental levels by extrapolation of standard curves found at higher concentrations. Cheam (1973) and Cheam and Gamble (1974) studied mercury, cadmium, and copper binding to solutions of fulvic acid to arrive at binding constant data. Bufflé and co-workers have studied the complexation of aquatic humic substances using the copper (Bufflé et al., 1977, 1980) and lead (Greter et al., 1979) ISE, and Bufflé (1980) has compared the results for copper with data from other researchers. Sposito and co-workers have likewise studied the complexation of sewage sludge using the calcium (Sposito et al., 1978) and copper (Sposito et al., 1979; Sposito and Holtzclaw, 1979) ISE. Weber and co-workers have studied water- and soil-derived fulvic acid complexation using the copper (Bresnahan et al., 1978), cadmium (Saar and Weber, 1979), and lead (Saar

and Weber, 1980) ISE.

Anodic stripping voltammetry (ASV) shares with ISE the features of low cost, operational ease, selectivity, ability to make direct measurements on environmental samples, and sensitivity to free metal ion. It has the additional advantage of detection limits for several trace metals to 10^{-10} M. Matson (1968) studied the application of ASV to environmental analysis, and Allen et al. (1970) studied free and acid-exchangable copper and lead in river and lake water. The use of ASV in complexation capacity studies and in various fractionation schemes has been discussed above. Direct titrations to determine the binding constants for copper-DOM complexes in natural waters have been reported by Shuman and Woodward (1977). O'Shea and Mancey (1976) used peak current and peak potential measurements during metal-humic acid titrations to show that copper forms a strong, nonlabile complex.

For the research to be conducted for this thesis, the miscellaneous techniques were rejected because of their experimental complexity. Separation techniques utilizing ion exchange or chelating resins could not be used because of the significant adsorption of humic substances onto such resins (MacCarthy, 1974). While ASV has a distinct advantage over ISE in detection limits, it possesses several important disadvantages. One is that metal complexes with binding constants less than 10^{13} are dissociated, and the previously-complexed metal is measured as free metal ion (Matson, 1968; Chau et al., 1974). A second disadvantage is that humic substances adsorb onto mercury, causing spurious results (Allen et al., 1976; Bufflé and Cominoli, 1981), although Weber and Cheng (1979) have shown that this problem is lessened

by using wax-sealed graphite electrodes. While the ISE does not suffer from a severe adsorption problem and is sensitive only to free metal ion, its higher detection limit and slow response times at low ion levels are important drawbacks (Blaedel and Dinwiddie, 1974). However, linear response can be lowered to 10^{-12} M through the use of metal-ion buffers (Sunda and Lewis, 1978; Stella and Granzerli, 1979). Thus it was felt that the copper ISE would offer the maximum information with the minimum number of problems.

EXPERIMENTAL

Reagents

Acetonitrile. Acetonitrile (Matheson, Coleman, Bell-Spectra Quality) was used without further purification.

Acetyl Chloride. Acetyl chloride (Baker - Instra-analyzed) was refluxed one hour with dimethylaniline and then distilled. The 51.0°C fraction was taken (Vogel, 1974).

Amino Acids. Amino acids (Sigma) were used without further purification.

Ammonia. Aqueous ammonia, 28.5% (Baker - Reagent grade), was used to prepare a 2.0 M solution.

Cupric Nitrate. Cupric nitrate solutions, 0.1000 M and 0.01000 M, were prepared directly by dissolving primary-standard copper metal in concentrated nitric acid (Kennish, 1979).

Dimethylaniline. Dimethylaniline (Baker - Reagent grade) was passed through two alumina columns (M. Woelm) and used immediately.

Dowex 50W-X4, 200/400. Dowex 50W-X4 (Biorad) was purified by the

method of Kaiser et al. (1974) and stored at 5.0°C.

Ethyl Acetate. Ethyl acetate (Baker - Instra-analyzed) was used without further purification.

Heptafluorobutyric Anhydride. Heptafluorobutyric anhydride (Pierce - 1 mL ampules) was stored at -10°C and used without further purification.

Humic Substances Solutions. Solutions of humic substances with nominal concentrations of 1.0 g/L and 10.0 g/L were prepared by dissolving freeze-dried aquatic humus isolated from the Williamson River in purified water. The pH was adjusted to 6.0 and the solutions stirred at room temperature for two hours and then refrigerated at 5°C overnight. The solutions were then centrifuged at 2.5K rpm for 0.5 hour, and the supernatants filtered through 0.45 μ m Millipore filters. The filtrates were quantitatively transferred to volumetric flasks and the flasks filled to the mark. The 500 mL, 1.0 g/L solution was stored at 5°C in a one liter, brown linear polyethylene bottle, after degassing with pre-purified N₂. The 50 mL, 10 g/L solution was stored at 5°C. The solutions were analyzed on a Dohrman total organic analyzer and found to be 382 mg C/L and 4500 mg C/L (Hedges, 1982).

Hydrochloric Acid. Hydrochloric acid, 38% (Baker - Reagent grade), was used directly for the hydrolysis reactions and used to prepare a 2.0 M solution.

Isopentyl Alcohol. Isopentyl alcohol (Baker - Reagent grade) was refluxed over magnesium turnings for one hour, then distilled. The 127.0°C. fraction was taken and stored over activated molecular sieve pellets, type 13X (Matheson, Coleman, Bell). The brown bottles were

stored at room temperature in a desiccator over indicating Drierite.

Isotopes. Carbon-14 labeled algal protein and algal protein hydrolysate (ICN Pharmaceuticals) and tritium-labeled glycine and glutamic acid (New England Nuclear) were stored at 5°C.

Methanol. Methanol (Baker - Reagent grade) was refluxed one hour over magnesium turnings and then distilled. The 65.0°C fraction was taken and stored identically to the isopentyl alcohol.

Nitric Acid. Nitric acid, 70.5% (Baker - Ultrex grade), was used to prepare an approximately 35% solution.

Perchloric Acid. Perchloric acid, 70% (Mallinckrodt - AR grade), was used to prepare solutions of about 1.0 M and 5.0 M which were standardized at 0.9995 ± 0.0005 M and 5.56 ± 0.01 M by volumetric titration against standardized sodium hydroxide (Perdue, 1982).

Sodium Azide. Sodium azide (Mallinckrodt - AR grade) was used without further purification.

Sodium Hydroxide. Sodium hydroxide, 50% w/w (Baker - Reagent grade) was used to prepare solutions of about 0.1 and 2.0 M, which were standardized at 0.0964 ± 0.0002 M and 1.516 ± 0.002 M by volumetric titration against primary standard potassium hydrogen phthalate (Kohltoff *et al.*, 1969). A 10 M solution was prepared by simple dilution.

Sodium Oxalate. Sodium oxalate (Merck - Primary standard grade) was dried for two hours in *vacuo* at 25°C over P_2O_5 and stored at room temperature in a desiccator over indicating Drierite.

Sodium Perchlorate. Sodium perchlorate was prepared by titrating 250.0 mL of standardized 5.56 M perchloric acid with a 10 M sodium hydroxide solution to pH 7.00 and diluting the solution to 500.0 mL.

The final concentration was 2.496 M.

Triethylamine. Triethylamine (Baker - Reagent grade) was redistilled, taking the 88-89°C fraction.

Water. All solutions were prepared with water that had passed through a Barnstead Nanopure water purification system consisting of a reverse-osmosis pretreatment followed by a granulated carbon organic cartridge, two mixed bed ion exchange cartridges, and a 0.22 μm membrane filter. The product water was regularly checked with a Chemtrix conductivity meter, Model 70, and the cartridges replaced if the water was above 2.0 $\mu\text{MHO}/\text{cm}^2$. Typical values were in the range 0.2 to 0.8 $\mu\text{MHO}/\text{cm}^2$.

XAD-7 Resin. XAD-7 macroreticular resin (Rohm and Haas) was soxhlet extracted with methanol as recommended by the manufacturer (Rohm and Haas, 1971).

Apparatus

Atomic Absorption Spectrophotometry. AAS measurements were made on an Instrumentation Laboratory Model 551 equipped with a Model 555 graphite furnace.

Calculations. Calculations requiring a computer were run on a Hewlett-Packard Model 85, a Rockwell AIM-65, a Tektronix Model 31, or the PSU Honeywell Model 66/20.

Centrifugation. Centrifugation of Williamson River water was accomplished in a Sorvall SS-3 centrifuge equipped with a Model KSB-3 continuous flow system.

Derivatization System. Derivatizations for the amino acid analysis

were done in 3 mL Reacti-vials (Pierce Chemical Company) equipped with Teflon-lined screw caps. The vials were heated in a milled aluminum block mounted on a Corning Model PC-35 hotplate.

Gas-Liquid Chromatography. GLC measurements were made on a Hewlett-Packard Model 5750 equipped with flame ionization detection. The column used for the amino acid analyses was 12-FT, 2-mm I.D. glass (Supelco), Packed with 3% SE-30, 100/120 Gaschrom-Q (Applied Science Laboratories) using the method of Leibbrand and Dunham (1973). Chromatographic peak areas were automatically calculated by a Hewlett-Packard reporting integrator, Model 3380A.

Glassware. Glassware for the amino acid study was washed in detergent, rinsed with purified water, soaked overnight in hot, alcoholic potassium hydroxide, and rinsed with purified water. Labware for the copper-humic substances studies was Nalgene linear polyethylene (LPE). LPE was chosen because it has been shown to cause the least loss of trace metals when compared to pyrex and Teflon (Batley and Gardner, 1977; Subramanian et al., 1978). There is some disagreement on the best cleaning procedure (Mart, 1979; Laxen and Harrison, 1981). For these studies, all LPE labware was washed in detergent, rinsed with purified water, soaked overnight in 35% nitric acid, and rinsed with purified water. For work involving the AAS, LPE labware was given a final rinse with 70% nitric acid (Roe, 1981). Volumetric labware was calibrated by quadruplicate weighings of water contained or delivered (Kolthoff et al., 1969).

Potentiometry. Potentiometric measurements of pH were made with either a Ross combination electrode (Orion Model 81-02) or a glass

electrode (Corning Model 476022) and a double-junction reference electrode with 1.0 M NaNO_3 in the outer chamber (Orion Model 90-02). The electrodes were attached to an Orion Model 611 digital pH/mv meter. Copper ion measurements were made with a copper ISE (Orion Model 94-29) and the above double-junction reference electrode attached to a Hewlett-Packard Digital Multimeter, Model 3490A.

Liquid Scintillation Counting. All liquid scintillation counting was done in 10 mL Aquasol (New England Nuclear) in 20 mL disposable glass scintillation jars (Kimble) in a Beckman Model LS9000 liquid scintillation counter using standard window settings for tritium and carbon-14. The use of the counter was generously provided by the Department of Neurology, Oregon Health Sciences University.

Titration System. Titrations to determine the copper-oxalate binding constants, the complexation capacity of isolated Williamson River humic substances, and the copper-humic substances stability function were conducted in a constant temperature bath equipped with a Brinkmann Model K-21R circulator/refrigerator and a Tronac Precision Temperature Controller Model PTC-40. Temperature was maintained at 25.01°C . Titrant was added by a Gilmont 2.5 mL Ultraburet Model 53200A with micrometer readout to $0.1 \mu\text{L}$, motor-driven by a Rockwell Aim-65 microcomputer. The titration vessel was milled from solid Teflon with a screw cap drilled out to accept pH, reference, and copper electrodes. Three other ports allowed for bubbling or sweeping the top of the solution with gas and the addition of titrant. A schematic of the system is shown in Figure 1.

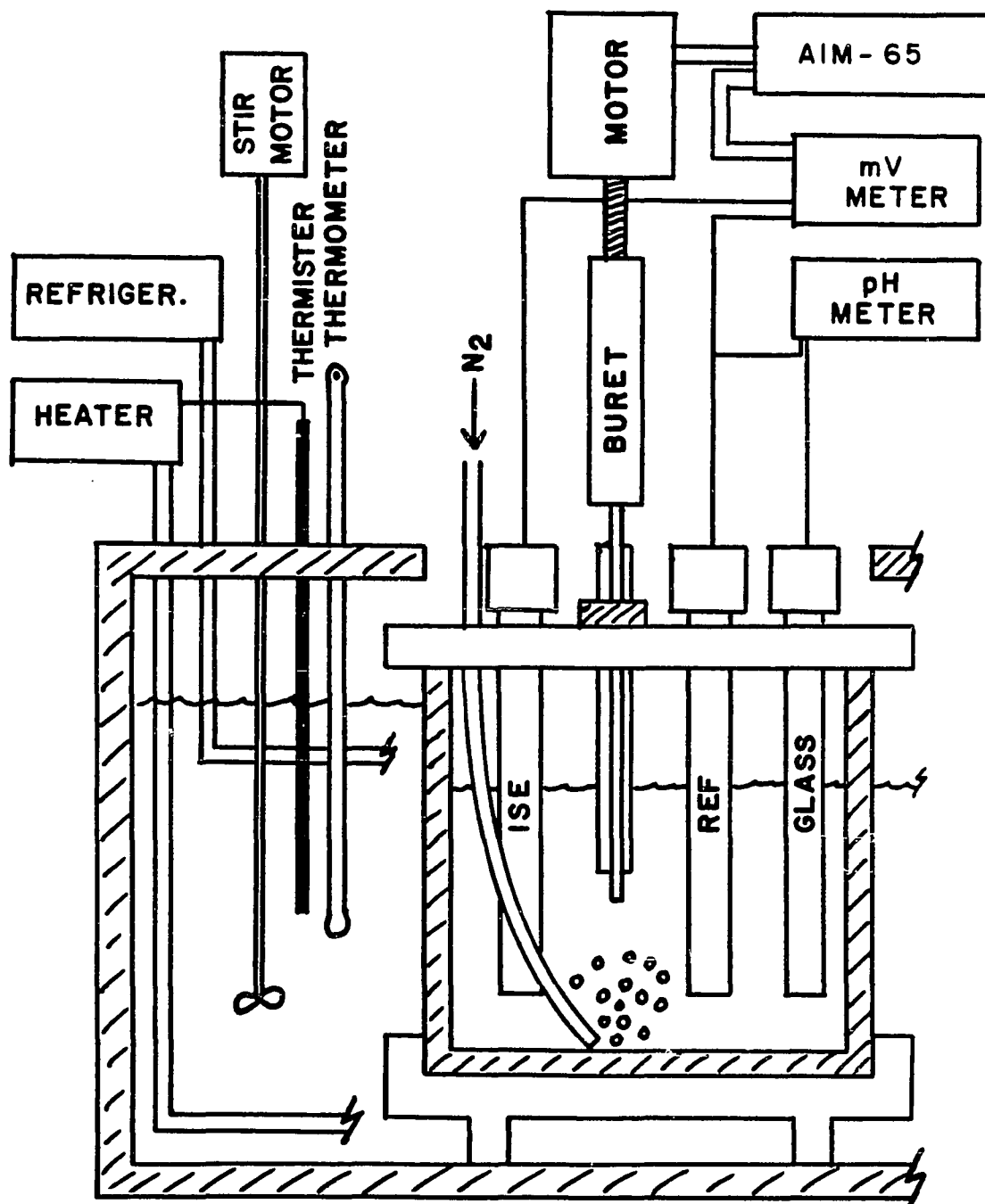


Figure 1. Constant-Temperature Titration System.

Methods For Amino Acid Analysis.

Sampling. From September, 1977 through September, 1979, samples were taken monthly from twelve sampling sites along the Williamson River and its tributaries. The approximate location of these sites is shown in Figure 2.

Abnormally low snow pack in the Cascade Mountain Range prior to and during this study lowered discharge into Klamath Marsh to the extent that no flow occurred out of the marsh through sampling site WR-50 during the three fall seasons observed. Flow did occur at site WR-56 because of numerous springs along the river between WR-50 and WR-56. No samples were taken at BS-10 during the winter months because of inaccessability due to snow.

Stream samples for the monthly survey were collected in 65-mL LPE bottles and preserved in 3 mM sodium azide and stored at 5°C until derivatization. Samples for the fractionation study were taken in January, 1980 at WR-21, WR-32, and WR-50 in 4-L LPE bottles and preserved in 3 mM sodium azide. Before storage at 5°C, the samples were deaerated for 15 minutes with prepurified nitrogen.

Chromatography. Elution regimes for the Dowex 50W-X4, 200/400 columns used in desalting hydrolysates were determined by the liquid - scintillation monitoring of tritiated glycine and glutamic acid test elutions. The desalting scheme for the 0.5 mL columns is given in Table I.

Recovery off the 0.5 mL Dowex 50-W-X4 columns was determined in triplicate by GLC analysis of a known, seventeen-amino acid mixture. The average column yield was $75 \pm 3\%$.

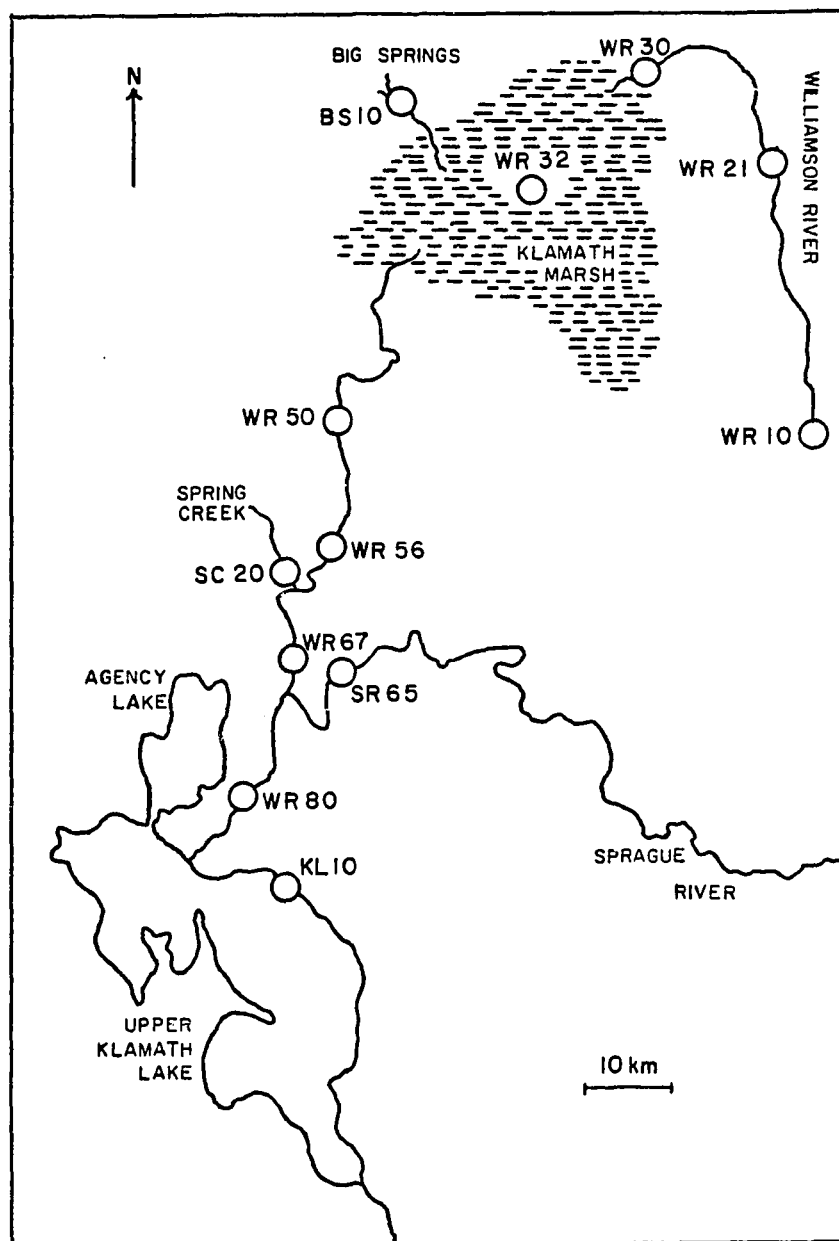


Figure 2. Location of Sampling Sites.

TABLE I

DESALTING SCHEME FOR 0.5 mL DOWEX 50W-X4 COLUMNS

| STEP | RESULT | VOLUME | REAGENT |
|------|--------|----------|-------------------------------|
| 1 | Wash | 5 mL | Water |
| 2 | Elute | 9 Drops | 2 M NH_3 (Discarded) |
| | Elute | 12 Drops | 2 M NH_3 (Sample) |
| 3 | Wash | 3 mL | 2 M NH_3 |
| 4 | Wash | 5 mL | Water |
| 5 | Wash | 5 mL | 2 M HCl |
| 6 | Wash | 5 mL | Water |

The separation efficiency of the 15 mL XAD-7 columns was determined by the liquid-scintillation monitoring of carbon-fourteen-labeled algal protein and algal protein hydrolysate. For triplicate runs at pH 2, $83.2 \pm 0.2\%$ of the algal protein and $88.3 \pm 0.3\%$ of the algal protein hydrolysate were nonretained by the resin. The efficiency of removal of humic substances was determined to be $\geq 99\%$ by monitoring adsorbance at 420 nm, pH 10.

The conditions for gas-liquid chromatography are given in Table II.

Assay for Humic Carbon. A sample of humic substances had been isolated from the Williamson River by Blunk (1982), and its carbon content analyzed. Various known weights were dissolved in purified water, buffered to pH 10, and the absorbances measured at 420 nm. A linear-regression analysis gave humic carbon as a function of absorbance at 420 nm (Blunk,

TABLE II

CONDITIONS FOR GAS-LIQUID CHROMATOGRAPHY

| | |
|---------------------------------|--------------------------------|
| Column Temperatures: | 90°C Initial |
| | 250°C Final |
| Programming: | 5 min. Initial Isothermal Hold |
| | 2°C/min. to 140°C |
| | 4°C/min. to 250°C |
| Injector Temperature: | 275°C |
| Detector Temperature: | 275°C |
| Carrier Flow (N ₂): | 30 mL/min. |
| Sample Volume: | 1 μ L |

1982): $\text{mg humic carbon/L} = (61.55 A_{420} + 0.655)/2$ for a 5 cm cell.

Field samples were collected in LPE bottles and deaerated with prepurified nitrogen at the sampling sites.

Total Organic Carbon. Total organic carbon (TOC) analyses were conducted at the Durham Water Treatment Laboratory of the United Sewage Agency, Durham, Oregon. Triplicate determinations were made using a Dohrman Model DC-50 Carbon Analyzer. Field samples were collected in 20 mL glass bottles and stored at 0°C.

Iron. Iron was analyzed by AAS on samples with pH adjusted to pH 2 with 35% nitric acid. Field samples were collected as for humic carbon.

Discharge and Flowrates. Most sample sites were located at river bridges. At such sites, a stream bed profile could be approximately mapped by measuring the vertical distance from the stream bed to a fixed

reference line. The vertical measurements were made with a 50-foot steel tape with an attached lead weight or with a rigid steel rod. The resulting profile was used to construct a graph of stream cross-sectional area versus water level, expressed as the vertical distance from the fixed reference line to the water surface. After the initial measurement of stream bed profiles, only the water level was monitored, the cross-sectional area being then estimated graphically. Because some bridges were not level, a reference point was established along the reference line of each bridge for all water level measurements. This same reference point was used for flow rates, which were determined with a General Oceanics 2031 Digital Flowmeter and 2035 Flowmeter Readout.

At WR10 and BS10, stream bed profiles were directly measured from water depth measurements at three-foot intervals along a line perpendicular to the stream bed. Water level was directly measured at a reference point. Flow rates at these two sites were determined by measuring the time required for a floating object (orange, stick, pumice, deer droppings, etc.) to travel a distance of thirty feet. At WR32, the stream flows through a large conduit which passes beneath road C-676. The water level was measured relative to the top of the conduit and an appropriate fraction of the cross-sectional area of the conduit was calculated as the stream bed cross-sectional area. Flow rates were measured as previously described with the digital flowmeter. No water level or flow rate data were obtained on Klamath Lake (KL10).

Derivatization For GLC Analysis. After the volumes were carefully measured, the water samples for the routine monthly survey were acidified to pH 2 with 6M HCl and evaporated under a stream of dry, pre-

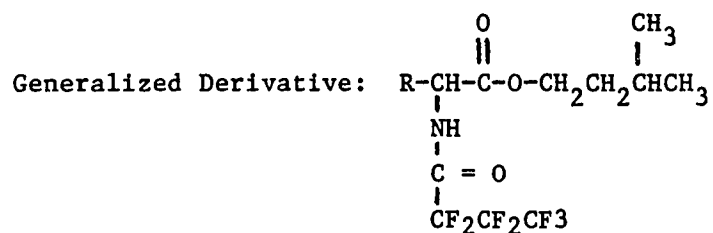
purified N_2 at $50^{\circ}C$ to a final volume of 2 mL. The samples were then quantitatively transferred to 3-mL Reacti-vials and evaporated to 1 mL. An equal volume of 12 M HCl was added, and the samples were hydrolyzed, under nitrogen, for 22 hours at $110^{\circ}C$. The hydrolysates were evaporated under a stream of nitrogen at $50^{\circ}C$ and taken up in 1 mL of 0.1 M HCl. They were then desalted on 0.5 mL columns of Dowex 50W-X4. Five thousand nanograms (50 μ L of a 100 ng/ μ L stock solution) of gamma-aminobutanoic acid (GABA) were added as an internal standard to the 2 M NH_3 eluants. The NH_3 fractions were evaporated under a stream of nitrogen at $50^{\circ}C$. Derivatization was accomplished by the method of Zanetta and Vincendon (1973) with the modification of using acetyl chloride instead of gaseous HCl to prepare the acidic alcohols (Felker and Bandurski, 1975). The basic steps for a run of six samples were as follows: (1) in a separate vial, 0.1 mL acetyl chloride was mixed with 1.1 mL methanol; (2) 0.2 mL acid-methanol was added to each vial, the vials swept with nitrogen and tightly capped, and the samples reacted 10 min. at room temperature; (3) the samples were blown dry at room temperature with nitrogen; (4) in a separate vial, acid-isopentanol was prepared exactly as the acid-methanol; (5) 0.2 mL acid-isopentanol was added to each vial, the vials swept with nitrogen and tightly capped, and the samples reacted 2 hours at $110^{\circ}C$; (6) the samples were cooled to room temperature, centrifuged briefly to remove solvent from the sealing disc, and blown dry at $50^{\circ}C$ with nitrogen; (7) 0.1 mL acetonitrile was added to each vial, followed by 0.02 mL heptafluorobutyric anhydride, the vials were swept with nitrogen and tightly capped, and the samples reacted 10 min. at $150^{\circ}C$; (8) the samples were cooled to room

temperature, spun briefly, and blown dry at 50°C with nitrogen;
 (9) 0.05 mL ethyl acetate was added, and the vials tightly capped; (10)
 samples were run immediately on GLC. The general form of the derivative
 is shown at the bottom of Table III.

TABLE III

STANDARD CURVES: ng AMINO ACID VERSUS
 AREA AMINO ACID/AREA 50 ng GABA

| Amino Acid | Slope | Intercept | Correlation Coefficient |
|------------|-------|-----------|-------------------------|
| ALA | 54.8 | 0.3 | 0.999 |
| GLY | 50.0 | -0.4 | 0.998 |
| VAL | 58.5 | -1.8 | 0.999 |
| THR | 52.2 | -3.9 | 0.999 |
| SER | 52.4 | -1.6 | 0.984 |
| LEV | 59.4 | -2.8 | 0.997 |
| ILE | 69.5 | -7.5 | 0.993 |
| PRO | 63.2 | -6.2 | 0.988 |
| MET | 90.8 | 2.4 | 0.994 |
| PHE | 41.2 | -5.7 | 0.994 |
| ASP | 58.6 | -4.1 | 0.999 |
| LYS | 75.6 | -2.2 | 0.990 |
| TYR | 61.1 | -1.1 | 0.991 |
| GLU | 61.5 | -4.1 | 0.994 |
| ARG | 192 | -4.5 | 0.977 |
| HIS | 219 | 11 | 0.979 |
| TRP | 232 | 11 | 0.990 |



Fractionation Study. The steps in the fractionation scheme are outlined in Figure 3. Each of the three river samples was analyzed in triplicate; 100 mL portions of each sample were analyzed directly for total amino acids; 1100 mL portions were filtered through prewashed, 0.45 μ m filters (Millipore), and 100 mL of this filtrate was analyzed for dissolved amino acids. Because of irreproducibility of results on samples eluted off the filters (presumably due to microbial growth on the filters and/or inability to quantitatively wash amino acids off the filters), particulate amino acids were calculated as the difference between total and dissolved amino acids. The remaining 1000 mL of filtered sample was acidified to pH 1.9 with 12 M HCl and passed through a 15 mL column of XAD-7. The humic substances were eluted with 40-50 mL of 0.1 M NaOH. A portion of the 0.1 M NaOH eluant was analyzed for humic amino acids. The 1000 mL of filtered sample that passed through the XAD-7 column was rotary evaporated to a final volume of 40 mL, desalted on a 1.5 mL Dowex 50W-X4, 200/400 column, and split into two equal portions. One portion was analyzed as previously described for free plus protein amino acids. The second portion was derivitized without a preceding hydrolysis step to give free amino acids only. Protein amino acids were calculated to be the difference between free-plus-protein and free amino acids.

Standards. A standard mixture of 17 amino acids was prepared to be 100 ng/ μ L in each amino acid. Volumes were added to derivatization vials so that injecting 1 μ L into the GLC would yield 6-80 ng of each amino acid. Each vial was spiked with GABA so this same injection volume would yield 50 ng GABA. Triplicate samples at each level of

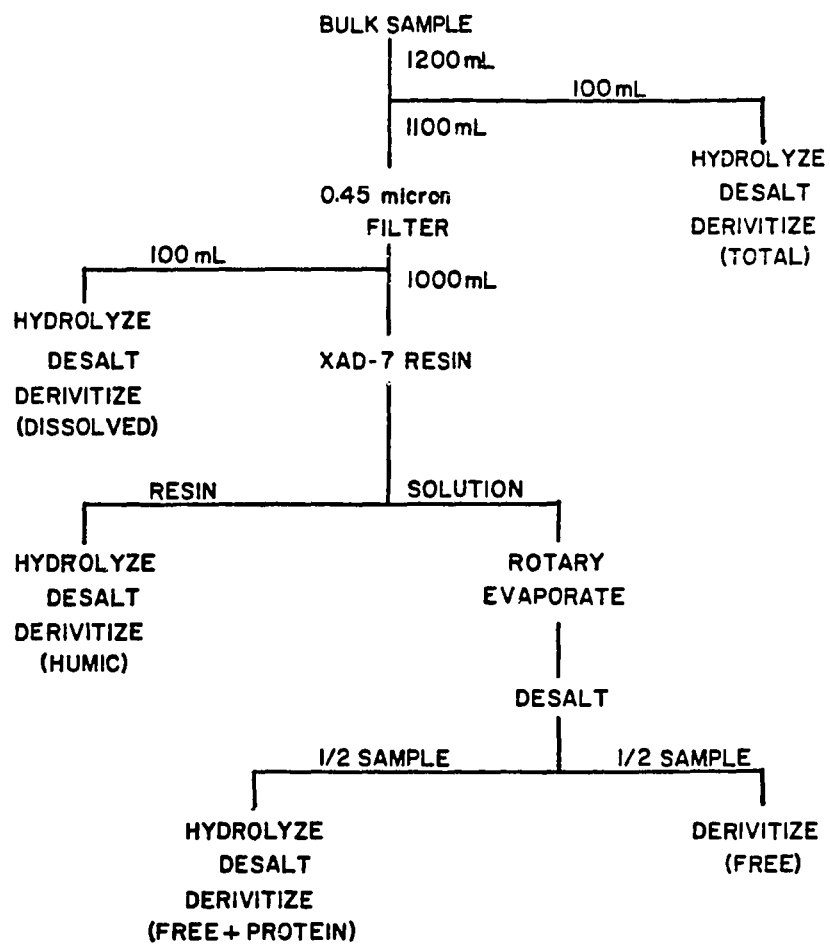


Figure 3. Fractionation Scheme.

amino acid were run and the data for each amino acid combined in an overall plot of ng amino acid vs. amino acid area/area of 50 ng GABA. The results are summarized in Table III. Reproducibility in amino acid area/area of 50 ng GABA was \pm 6%.

The hydrolysis, desalting, and derivatization steps were checked as an entire procedure by analyzing a known protein, the first 31 residues of raccoon alpha-hemoglobin. The results are summarized in Table IV.

TABLE IV
RACCOON ALPHA-HEMOGLOBIN, FIRST 31 RESIDUES

| Amino Acid | Residues Expected | Residues Found* |
|------------|-------------------|-----------------|
| ALA | 5 | 5.0 |
| GLY | 5 | 4.8 |
| VAL | 1 | 1.0 |
| THR | 1 | 1.3 |
| SER | 1 | 1.3 |
| LEV | 2 | 2.0 |
| ILE | 2 | 1.5 |
| PRO | 1 | 1.2 |
| MET | 0 | 0 |
| PHE | 0 | 0 |
| ASP | 3 | 3.1 |
| LYS | 3 | 2.2 |
| TYR | 1 | 0 |
| GLU | 3 | 3.1 |
| ARG | 1 | 0.5 |
| HIS | 1 | 0.7 |
| TRP | 1 | ** |

* Relative to ALA = 5, average of two analyses.

**TRP is lost during acid hydrolysis.

Methods for Complexation Capacity Study

Isolation of Williamson River Humic Substances. Twelve, five-gallon polyethylene carboys were filled with water from WR-50. After being transported to the lab, each carboy was deaerated 15 min. with

prepurified nitrogen and then stored at 5°C. Four carboys, in turn, were centrifuged with the continuous flow system at 10K RPM at a flow rate of about 150 mL/min., acidified to pH 2 by the addition of 12 M HCl, and applied to a 6.7 L column of XAD-7 resin. The humic substances were eluted with 1300 mL 0.15 M triethylamine, which was rotary evaporated at 30°C to a final volume of about 250 mL. This process was repeated twice, the evaporated column eluants pooled, and the volume brought to 4 liters by the addition of purified water. Triethylamine was used as the eluting base because, unlike sodium hydroxide, it is volatilized off during rotary evaporation. The 4 liters of solution were passed through a 400 mL column of Amberlite IR-120 (Rohm and Haas) in the hydrogen form. The prior dilution was necessary to prevent the precipitation of humic acid as the pH drops as hydrogen ion on the column is displaced. The desalted humics solution was rotary evaporated at 30°C to a final volume of 470 mL. This solution was freeze-dried, and the final product weighed. The original sixty gallons of river water yielded 4.8 g isolated humic materials. An elemental analysis was done on the freeze dried product (Hedges, 1982): 48.3% C, 3.86% H, 1.96% N, 4.76% ash.

Complexation Capacity Titrations. All complexation capacity experiments were carried out at 25.0°C in the constant temperature titration system previously described. The AIM-65 microprocessor was interfaced to the Hewlett-Packard multimeter and the Gilmont buret. The course of each titration was under the control of the microprocessor, utilizing the program HATIT. Basically, the program is in two parts. The first part accepts any number of metal ion standards, the concentration of each being keyboard entered. The program then reads the

voltages from the multimeter after a user-set delay time. A reading is taken every five seconds until at least 10 successive readings agree within a user-set range, here ± 0.2 mV. The average of the ten voltage readings is computed and entered for that particular standard. The program then computes a standard linear least squares analysis of the data and prints the results. The second part of the program drives a motorized precision buret to deliver titrant to the titration vessel. The number of titration points, delay time, and volume increment between each point is keyboard entered. Voltage readings are taken as before for the standards. At the end, the program will do selected calculations on the raw data, based on the standardization. A listing of HATIT is given in Appendix A.

The accuracy and reproducibility of the motor-driven buret was examined. For increments of 20 μL (the same size increment used in this study), the motor drive is accurate to ± 0.4 μL (Iliaifar, 1982).

Standards for the complexation capacity study ranged from 10^{-3} M to 10^{-6} M total copper in 0.1 M NaClO_4 at pH 4.5. For the triplicate titrations at pH 5.0, 5.5, and 6.0, 0.1000 M copper titrant was added in 20 increments of 20 μL each, and the humus solutions were 38.2 mg C/L. For the triplicate titrations at pH 6.5, 0.01000 M copper titrant was added in 20 increments of 20 μL each to a 3.82 mg C/L humus solution. The pH was monitored and adjusted with 2 - 4 μL aliquots of 0.1 M NaOH.

The raw data were plotted as complexed metal versus total metal and fitted with a simplex program, SMPLX, to the equation

$$Y = A [1 - \exp(BX)] \quad (1)$$

where Y = complexed metal and X = total metal. The parameter A

represents the limiting value of Y as X approaches infinity. A listing and description of SMPLX is given in Appendix B.

Methods for Copper-Humic Substances Stability Function Study

Determination of Copper-Oxalate Constants. Since the ISE cannot measure copper ion at the levels found in natural waters, it was decided to attempt humic substances titrations into copper, metal-ion buffers. Oxalic acid was chosen because, as sodium oxalate, it can be obtained in primary standard purity and because it's reported binding constants for copper ($\log K_1 = 4-6$, $\log K_2 = 3-5$) fall into a range expected for copper-humic substances (Sillen and Martell, 1964). To determine a best value for the two binding constants for the $\text{Cu}(\text{OX})$ and $\text{Cu}(\text{OX})_2$ species, three sets of titrations were done in triplicate at pH 6.0. All titrations were done in the constant temperature system at 25.01°C and under microprocessor control using the program HATIT, both previously described. Standards for the titrations ranged from 10^{-3} M to 10^{-6} M copper in 0.1 M NaClO_4 at pH 4.5. For the 10^{-3} M oxalate solution in 0.1 M NaClO_4 , 0.1000 M copper titrant was added so that total copper ranged from 10^{-5} M to 10^{-3} M. For the 10^{-4} and 10^{-5} M oxalate solutions in 0.1 M NaClO_4 , 0.01000 M copper titrant was added so that total copper ranged from 10^{-6} to 10^{-4} M. The free metal ion data were fit on the simplex program, SMPLX. Since free oxalate concentration is not known, it was calculated from the ligand mass balance quadratic equation using an initial guess for K_1 and K_2 :

$$[(\text{M})2K_2](\text{L})^2 + [1 + K_{a1}(\text{H}) + K_{a2}(\text{H})^2 + K_1(\text{M}) + K_4(\text{M})(\text{H})](\text{L}) - L_T = 0 \quad (2)$$

where (M) is the experimental free metal ion concentration, (L) is free oxalate concentration, L_T is total oxalate concentration, (H) is hydrogen ion concentration, K_{a1} and K_{a2} are the proton binding constants for oxalic acid, and K_4 is the binding constant for the species CuHOX. The ligand value thus calculated is then put into the metal mass balance equation (Equation 3), which is fitted for best values of K_1 and K_2 . The new K_1 and K_2 are put into Equation 2, which is re-solved for (L). The process is repeated until the change in successive values of K_1 and K_2 is less than a user-selected value.

$$(M)[1+K_1(L)+K_2(L)^2+K_4(H)(L)+K_3(OH)] - M_T = 0 \quad (3)$$

where M_T is the total metal concentration and K_3 is the binding constant for the species $Cu(OH)^+$.

Feasibility of the Metal-Ion Buffer System. It is known that the presence of a metal-buffering ligand in excess of total metal concentration can extend the working range of the ISE many orders of magnitude below its normal operating limit, as long as total metal is within the linear range of the ISE in an unbuffered solution (Blaedel and Dinwiddle, 1974). A set of copper standards in 0.1 M $NaClO_4$, pH 4.5 were prepared from 10^{-3} M to 10^{-8} M and analyzed in the constant temperature system to determine the feasible lower limit of linear response. The 0.1000 M oxalate was titrated in duplicate into 10^{-6} and 10^{-7} M total copper solutions at pH 5.0. The free copper concentration found was compared to that calculated for the system using the program MLEQL. MLEQL, based on the calculation methods used in the Fortran program MINEQL (Westall et al., 1976), is a program written for the Tektronix Model 31 desktop computer that will calculate the equilibrium

concentrations for all species in a system of a single metal, a single ligand of interest, and four metal-hydroxy complexes.

Humic Substances Titrations into a Copper, Metal-Ion Buffer. To determine the amounts of copper and humic substances necessary to duplicate the DOM: copper ratio at the most humus-rich site on the river, WR-50, total copper was determined by quadruplicate graphite furnace AAS analysis of acidified river water samples. The amount of humic substances in mg C/L was estimated from absorbance measurements (Perdue *et al.*, 1981), converted to eq copper/L via the complexation capacity measurements done for this research, and corrected by subtracting the equivalents of iron found in each sample (Perdue *et al.*, 1981). The ratio of DOM:copper was then calculated for each of sixteen sampling dates for WR-50, and these results averaged.

All titrations to determine the copper-humic substances binding function were carried out at 25.01°C in the constant temperature system and under microprocessor control using the program HATIT, both previously described.

Duplicate titrations were done at pH 5.0, 5.5, 6.0, and 6.5, to match the complexation capacity studies done earlier. Because the metal-ion buffer solution to be titrated would be the same in all cases except for pH, it was made in a single, 2 L batch to be 10^{-5} M in oxalate, 10^{-7} M in total copper, and 0.1 M in NaClO_4 . In all cases, the 4500 mg C/L humic substances solution was added in 50 increments of 0.050 mL each. The pH was monitored and adjusted with 2 - 4 μL aliquots of 0.1 M NaOH.

CHAPTER III

CHEMICAL EQUILIBRIA IN MULTILIGAND MIXTURES

The fact that metal complexation by humic substances results in the release of protons indicates that, to some extent, the same ligands are involved in proton and metal binding. While proton binding can be studied in the absence of competitive metal binding in metal-free solutions, metal complexation must inevitably be studied in competition with proton binding. It is therefore highly advantageous to use conditional stability constants to describe metal binding at constant pH. In the following equations, all charges are omitted and only 1:1 metal-ligand complexes are explicitly considered. For complexation of a metal, M, by the i^{th} deprotonated ligand or binding site in a multiligand mixture at constant pH,



and

$$K_i = \frac{[ML_i]}{[M][L_i]} \quad (5)$$

It is more convenient to define a conditional stability constant

$$K'_i = \frac{[ML_i]}{[M][H_{X_i}L_i]} = K_i \left(\frac{[L_i]}{[H_{X_i}L_i]} \right) \quad (6)$$

where $[H_{X_i}L_i]$ is the concentration of all forms of the i^{th} ligand that are not bound to M. The conditional stability constant, K'_i , is thus equal to the thermodynamic stability constant, K_i , times the fraction of $H_{X_i}L_i$ that is not protonated. This fraction is constant at constant pH.

In a complex multiligand mixture, both an average stability "constant", \bar{K} , and a conditional average stability "constant", \bar{K}' , can be defined, the latter being readily calculated from experimental data.

$$\bar{K} = \frac{\sum_i [ML_i]}{[M] \sum_i [L_i]} = \frac{\sum_i K_i [L_i]}{\sum_i [L_i]} \quad (7)$$

$$\bar{K}' = \frac{\sum_i [ML_i]}{[M] \sum_i [H_{X_i}L_i]} = \frac{\sum_i K'_i [H_{X_i}L_i]}{\sum_i [H_{X_i}L_i]} = \frac{(C_M - [M])}{[M] (C_L - C_M + [M])} \quad (8)$$

where C_L is the stoichiometric concentration of ligand and C_M is total metal minus hydroxy complexes. Dividing the numerator and denominator of Eq. 8 by the concentration of all uncomplexed forms of an arbitrarily selected reference ligand $[H_{X_r}L_r]$, Eq. 9 is obtained.

$$\bar{K}' = \frac{\sum_i K'_i \left(\frac{[H_{X_i}L_i]}{[H_{X_r}L_r]} \right)}{\sum_i \left(\frac{[H_{X_i}L_i]}{[H_{X_r}L_r]} \right)} \quad (9)$$

This particular form for \bar{K}' is introduced here to more amply illustrate the fact that \bar{K}' is not a constant, but rather a function whose value changes continuously as metal is added to a multiligand mixture. For purposes of discussion let us assume that $H_{X_R}L_R$ is the weakest metal-binding ligand in the mixture. Consider then the behavior of \bar{K}' as metal ions are added to this ligand mixture. The form of \bar{K}' is that of a weighted average. The weighting factor ($[H_{X_I}L_I]/[H_{X_R}L_R]$) of the ligand with the largest K'_I value will be greatest at the lowest levels of added metal and will decrease steadily as C_M increases. The inevitable result is that \bar{K}' must decrease as C_M increases and cannot be regarded as a constant at all. The functional nature of \bar{K}' has been clearly recognized by previous researchers (MacCarthy and Smith, 1979; Gamble et al., 1980).

For reasons that will become more apparent later, it is useful to examine the general equilibrium description of a multiligand mixture that contains two distinct classes of ligands (classes I and II). In this case, equations analogous to Eq. 8 can be derived for each class of ligands.

$$\bar{K}'_I = \frac{\left(\sum_i K'_i [H_{X_i} L_i]\right)_I}{\left(\sum_i [H_{X_i} L_i]\right)_I} \quad \text{and} \quad \bar{K}'_{II} = \frac{\left(\sum_i K'_i [H_{X_i} L_i]\right)_{II}}{\left(\sum_i [H_{X_i} L_i]\right)_{II}} \quad (10)$$

Unfortunately, only the overall \bar{K}' given by Eq. 8 can be calculated from experimental data. The experimental \bar{K}' is a weighted average of \bar{K}'_I and \bar{K}'_{II} .

$$\bar{K}' = \frac{\bar{K}'_I \left(\sum_i [H_{X_i} L_i] \right)_I + \bar{K}'_{II} \left(\sum_i [H_{X_i} L_i] \right)_{II}}{\left(\sum_i [H_{X_i} L_i] \right)_I + \left(\sum_i [H_{X_i} L_i] \right)_{II}} \quad (11)$$

More importantly, it must be recognized that, like \bar{K}' , \bar{K}'_I and \bar{K}'_{II} are functions that change continuously as the ligands within each class react sequentially with added metal. Any type of average stability "constant" will have a fixed value only at a single C_M value during the titration of metal into a multiligand mixture.

The third general subject is the treatment of complexes with other than 1:1 stoichiometry. This subject has been rigorously examined by MacCarthy and coworkers (MacCarthy, 1977; MacCarthy and Smith, 1979). The conclusions reached above with regard to 1:1 metal-ligand complexes also apply to other possible stoichiometries. Thus, while average "constants" for 1:1 and 1:2 complexes can be defined mathematically, those "constants" will change continuously as metal is added to a multiligand mixture.

DISCRETE MULTILIGAND MODELS

Multiligand models or the analogous multiple binding site models usually assume 1:1 stoichiometry for all metal-ligand complexes. It is convenient to combine Eq. 6 with the ligand mass balance equation for the i^{th} ligand or binding site,

$$C_i = [H_{X_i} L_i] + [ML_i] \quad (12)$$

where C_i is the stoichiometric concentration of the i^{th} ligand or binding site, to obtain

$$v_i \equiv [ML_i]/C_i = \left(\frac{K'_i[M]}{1 + K'_i[M]} \right) \quad (13)$$

When summed for all ligands, Eq. 14 is obtained.

$$\bar{v} \equiv \frac{\sum_i v_i C_i}{\sum_i C_i} = \sum_i \left(\frac{K'_i[M]}{1 + K'_i[M]} \right) \left(\frac{C_i}{C_L} \right) = \frac{C_M - [M]}{C_L} \quad (14)$$

Experimental \bar{v} values, calculable from C_M , C_L , and $[M]$ via Eq. 14, can be used to calculate K .

$$\bar{K}' = \frac{1}{[M]} \left(\frac{\bar{v}}{1 - \bar{v}} \right) \quad (15)$$

As indicated previously, \bar{K}' values are not constant in multiligand mixtures. The only real constants in such systems are the K'_i values of the individual ligands or binding sites, and then only at constant pH. There is no direct method by which those K'_i values can be extracted from experimental \bar{K}' values for multiligand mixtures such as humic substances, simply because there are potentially so many different metal binding groups to be characterized.

In several recent studies of metal complexation by humic substances (Mantoura and Riley, 1975; Guy and Chakrabarti, 1976; Bresnahan et al., 1978; Sposito et al., 1979; Hirata, 1981; Sohn and Hughes, 1981;

Sposito, 1981; McKnight et al., 1982), a serious conceptual error has resulted from attempts to use the Scatchard equation (Scatchard, 1949; Scatchard et al., 1957) or other related equations to extract equilibrium constants and ligand concentrations from experimental data. In those studies, it is assumed that the K'_1 values for discrete, individual ligands in Eq. 14 can alternatively be assumed to represent average stability constants for distinct classes of ligands. The mathematical fallacy in this assumption is best illustrated by examining the pertinent equations.

The first term of Eq. 14

$$\bar{v} = \left(\frac{K_1 (M)}{1 + K_1 (M)} \right) \left(\frac{C_1}{C_L} \right) \quad (16)$$

is easily rearranged to yield the one-component Scatchard equation Eq. (17).

$$\frac{\bar{v}}{(M)} = K_1 \left(\frac{C_1}{C_L} \right) - K_1 \bar{v} \quad (17)$$

This simple equation, which predicts a linear relationship between $\bar{v}/(M)$ and \bar{v} , never adequately describes the curvilinear plots of $\bar{v}/(M)$ versus \bar{v} that are obtained in studies of metal-humus complexation equilibria. Almost without exception, those researchers who use the Scatchard equation to analyze experimental data respond to the failure of the linear one-component model (Eq. 17) by including the second term from Eq. 14, yielding

$$\bar{v} = \left(\frac{K_1 (M)}{1 + K_1 (M)} \right) \left(\frac{C_1}{C_L} \right) + \left(\frac{K_2 (M)}{1 + K_2 (M)} \right) \left(\frac{C_2}{C_L} \right) \quad (18)$$

The addition of the second term (which increases the number of curve-fitting parameters from two to four) generally results in a greatly improved fit of the experimental data, leading to the conclusion that humic substances contain two "classes" of metal complexing ligands. It should be noted that McKnight and co-workers (McKnight et al., 1982), were unable to fit their data for one type of aquatic humis to a two-component model and added a third term to Eq. 18. The curve-fitting constants K_1 , C_1/C_L , K_2 , and C_2/C_L are treated as average stability constants and ligand concentrations for the two presumed classes of ligands. The best values of K_1 , C_1/C_L , K_2 , and C_2/C_L are necessarily obtained by curve-fitting experimental data over a range of \bar{v} values. So C_M and/or C_L are varied considerably, usually by titration of metal into a humus solution, to generate the data set. In the previous discussion, the functional nature of average stability "constants" was demonstrated. Even if humic substances did contain two distinct classes of ligands, \bar{K}_I' and \bar{K}_{II}' would vary continuously during a titration. Thus, \bar{K}_I' and \bar{K}_{II}' cannot be equated with K_1 and K_2 , both of which are implicitly assumed to be independent of C_L and C_M in Eq. 18. The whole idea of extracting average stability constants from Eq. 18 is clearly erroneous. The four constants that are obtained from the two-component Scatchard equation must therefore be regarded as empirical curve-fitting parameters with no chemical significance.

The conclusions given in the preceding paragraph are not intended as a criticism of the Scatchard equation per se, but rather the erroneous assumption that K_1' could represent a class of related ligands rather than one discrete ligand. The Scatchard equation has been widely and successfully applied to biomolecules such as proteins and nucleic acids. It is not at all unreasonable to expect that a purified protein molecule might have only one or two distinct sites for binding of a metal ion or other substrate.

The same conceptual error has also been made in studies where other types of equations have been used to analyze experimental data. For example, if Eq. 16 is inverted and rearranged, Eq. 19 is obtained.

$$\frac{1}{\bar{v}} = \left(\frac{C_L}{C_1} \right) + \left(\frac{C_L}{C_1} \right) \left(\frac{1}{K_1(M)} \right) \quad (19)$$

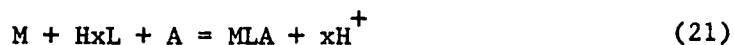
By plotting $1/\bar{v}$ versus $1/(M)$, C_1/C_L and K_1 can be obtained for a system that contains only one discrete ligand. This equation has been in use for as long as the Scatchard equation for describing equilibria in biochemical systems (Karush and Sonenberg, 1949). More recently, Eq. 19 has been used by Bufflé and co-workers (Bufflé et al., 1977) in an attempt to describe metal binding by humic substances, with the erroneous assumption that K_1 could represent the average stability constant (\bar{K}_1') for a class of ligands. In this case, the failure of Eq. 19 to describe metal complexation by humic substances was attributed to the formation of both 1:1 and 1:2 metal-ligand complexes. The addition of an additional curve-fitting parameter (the average stability constant

for 1:2 complexes) resulted in a greatly improved fit of the experimental data. As in the case of the two-component Scatchard equation, however, the curve-fitting parameters of the two-stoichiometry model are simply empirical constants. While it is indeed possible that 1:1 and 1:2 complexes are formed, it is not possible to determine the relevant average equilibrium constants because those "constants" change continuously as C_L and/or C_M are changed.

In further work, Bufflé and co-workers (Bufflé et al., 1980) found that their two-stoichiometry model would not fit their data. After ruling out the possibility of polynuclear complexes with humus and copper hydrolysis products because of experimental conditions, they first added a third set of terms to Eq. 19 to account for a proposed copper binding to a humus aggregate:



where n is arbitrarily ≤ 2 . While the expanded model enhanced the data fit, the authors admitted that "the errors incurred in the calculated constants become so large that it is difficult to tell whether or not they have any significance" (Bufflé et al., 1980). When work done at various levels of C_L gave data that could not all be fit with the same three-component equation, a fourth reaction was proposed, involving mixed ligand complexes, (Bufflé et al., 1980):



where A is an unspecified inorganic ligand. Data were presented to show that the MLA binding constant can be reasonably estimated as the product

of the binding constants for ML and MA.

The end result was a model involving the equilibrium binding expressions for four species: ML, $M(L)_2$, $M(L_2)$, and MLA. Below 30 mg humus/L, only ML, $M(L)_2$, and MLA were considered. Above 30 mg humus/L, ML, $M(L)_2$, MLA, and $M(L_2)$ were considered. In both cases, the rationale for the existence of the extra species was the failure of the original, two-component model, not rigorous knowledge of the chemical system. The possible artificiality of this approach was recognized at the time when the authors, in a discussion of the proposed $M(L_2)$ species, admitted that their method "does not allow these $[M(L_n)]$ complexes and the classical ML_n complexes to be distinguished" (Bufflé et al., 1980).

The discussion in the preceding paragraphs may have given the impression that there is no rigorous method by which metal complexation by humic substances can be efficiently described. However, MacCarthy and Smith (1979) have shown that, under severely limiting conditions of excess ligand that may not be attainable in laboratory studies or in the environment, the average stability "constants" for 1:1, 1:2, and higher complexes in multiligand systems approach constant limiting values. While this conclusion is of theoretical interest, it is of little practical importance, given the analytically imposed limitations on C_M and C_L values in laboratory studies.

CONTINUOUS MULTILIGAND MODELS

The most rigorous method for describing proton and metal binding by humic substances has been developed by Gamble and co-workers (Gamble, 1970, 1972; Burch et al., 1978; Gamble et al., 1980), who have clearly

recognized the functional nature of \bar{K}' values. They have suggested that humic substances contain a continuous distribution of non-identical functional groups that can bind protons or metal ions. Even when they have subdivided proton binding sites into two classes, the \bar{K}'_I and \bar{K}'_{II} values have been consistently treated as functions rather than constants. In some instances, they have used polynomial equations to empirically describe the variation of \bar{K}' values with solution composition. While Gamble and co-workers have not calculated discrete K'_i values from experimental data, they have demonstrated that the instantaneous \bar{K}' values for the group which is reacting at a particular set of C_L and C_M values can be estimated mathematically by partial differentiation of appropriate polynomial equations.

The principal objection to the rigorous treatment proposed by Gamble is probably more psychological than technical. There is no efficient method for describing the variation of \bar{K}' with solution composition, so the \bar{K}' results must be either tabulated or presented graphically. A simple set of constants that could be used to reconstruct the data set is not obtainable by Gamble's method.

Posner (1964, 1966) showed that proton binding by humic substances was efficiently described by a continuous multiligand distribution model, the relative concentration of each discrete ligand being normally distributed relative to the pK_i of the ligand. In a normal distribution of ligands,

$$\frac{C_i}{C_L} = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{\mu - pK_i}{\sigma}\right)^2\right] dpK \quad (22)$$

where C_1/C_L is the mole fraction of ligands in the interval $d\text{pK}$ whose acid dissociation constant is expressed as a negative logarithm (pK_1), and σ is the standard deviation for the distribution of pK_1 values about the mean pK values (μ) for the mixture of ligands. Similar models have been used to describe the binding of anions to proteins (Karush and Sonenberg, 1949) and the adsorption of gases on catalytic surfaces (Sips, 1948). This general approach seems to be an excellent alternative to Gamble's method in that an entire titration curve can be summarized by the μ and σ values for the multiligand mixture. The somewhat objectionable procedure of assuming a normal distribution can be overcome by the use of more sophisticated statistical methods that actually determine the shape of the best ligand distribution curve from experimental data (Parrish, 1982).

For this research, only the normal distribution model will be examined. This distribution model is easily combined with Eq. 14 to yield:

$$\bar{v}_{\text{calc}} = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \left(\frac{[M]10^{\log K'}}{1 + [M](10^{\log K'})} \right) \exp \left[-\frac{1}{2} \left(\frac{\mu - \log K'}{\sigma} \right)^2 \right] d \log K' \quad (23)$$

Numerical methods (e.g., Gaussian quadrature, Simpson's method) can be used to evaluate the integral in Eq. 23 for any values of $[M]$, given a set of μ and σ values. Given a set of experimental \bar{v} and $[M]$ values, nonlinear regression techniques can be used to determine the best values of μ and σ for the set of data. Either \bar{v} or corresponding $\log \bar{K}'$ values can be used for regression.

In proton binding by humic substances, both carboxylic acid and

phenol functional groups must be considered. Likewise, the possibility of two classes of metal binding ligands should be considered.

Accordingly, a bimodal normal distribution model will be used in some of the applications that follow. The extension from Eq. 23 to a bimodal model is easily accomplished. Equations that are analogous to Eq. 23 can be written for \bar{v}_I (given μ_I and σ_I) and for \bar{v}_{II} (given μ_{II} and σ_{II}). Then for $C_L = (C_I + C_{II})$, where C_I and C_{II} are stoichiometric concentrations of two classes of ligands (e.g., carboxyl and phenolic groups),

$$\bar{v} = \theta \bar{v}_I + (1 - \theta) \bar{v}_{II} \quad (24)$$

where $\theta = C_I/C_L$ and $(1 - \theta) = C_{II}/C_L$. The bimodal distribution model thus has five curve-fitting parameters (θ , μ_I , σ_I , μ_{II} , and σ_{II}) that must be determined by nonlinear regression.

CHAPTER IV

RESULTS AND DISCUSSIONS

Amino Acid Study

As shown in Figure 4, 15 major amino acids were separated. Asparagine and aspartic acid yield a single peak, as do glutamine and glutamic acid. On a few occasions when total amino acids were above 10 μ M, other amino acids gave separate but very small peaks. These included β -ALA, HYP, ORN, and TYR.

The results for individual amino acids at each sampling site for the two-year study are given in Appendix D. The mole percentages of the individual amino acids, averaged over all of the sampling sites, are given in Table V.

TABLE V

MOLE PERCENT OF TOTAL AMINO ACIDS IN THE
WILLIAMSON RIVER, AVERAGED OVER TWO YEARS

| <u>Amino Acid</u> | <u>Mole %</u> | <u>Amino Acid</u> | <u>Mole %</u> |
|-------------------|---------------|-------------------|---------------|
| Glycine | 18.7 | Leucine | 4.7 |
| Aspartic acid | 13.6 | Methionine | 3.6 |
| Alanine | 11.8 | Proline | 3.5 |
| Glutamic acid | 9.6 | Lysine | 3.0 |
| Serine | 9.5 | Isoleucine | 2.3 |
| Threonine | 6.1 | Histidine | 2.2 |
| Phenylalanine | 5.2 | Arginine | 1.2 |
| Valine | 4.7 | | |

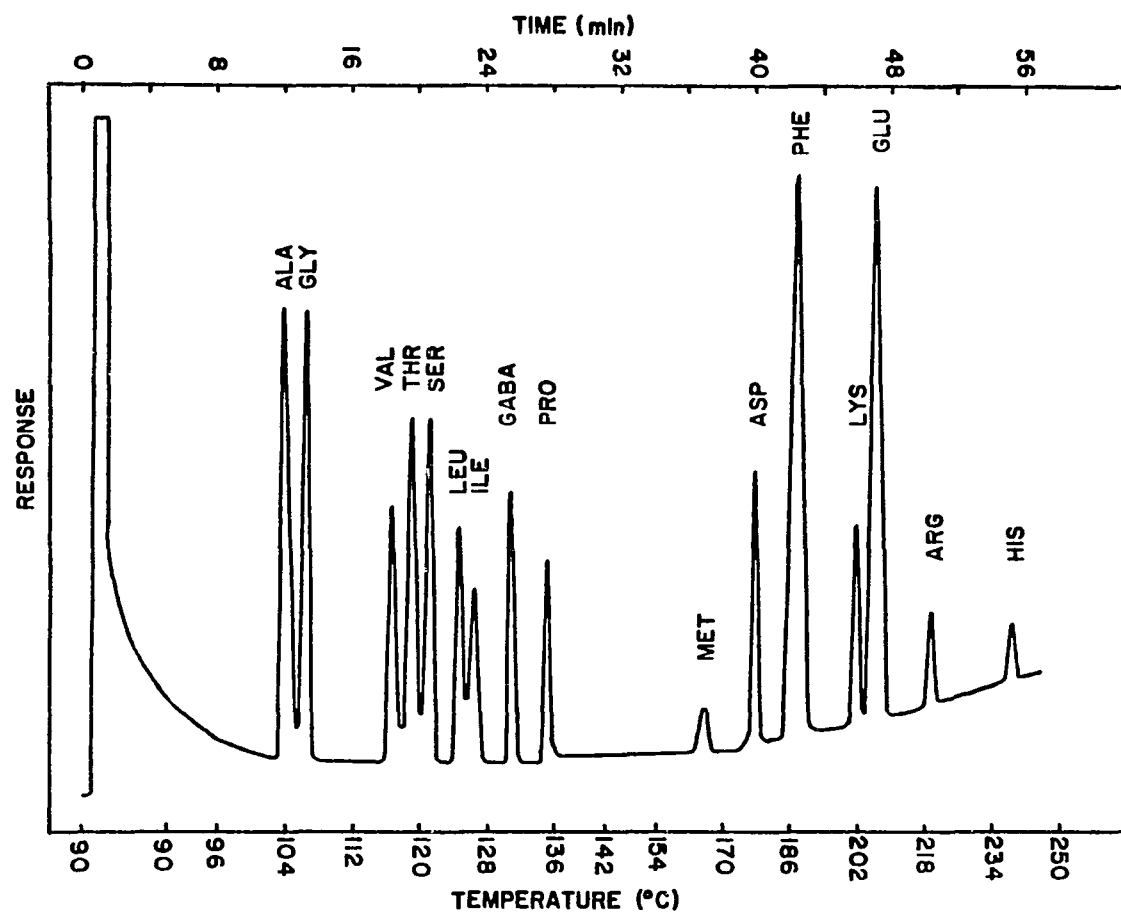


Figure 4. Typical chromatogram: total amino acids at sampling site KL-10, September, 1978.

The seasonal variation in the amounts of the five most abundant amino acids at six selected sampling sites is shown in Figure 5. In general, the five most abundant amino acids were Gly > Asp > Ala > Ser \cong Glu. This order showed no significant variation through the river system through the two years. This relative order has been found in other river systems by other workers (Peake et al., 1972; Beck et al., 1974). The two-year averages for total amino acids at each sampling site are shown in Figure 6. The input from the marsh is clearly seen, as are the diluting effects of the many riverbed springs between WR-50 and WR-56 and of Spring Creek. At WR-50, the value shown is the average for only those months that water was flowing out of the marsh. The range at WR-50 was from 1.5 to 15.9 μ M.

While charge considerations may cause concern for the kind of bonding occurring between the amino acids found in this research and humus, the presence of the acidic amino acids is not unexpected. Stevenson (1982) reports that the distribution of amino acids found in soils closely resembles that found for the bacterial cells in soils, Gly, Ala, Asp, and Glu being predominant. Carter and Mitterer (1978) report a relative abundance for decomposing aquatic plant debris of Gly > Asp > Glu > Ala > Ser. Thus the relative distribution of amino acids associated with Williamson River humus closely resembles that found in two important possible sources of aquatic humus.

The two-year averages for humic carbon are also shown in Figure 6 and are seen to closely follow the average total amino acid concentrations. KL-10 shows anomalous behavior that is most probably due to the high concentration of algae present in Klamath Lake during the

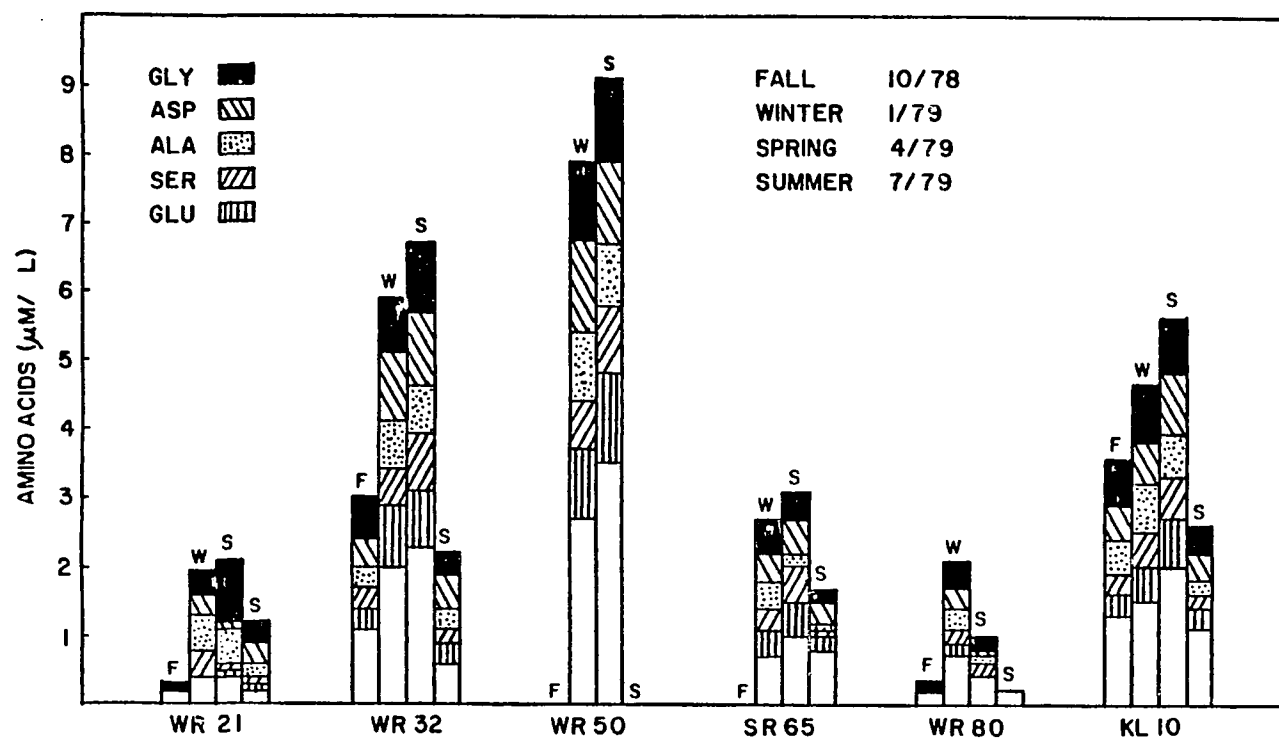


Figure 5. Seasonal amino acid distribution at selected sampling sites.

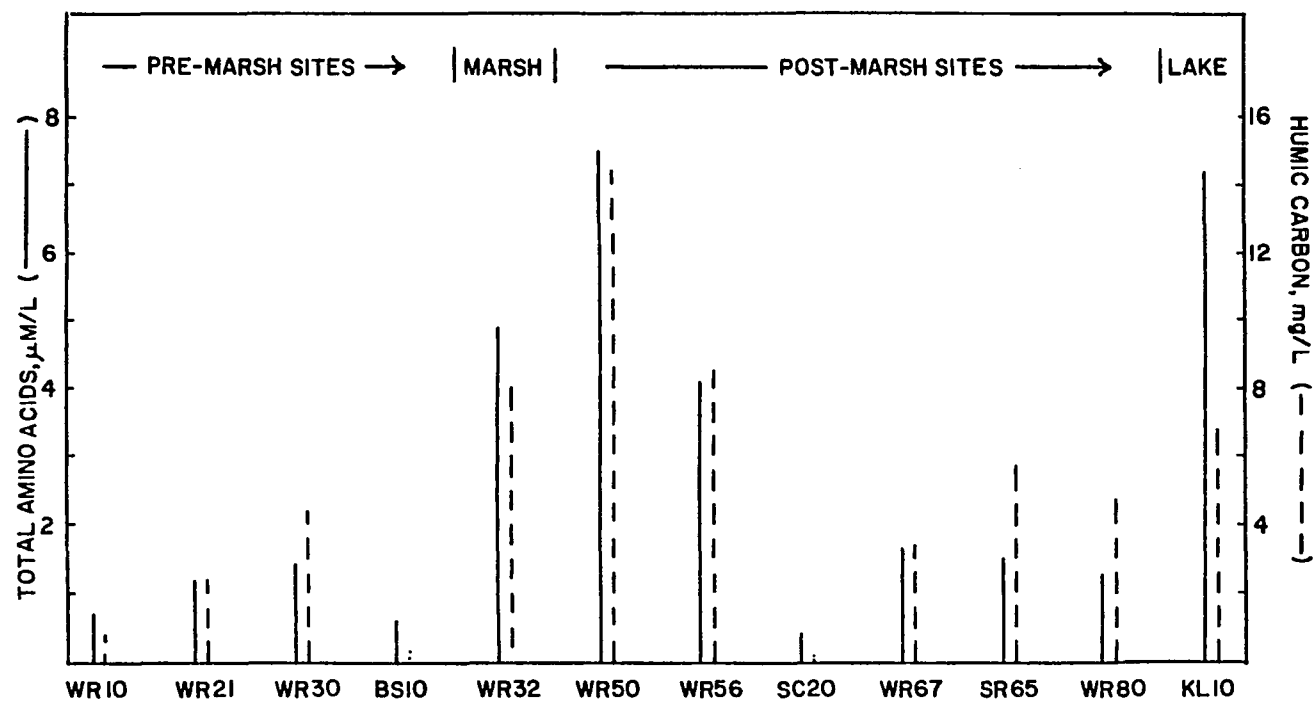


Figure 6. Two-year averages for total amino acids and humic carbon.

summer, fall, and early winter months ($>30,000$ cells/mL of Aphanizomenon flos-aquae). When total amino acid concentrations are plotted against humic carbon, significant r^2 corrections are found. For example, Figure 7 shows such a plot for the December, 1978 field trip. Disregarding the anomalous KL-10 point, the least-squares line gave an r^2 of 0.976. This evidence suggested that most of the amino acids were associated with humic substances.

Generally, total amino acid concentrations were several times higher in the winter and spring months than in the summer and fall months. This seasonal pattern was also observed for humic carbon and discharge. The positive correlation among these parameters is exemplified in Figure 8, which shows the data from the Sprague River sampling site (SR-65). On the basis of these results, it appears likely that the principal source of humic carbon and amino acids is surface runoff, which flushes these components from water-saturated soils during periods of high discharge. Similar variations of total organic carbon with discharge have been previously noted (Beck et al., 1974; Dahm, 1980).

In view of the biological lability of free amino acids, proteins, etc., it seems likely that those amino acids which are mobilized from soils are already associated with humic substances. Thus the overall seasonal variability of amino acid concentrations in this river system is probably best explained by a predominant discharge-related pattern on which relatively minor biological perturbations are superimposed.

To test the hypothesis that the amino acids were associated with humic substances, a fractionation scheme centering on the use of the

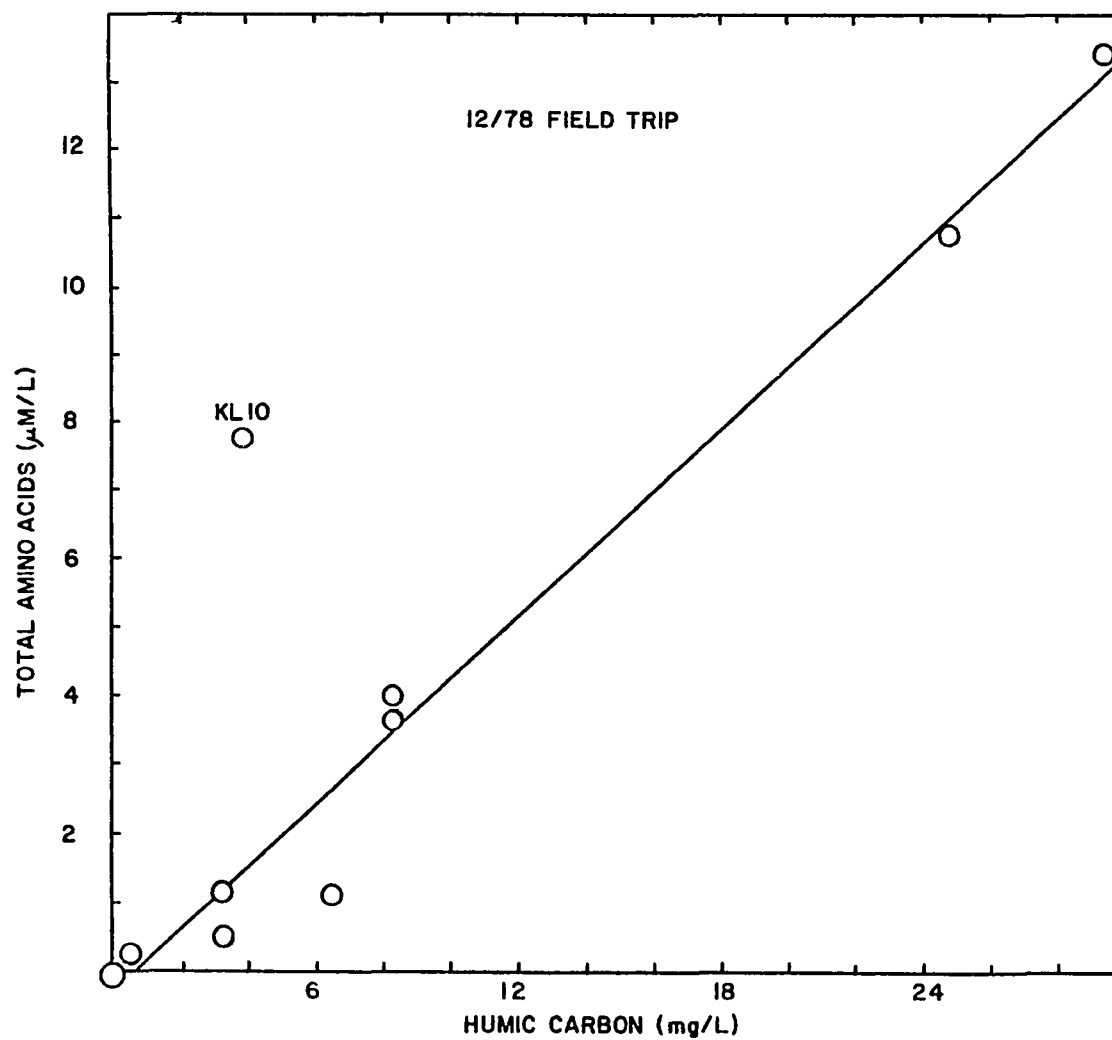


Figure 7. Total amino acids versus humic carbon for the December 1978 field trip.

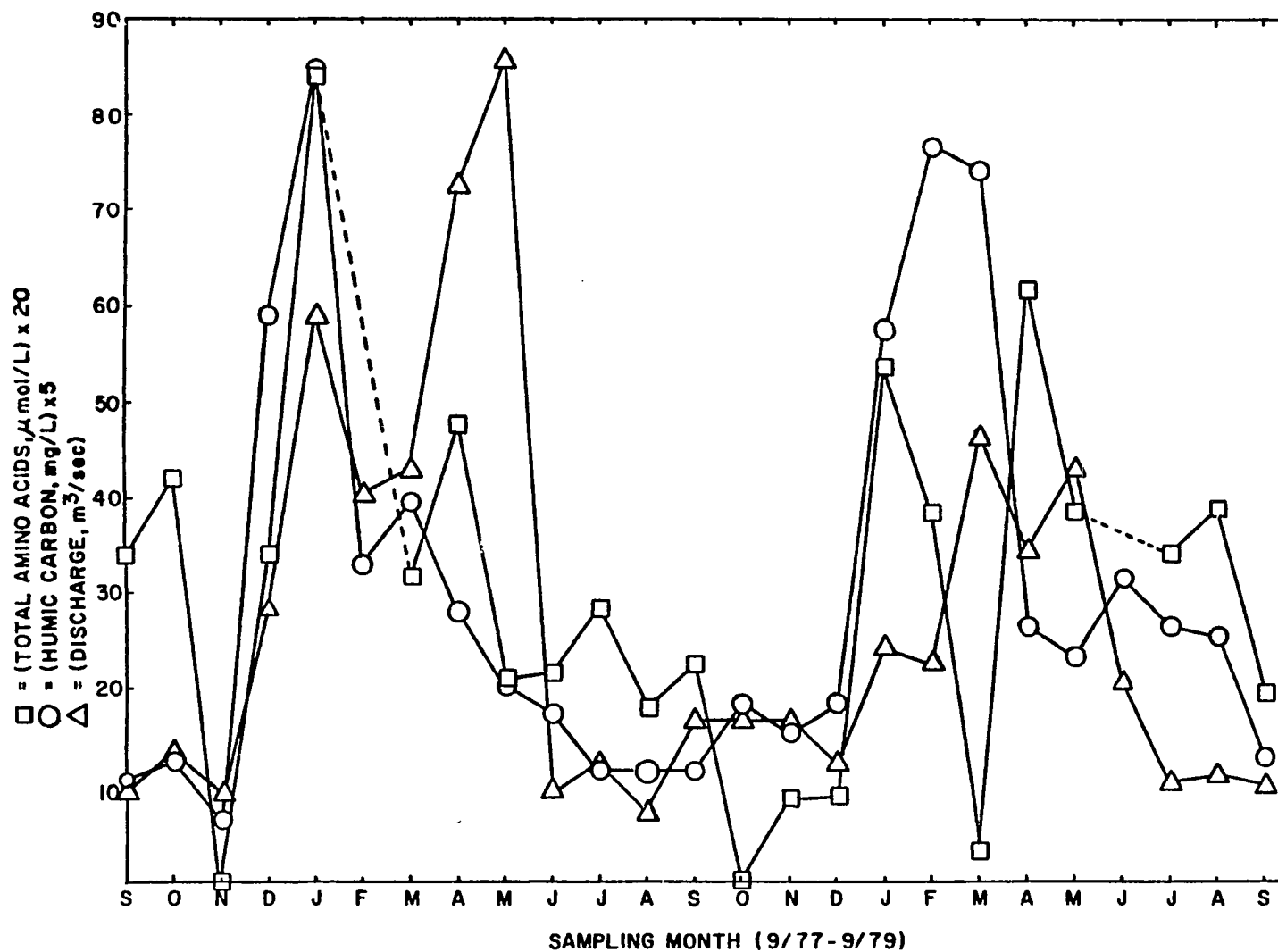


Figure 8. Discharge, humic carbon, and total amino acids at Sprague River over two years.

macroreticular resin XAD-7 was devised. Macroreticular resins have been used to isolate humic substances from seawater (Mantoura and Riley, 1975; Stuermer and Harvey, 1978) and freshwaters (Weber and Wilson, 1975), and their relative performances have been recently compared (Aiken et al., 1979). Three sampling sites were chosen for the study: WR-21, well before the marsh where the Williamson is a clear, dilute stream; WR-32, directly in the marsh; and WR-50, immediately after the marsh. The results of the fractionations are shown in Figure 9. In all three cases, the humic-associated amino acids were $\geq 96\%$ of the total dissolved amino acids. The amounts of free amino acids were below detection limit, implying that the remaining dissolved amino acids were either proteinaceous or possibly humic-associated amino acids that had bled through the XAD-7 column. The high percentage of particulate amino acids at WR-21 can be accounted for by the comparatively high concentration of diatoms at this site. Cell counts average about 8,000 cells/mL (mostly Navicula ssp.), while WR-32 and WR-50 average about 1,700 cells/mL (mostly Fragilaria ssp.). At all three sampling sites, humic-associated amino acid carbon accounted for about 1% humic carbon.

The Application of the Two-Component Scatchard Equation to Defined Ligand Mixtures.

Assuming that the metal binding properties of aquatic humic substances could be approximated by a continuum of binding sites, the two-component Scatchard equation (Eq. 18) was used to analyze a hypothetical ligand mixture in which the ligand concentrations, C_i , were normally distributed. This was accomplished by solving Eq. 23 for \bar{v} over

| FRACTION | SAMPLING SITES | | |
|-------------------------------------|----------------------|----------------------|----------------------|
| | WR21 | WR32 | WR50 |
| UNFILTERED | 0.78±0.03 | 1.20±0.14 | 2.38±0.11 |
| PARTICULATE" (% of unfiltered) | → 0.32 (41%) | → 0.20 (16%) | → 0.15 (6%) |
| FILTERED | 0.46±0.05 | 1.00±0.08 | 2.23±0.07 |
| HUMIC-ASSOCIATED (% of filtered) | → 0.44±0.04 (96%) | → 0.98±0.08 (98%) | → 2.21±0.06 (99%) |
| FREE+PROTEIN | 0.007±0.003 | 0.025±0.005 | 0.026±0.003 |
| PROTEIN" | → 0.007 | → 0.025 | → 0.026 |
| FREE | 0 | 0 | 0 |

"found by difference

Figure 9. Amino acid concentrations (μM)
in river water fractions.

the range $-11.0 \leq \log [M] \leq -3.0$ in increments of 0.1 log units with a constant $C_L = 2.0 \times 10^{-4}$ M and with $\mu = 2.00$ and $\sigma = 3.00$. \bar{K}' was then calculated for each value of $[M]$ using Eq. 15. The corresponding C_M at each value of $[M]$ is given by

$$C_M = [M] + \bar{\nu} C_L \quad (25)$$

The resultant $[M]$ and C_M data were treated as if from a metal-into-ligand titration and subjected to a two-component Scatchard analysis. The point of the exercise was to examine the sensitivity of the Scatchard analysis to the range of C_L , C_M , and $[M]$ values used to obtain the four curve-fitting parameters of the Scatchard equation: K_1 , C_1/C_L , K_2 , C_2/C_L .

To simulate experimental limits of detection of $[M]$, three plots of $\bar{\nu}/[M]$ versus $\bar{\nu}$ were constructed (Eq. 17) assuming that the analytical detection limits for $[M]$ were 1.26×10^{-8} M (Fit 1), 1.26×10^{-7} M (Fit 2), and 1.26×10^{-6} M (Fit 3). Using all the data points from the assumed "detection limit" to the highest value of $[M]$, a weighted, nonlinear regression procedure (Appendix C) was used to fit the resultant curved plots to the two-component Scatchard equation (Eq. 18), yielding the "best" values of K_1 , C_1/C_L , K_2 , and C_2/C_L . The calculated results are tabulated in Table VI and the calculated curves are superimposed on the data in Figure 10.

From the results in Table VI, it is apparent that the values of K_1 and C_1/C_L are particularly sensitive to the "detection limit," a four-fold change in C_M leading to a 20-fold change in K_1 . K_2 and C_2/C_L are relatively less sensitive to changes in C_M because the region

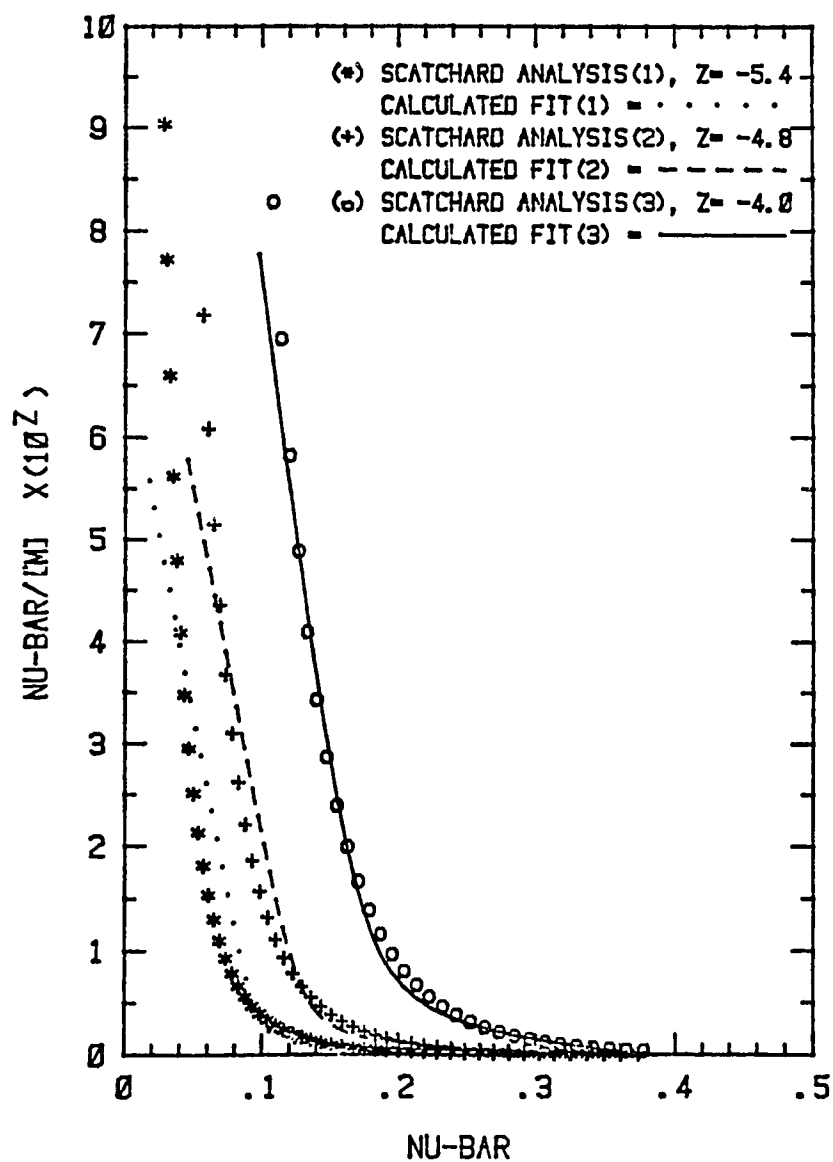


Figure 10. Synthetically-generated and calculated fits for the Gaussian ligand set using the two-component Scatchard equation. Lowest C_M for Fit 1 = $5.72 \mu\text{M}$, Fit 2 = $11.5 \mu\text{M}$, Fit 3 = $22.1 \mu\text{M}$.

TABLE VI

SENSITIVITY OF SCATCHARD EQUATION PARAMETERS TO
TITRATION RANGE FOR THE GAUSSIAN LIGAND SET

| Fit | Lowest [M] | Lowest C_M (μ M) | Log K_1 | C_1/C_L | Log K_2 | C_2/C_L |
|-----|-------------------------|-------------------------|-----------|-----------|-----------|-----------|
| 1 | 1.26×10^{-8} M | 5.72 | 7.26 | 0.094 | 4.52 | 0.243 |
| 2 | 1.26×10^{-7} M | 11.5 | 6.65 | 0.126 | 4.21 | 0.234 |
| 3 | 1.26×10^{-6} M | 22.1 | 6.03 | 0.166 | 3.89 | 0.220 |

of the data set that mainly determine their value (high \bar{v}) is unchanged in the three test cases. It is also clearly the case that K_1 , C_1/C_L , K_2 , and C_2/C_L have no chemical significance because, rather than consisting of two ligands or even two classes of ligands, the data were generated from a continuous Gaussian distribution of "ligands" with an average $\log \bar{K}' = 2.00$.

When the two-component Scatchard equation is applied to laboratory metal-humus titrations, the parameters found at the levels of C_M measurable (typically $\geq 10^{-6}$ M) are assumed to hold at levels of C_M found in the natural aquatic environment ($\leq 10^{-7}$ M), the assumption being that goodness of fit at laboratory levels of C_M will extend to levels of C_M that cannot be experimentally verifiable (Bufflé et al., 1977; Bresnahan et al., 1978; Sposito et al., 1979). This can be directly tested for the computer-simulated titration. In Figure 11, $\log \bar{K}'$ versus $\log (C_L/C_M)$ for the simulated data is plotted as a solid line. This line is still rising at $\log (C_L/C_M)$ values greater than two.

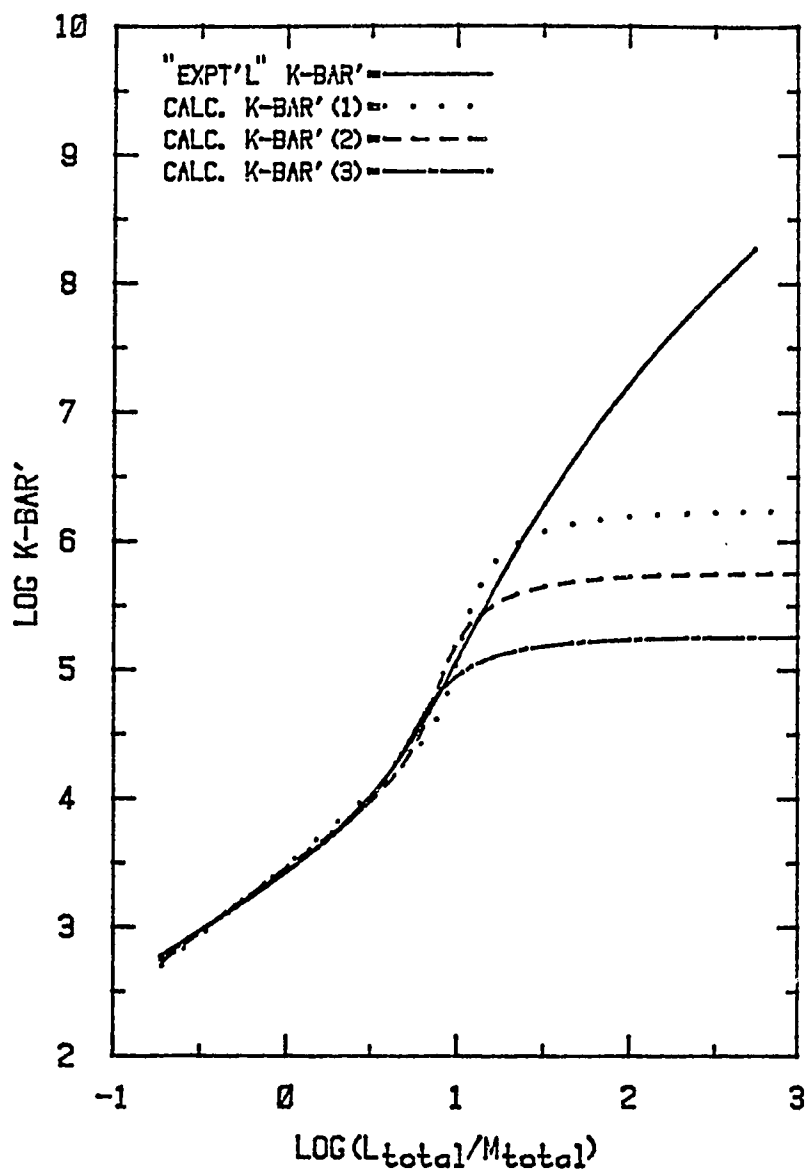


Figure 11. Synthetically-generated $\log \bar{K}'$ values for the Gaussian ligand set and $\log \bar{K}'$ values calculated from the two-component Scatchard equation. Lowest C_M for Fit 1 = $5.72 \mu\text{M}$, Fit 2 = $11.5 \mu\text{M}$, Fit 3 = $22.1 \mu\text{M}$.

(In natural waters containing 10 mg C/L of aquatic humus and $C_M = 10^{-8}$ M, $\log (C_L/C_M)$ is approximately three.) In contrast, all three Scatchard-fitting curves level off at $\log \bar{K}'$ values that are several orders of magnitude too low. As a result, the concentration of uncomplexed metal, $[M]$, at a $\log (C_L/C_M)$ of three (2.5×10^{-12} M) is not accurately predicted by the Scatchard fits. The best predicted $[M]$ value (1×10^{-9} M), which is obtained from Fit 1 with the lowest "detection limit," is still more than two orders of magnitude too high. In that fit, a departure from the gradual increase of $\log \bar{K}'$ of the Gaussian data set is seen. In fact, if the "detection limit" is lowered even further, the Scatchard curve will rise vertically near $\log (C_L/C_M) = 1$. It must be pointed out that the validity of this last criticism rests on the experimentally unproven assumption that the metal binding sites of aquatic humus can be approximated by a continuous function of individual binding sites whose concentrations are normally distributed with respect to some average $\log K'_i$.

To address this problem, a second test was conducted employing the Fortran program MINEQL (Westall et al., 1976). A copper titration was simulated into a twenty-three ligand mixture ($C_L = 2.0 \times 10^{-4}$ M) at pH 5.0 and $I = 0.1$ M NaNO_3 . Total copper was varied from 10^{-8} M to 10^{-3} M in steps of 0.1 log unit. The choice of ligands and concentrations was designed to produce a gradual titration curve and to avoid complexes of greater than 1:1 stoichiometry. Within these constraints, ligand concentrations were weighted normally about the average log copper binding constant (4.58). The individual ligands, K'_i , and C_i values are summarised in Table VII. The actual concentration of every chemical spe-

TABLE VII

LIGAND SET FOR THE MINEQL
SIMULATED COPPER TITRATION

| Ligand | Log K' | Conc. (μ M) |
|-----------------|--------|------------------|
| Acetate | 1.65 | 10.45 |
| Alamine | 3.58 | 11.01 |
| Arginine | 3.58 | 11.01 |
| Aspartate | 3.98 | 10.83 |
| Citrate | 6.37 | 9.05 |
| DCTA | 14.66 | 1.87 |
| EDTA | 12.38 | 5.41 |
| Ethylenediamine | 3.78 | 9.48 |
| Glutamate | 3.41 | 11.01 |
| Glycine | 3.58 | 11.01 |
| Histidine | 6.52 | 7.93 |
| Isoleucine | 3.68 | 10.92 |
| Leucine | 3.68 | 10.92 |
| Norcardamine | 3.10 | 6.72 |
| NTA | 8.34 | 3.64 |
| Ornithine | -2.42 | 0.09 |
| Oxalate | 6.14 | 8.67 |
| Phenylalanine | 3.68 | 10.92 |
| Phthalate | 2.99 | 11.01 |
| Salicylate | 2.26 | 10.45 |
| Sulfosalicylate | 3.44 | 11.01 |
| Tartrate | 3.37 | 10.55 |
| Valine | 3.48 | 11.01 |

cies at each "titration" point is given in the program output, so the system is essentially defined and is easily reproduced by other researchers with access to MINEQL. The given C_M and calculated $[M]$ data were subjected to a two-component Scatchard analysis for three "detection limits" for C_M : Fit 1 = 6.31 μ M, Fit 2 = 12.6 μ M, and Fit 3 = 20.0 μ M. Again, the $\bar{v}/[M]$ versus $[M]$ plots were analyzed with a weighted, nonlinear regression fit to Eq. 18, yielding the "best" values of K_1 , C_1/C_L , K_2 , and C_2/C_L . The calculated results are tabulated in Table VIII, and

the calculated curves are superimposed on the data in Figure 12. The three data subsets above were also analyzed with a weighted, nonlinear regression fit to the Gaussian equation (Eq. 23). The "best" values of μ and σ are also tabulated in Table VIII.

TABLE VIII

GAUSSIAN AND SCATCHARD FITS FOR THE COPPER-
TWENTY-THREE LIGAND MINEQL SIMULATED TITRATION

| Fit | Lowest C_M (μM) | Gaussian Fit | | Scatchard Fit | | | |
|-----|--------------------------|--------------|----------|---------------|-----------|-----------|-----------|
| | | μ | σ | Log K_1 | C_1/C_L | Log K_2 | C_2/C_L |
| 1 | 6.31 | 3.28 | 3.41 | 8.27 | 0.095 | 3.97 | 0.657 |
| 2 | 12.6 | 3.64 | 1.77 | 6.88 | 0.130 | 3.82 | 0.674 |
| 3 | 20.0 | 3.71 | 1.42 | 6.86 | 0.138 | 3.79 | 0.675 |

The predictions of the Scatchard and Gaussian models for $\log \bar{K}'$ for the MINEQL data set were compared using that portion of the data set for which the lowest $C_M = 6.31 \mu M$. The "best" values of the respective curve-fitting parameters are given in Table VIII, and the calculated $\log \bar{K}'$ versus $\log (C_L/C_M)$ for each model is shown in Figure 13.

Neither model is very effective at describing the simulated curve at both the low C_L/C_M values found in most laboratory studies and the high C_L/C_M values that typify natural waters. The Gaussian model is more successful in the extrapolation to high C_L/C_M values, even though it contains only half as many curve-fitting parameters as the two-component Scatchard model. This is interesting in view of the fact that the MINEQL ligand set is not continuous and non-Gaussian. The flat region

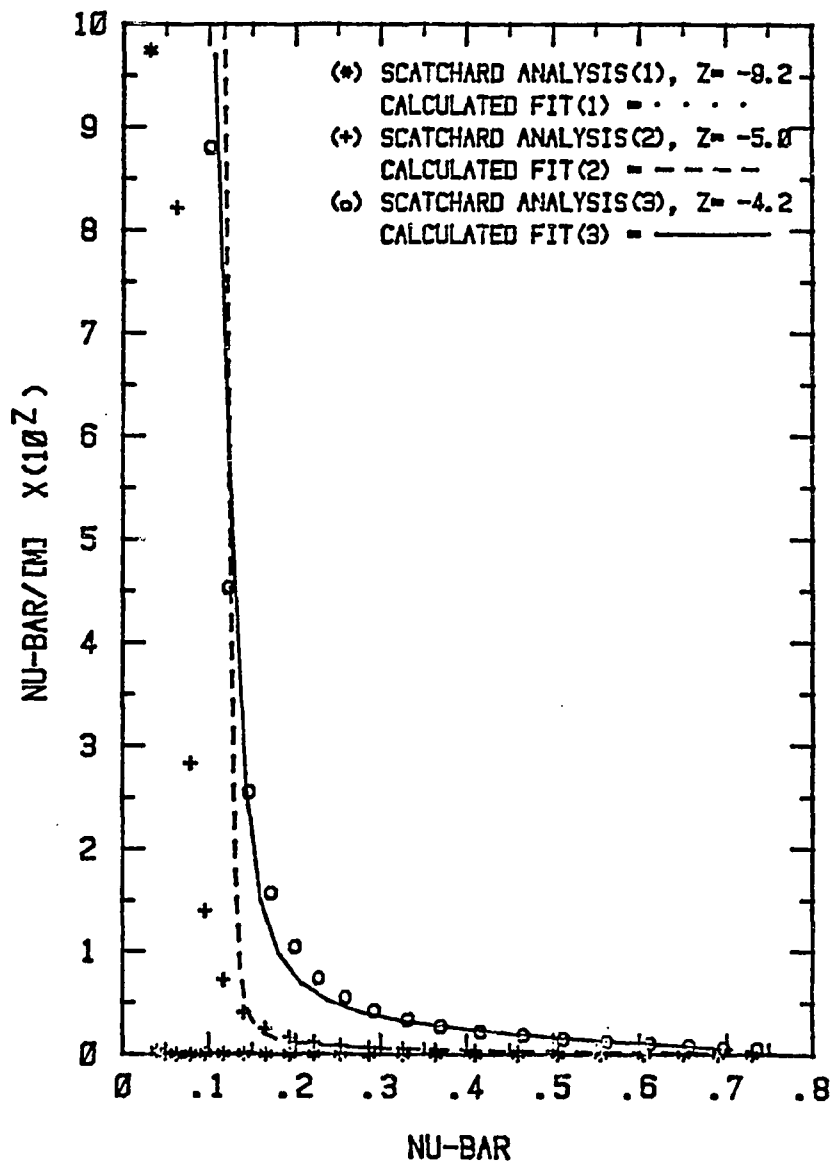


Figure 12. Synthetically-generated $\bar{\nu}$ and calculated fits for the MINEQL ligand set using the two component Scatchard equation. Lowest C_M for Fit 1 = $6.31 \mu\text{M}$, Fit 2 = $12.6 \mu\text{M}$, Fit 3 = $20.0 \mu\text{M}$.

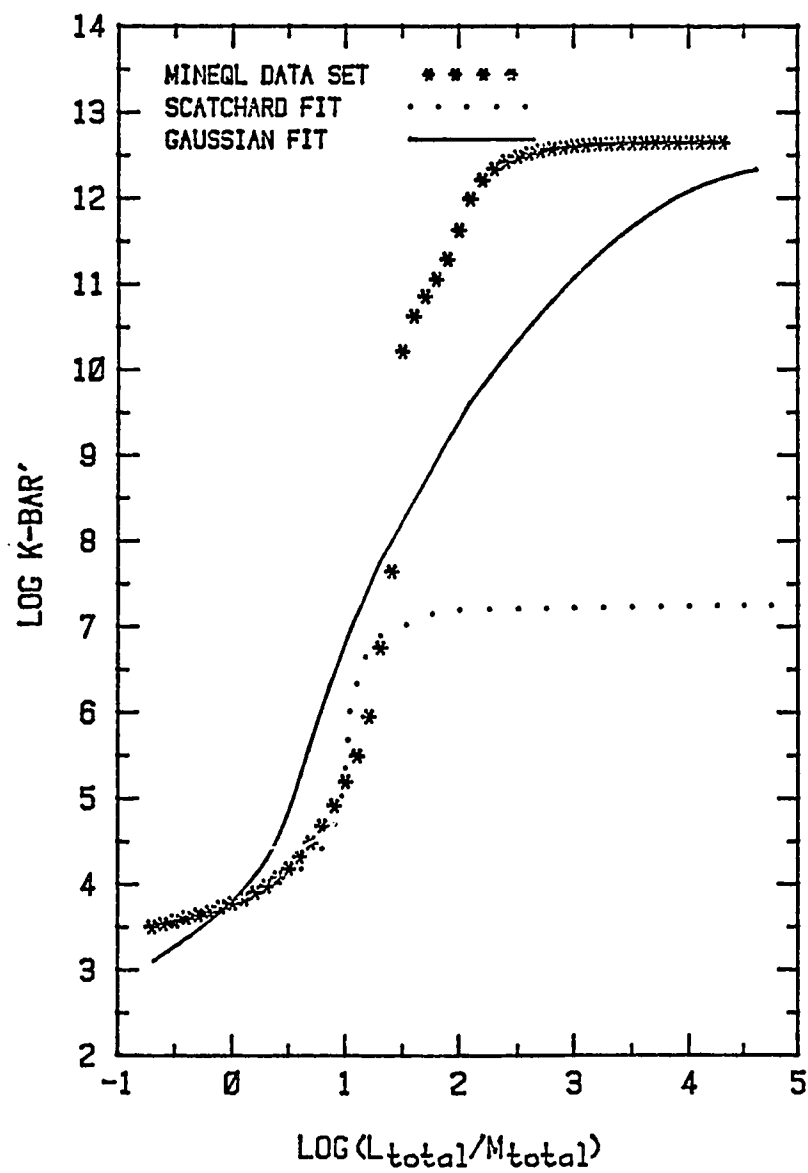


Figure 13. Simulated $\log \bar{K}'$ values and calculated fits for the MINEQL ligand set using the Scatchard and Gaussian models. Lowest C_M in data subset used to find fitting parameters = $6.31 \mu\text{M}$.

of the $\log \bar{K}'$ above a $\log (C_L/C_M)$ of three is due to the titration of the strongest ligand in the data set.

Humic substances are almost certainly intermediate in complexity between the discrete, twenty-three ligand MINEQL simulation and the continuous distribution of ligands exemplified by the Gaussian simulation. Intuitively, a distribution model is probably a better representation of humic substances, even if the actual binding site distribution in humus is non-Gaussian.

The last area to be examined in the application of the Scatchard model to metal binding with aquatic humus is the assertion that the successful application of the two-component Scatchard model results in two average constants for two types of binding sites (cf Sposito, 1981). The presence of two types of acidic functional groups in humus, carboxylic and phenolic/salicylic, has been well-established (Stevenson and Butler, 1969; Schnitzer and Khan, 1972). This knowledge has been used to support the idea that average metal binding constants for these two groups exist and can be successfully found via application of the two-component Scatchard equation to metal-humus titration data. The theoretical discussion in Chapter III showed that, while classes of binding sites may well exist in humic substances, any average binding "constant" will vary during the course of a metal-humus titration. The parameters derived from any truncated discrete model will be constant only if every binding site within a class has the same metal binding constant.

The postulated inadequacy of discrete models was tested by representing "classes" of ligands or binding sites as normal distributions. A system containing two discrete ligands ($\log K_1 = 6.16$, $C_1/C_L = 0.21$,

$\log K_2 = 4.22$, $C_2/C_L = 0.79$) and a system containing two normally distributed classes of ligands ($\theta = 0.21$, $\mu_1 = 6.16$, $\mu_2 = 4.22$, $\sigma_1 = \sigma_2 = 1.0$) are shown in Figure 14, in which the distribution of ligands with respect to $\log K_1$ values is given. The values for $\log K_1 = \mu_1$ and $\log K_2 = \mu_2$ are representative of values reported in the literature for Scatchard analyses of metal-humus complexation reactions (Mantoura and Riley, 1975; Bresnahan *et al.*, 1978; Alberts and Giesy, 1981; McKnight *et al.*, 1982). From Fig. 14, it is apparent that the discrete, two-ligand system becomes equivalent to the bimodal Gaussian system as σ_1 and σ_2 approach zero. Accordingly, the two-component Scatchard equation is expected to more accurately model the nature of the Gaussian system as both σ_1 and σ_2 in that system approach zero.

This expectation was confirmed by generating data sets derived from bimodal Gaussian distributions with variable σ values via Eq. 23, Eq. 24, and Eq. 17, and then fitting these data sets to the two-component Scatchard equation. The Gaussian parameters were as above: $\theta = 0.21$, $\mu_1 = 6.16$, $\mu_2 = 4.22$, and $0.1 \leq \sigma_1 = \sigma_2 \leq 4.0$. The titrations were simulated by allowing $\log [M]$ to vary from -11.0 to -3.0 in steps of 0.2 log units. The Scatchard parameters were determined on each entire data set using a weighted, nonlinear regression analysis to Eq. 18 (Appendix C).

The "best" Scatchard fitting parameters for each of the simulated titrations as a function of the ligand distribution σ value are given in Figure 15. The actual results showed minor random deviations from the smooth curves given in Fig. 15, probably due to the fairly coarse convergence criterion used to minimize computer time in the nonlinear

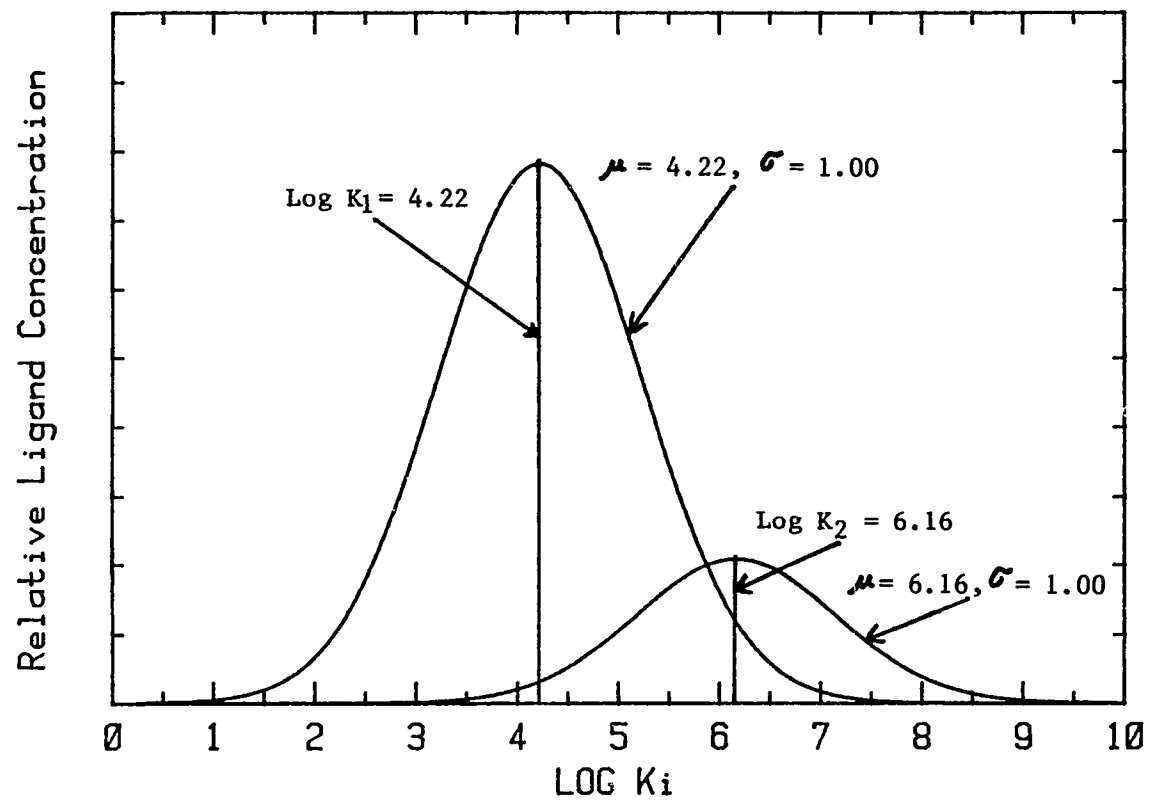


Figure 14. Comparisons of discrete and continuous ligand mixtures.

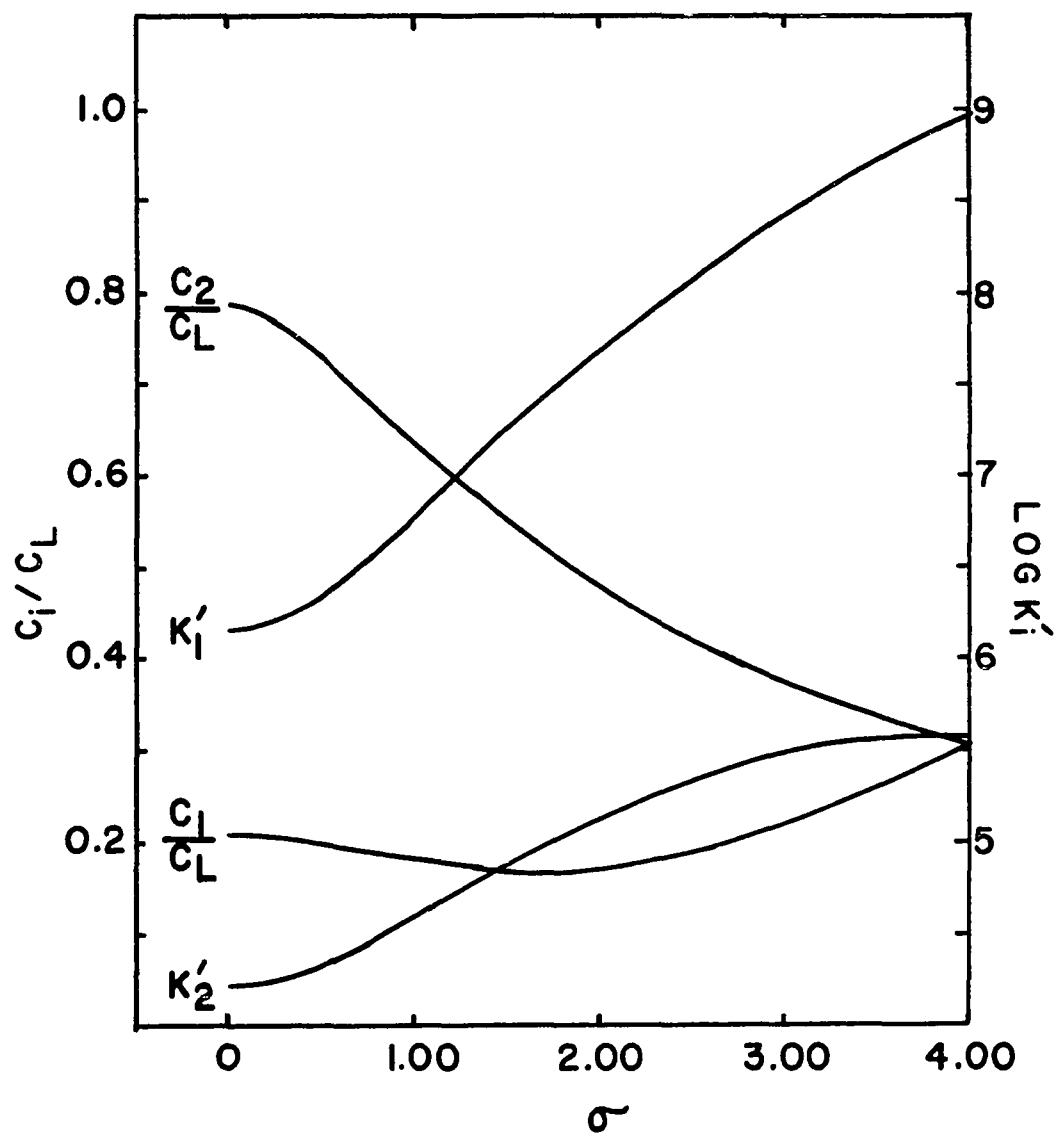


Figure 15. Variation of Scatchard parameters with the standard deviation of a continuous distribution of ligands.

regression program. At very small σ values, the calculated Scatchard parameters ($\log K_1$, C_1/C_L , $\log K_2$, C_2/C_L) are very close to the actual parameters used to generate the bimodal Gaussian distribution (μ_1, θ , $\mu_2, \theta-1$). As σ increases, the Scatchard parameters $\log K_1$ and C_2/C_L deviate dramatically from μ_1 , and $(1-\theta)$. It is clear that the two-component Scatchard model correctly predicts the average binding constants of the two distributions only when σ approaches zero, the limiting case in which a distribution does not in fact exist. For finite values of σ , the Scatchard parameters are in error. Thus the proposition that, even if classes of ligands exist with a finite range of binding constants, the Scatchard equation yields the average constants for classes (Sposito, 1981) is false.

By definition, the sum of the relative abundances of classes of ligands equals one. Likewise, the sum of C_1/C_L and C_2/C_L theoretically equals one if C_L is defined as the total concentration of binding sites. From Fig. 15, the sum of C_1/C_L and C_2/C_L is seen to gradually decrease from 1.0 to 0.6 as σ increases from 0.1 to 4.0. Thus, these fitting parameters cannot possibly represent actual abundances of binding sites.

It was shown above that the two-component Scatchard model does not correctly predict the average binding constants for two distributions of ligands that were used to simulate a metal-humus titration. It can also be shown that, given a system containing two classes of normally distributed ligands, the \bar{K}'_I and \bar{K}'_{II} calculated from the simulated titration data are not constant. A data set was generated using Eq. 24 and Eq. 23 and the parameters $\theta = 0.21$, $\mu_1 = 6.16$, $\mu_2 = 4.22$, $\sigma_1 = \sigma_2 = 1.00$, $C_L = 1 \times 10^{-4}$ M. The values of \bar{K}'_I , \bar{K}'_{II} , \bar{K}' , and \bar{v} were calculated for

the simulated titration for $10^{-11} \text{ M} \leq [\text{M}] \leq 10^{-3} \text{ M}$. The results, given in Figure 16, clearly show that \bar{K}'_I and \bar{K}'_{II} vary continuously and cannot be considered as constants except under extreme excess ligand concentration, as described by MacCarthy and Smith (1979). The $\log K_1$ and $\log K_2$ values from the Scatchard analysis of this data set are 6.77 and 4.60 and are not equivalent to $\log \bar{K}'_I$ and $\log \bar{K}'_{II}$ or to μ_1 , and μ_2 , the mean $\log \bar{K}'$ values of the two classes of ligands. (See Fig. 15.)

The Application of the Continuous Distribution Model to Proton and Copper Binding by Aquatic Humus.

To illustrate the ability of the bimodal Gaussian distribution model to describe proton binding by aquatic humus, the model was used to fit data from a titration of Williamson River humus in 0.1 M NaClO₄ with 1.516 M NaOH. In this case, the definition of \bar{v} , as given by Perdue et al. (1980), is

$$\bar{v} = \frac{(\text{OH}_a - \text{OH}) - (\text{OH}_a^* - \text{OH})}{C_L} \quad (26)$$

where OH_a is the mmols of base added, OH is the mmols of based found, (*) denotes values for a reagent blank titration, and C_L is the total molar acidity of the humus. For the titrations done in this study, (OH_a - OH*) was large and irreproducible at beyond pH 12, presumably due to the large sodium error of the glass electrode (Skoog and West, 1963; Laitinen and Harris, 1975). For this reason, \bar{v} was calculated only for titration points for which the blank was < 1% of (OH_a - OH).

One problem in the direct titration of humic substances is the

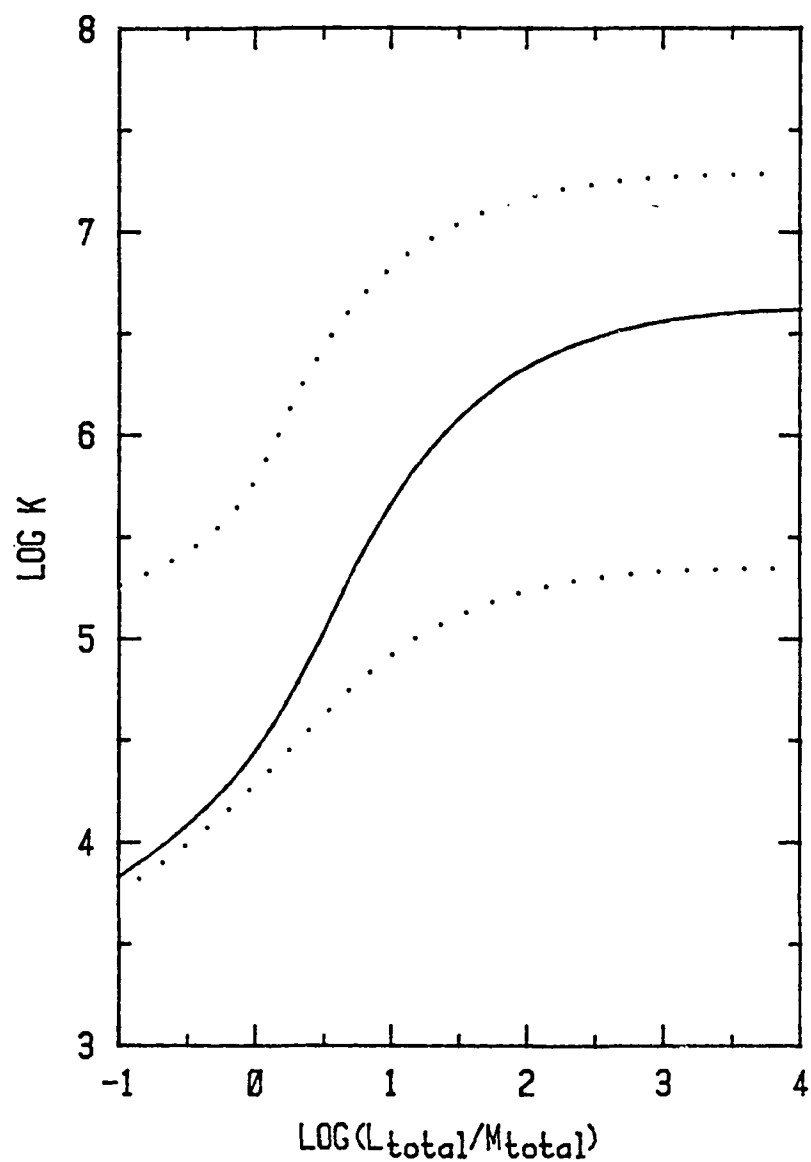


Figure 16. Computed values of \bar{K}' (—) and \bar{K}'_I and \bar{K}'_{II} (....) for a simulated metal titration into a continuous, bimodal Gaussian distribution of ligands.

operational nature of C_L used in Eq. 26. This problem has been thoroughly discussed by Perdue et al. (1980) and is clearly seen in Figure 17, which shows the numerator of Eq. 26 plotted against pH for a titration of the 4500 mg/L solution of Williamson River humus. In this case, C_L is taken as the value of $(OH_a - OH)$ at the last titration point: 0.372 mmols OH or 9.30 mmols OH/g humus. It can be clearly seen that at this point the titration curve is still rising. Thus, the C_L found in this way is at best a lower limit, and the value for C_L and the corresponding values for θ must be considered as operational only. It should be noted that the total acidity of a different sample of Williamson River humus was found to be 9.5 mmols H/g humus by Perdue (1979), using the barium hydroxide method. While this procedure has been historically accepted to yield a measure of all of the acidic hydrogens in humus (Schnitzer and Khan, 1972), it is now thought that this method also underestimates total acidity (Perdue et al., 1980).

An estimate of the upper limit of C_L can be obtained from examining the functional group analysis presented for Williamson River humus by Perdue (1979). Titration calorimetry, which gives a lower limit for carboxyl content (Perdue et al., 1980), yielded a value of 3.3 meq/g humus. The calcium acetate method, which gives a value for carboxyl content that is too high due to analytical problems in the procedure (Perdue et al., 1980), yielded a value of 5.1 meq/g humus. This yields a possible range for carboxyl content of 0.35 - 0.54 of the total acidity. The \bar{v} values from the humus proton titration were varied to reflect a range of C_L values so that the resulting fitting parameter, θ , fell within the above range. (θ is the fraction of C_L in the

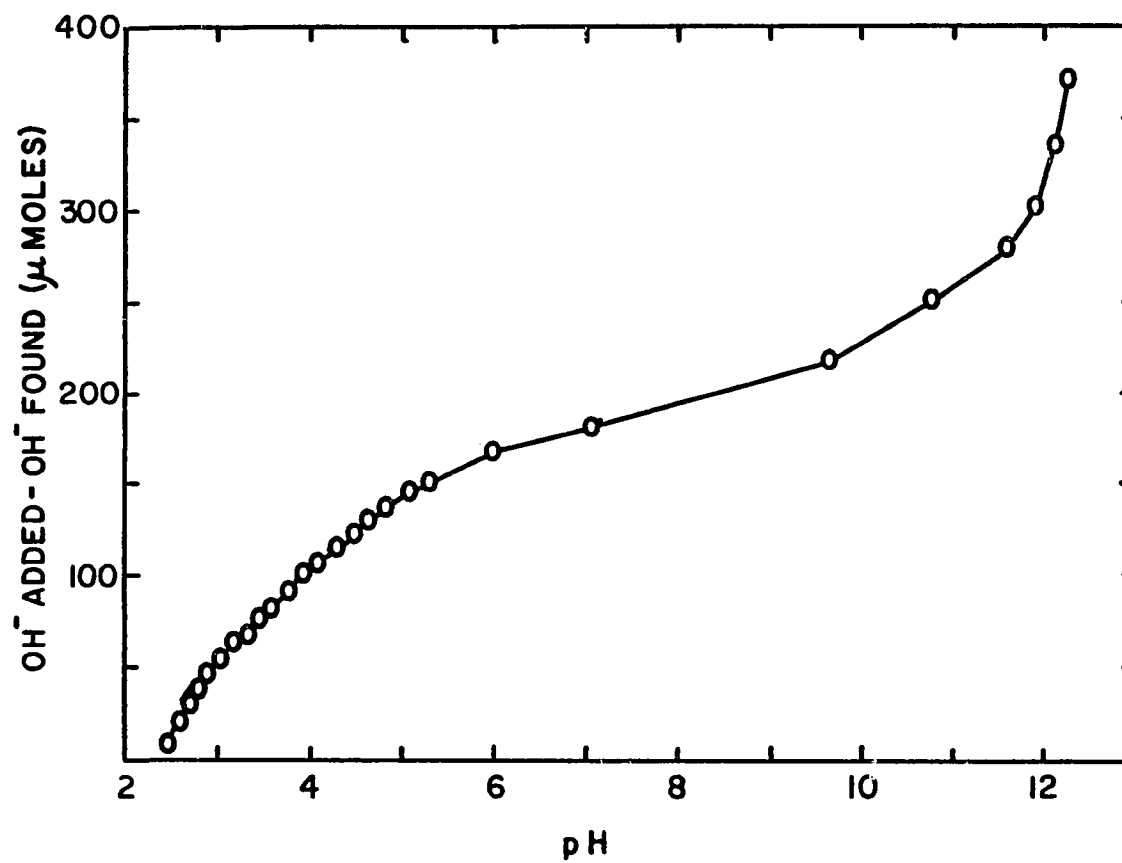


Figure 17. Base-titratable functional groups versus pH for a 4 g/L solution of Williamson River humus in 0.1 M NaClO₄.

first, or lower μ distribution.) The results are given in Table IX. It can be seen that the above range of carboxyl content is approximated by a total acidity range of about 9.0 to 15.0 mmols OH/g humus.

TABLE IX
VARIATION IN BIMODAL GAUSSIAN FITTING
PARAMETERS WITH CHANGE IN C_L

| C_L (mmols H/g humus) | θ | μ_1 | σ_1 | μ_2 | σ_2 |
|----------------------------|----------|---------|------------|---------|------------|
| 9.0 | 0.35 | 3.56 | 0.008 | 10.48 | 2.89 |
| 9.3 | 0.31 | 3.42 | 0.021 | 9.89 | 3.88 |
| 10.0 | 0.34 | 3.16 | 0.52 | 9.72 | 4.21 |
| 11.0 | 0.39 | 2.84 | 0.80 | 9.66 | 4.35 |
| 12.0 | 0.44 | 2.54 | 1.01 | 9.64 | 4.40 |
| 13.0 | 0.48 | 2.27 | 1.16 | 9.63 | 4.44 |
| 14.0 | 0.53 | 1.99 | 1.30 | 9.61 | 4.46 |
| 15.0 | 0.56 | 1.80 | 1.38 | 9.59 | 4.49 |

For the purpose of this research, total acidity will be taken as the midpoint of this range of values, 12.0. The \bar{v} , pH experimental data (assuming $C_L = 12.0$ mmols H/g humus) and the calculated fit (using the "best" parameter estimates for $C_L = 12.0$ given in Table X) are shown in Figure 18. The experimental data are fit to within a relative error of 3% to pH 7. From this point to pH 12, the data are fit less well, most probably due to the lack of data points through the inflection region between pH 7 and 10. Considering that the Gaussian function may not be the most appropriate description of proton binding sites in aquatic humus, the fit was considered to be satisfactory.

Examination of the Gaussian parameters themselves further substan-

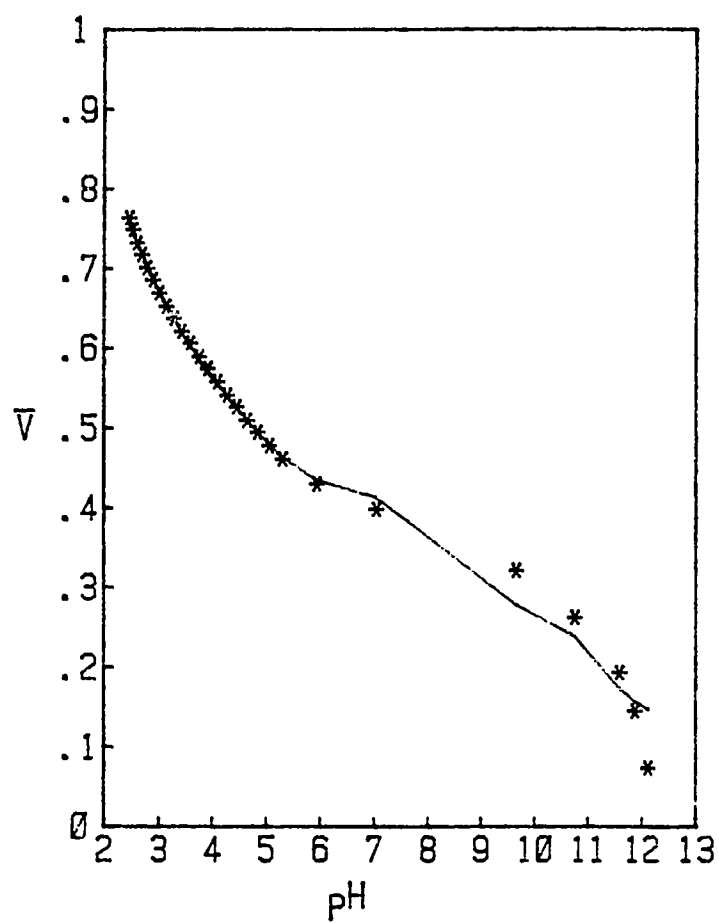


Figure 18. Experimental \bar{V} values (*) and calculated fit (—) for the bimodal Gaussian modeling of a proton titration of Williamson River humus.

tiates the general utility of the model. A plot of the distribution from the $C_L = 12.0$ fitting parameters is given in Fig. 19. The mean pK_a values of the two classes of functional groups are consistent with the known properties of carboxyl groups ($\mu_1 = 2.54$) and phenolic hydroxyl groups ($\mu_2 = 9.64$), both of which are thought to be responsible for the acidic properties of humic substances (Stevenson and Butler, 1969; Schnitzer and Khan, 1972). The mean pK_a of the phenolic group is lower than would be expected. This is again most probably due to the paucity of data in the inflection region of the titration curve. Finally, when the estimated total acidity (12.0 mmols H/g humus) is multiplied by theta, a carboxyl content of 5.3 mmols/g is obtained. Although this value is slightly higher than expected (Perdue et al., 1980), it is not unreasonable.

The applicability of the Gaussian distribution model to copper binding by humic substances was evaluated using two data sets for titration of humus with Cu(II) at pH 5.0 and $I = 0.1 \text{ M NaClO}_4$. The first data set was obtained in this laboratory using aquatic humus from the Williamson River, Oregon. The second data set was constructed from the Scatchard fitting parameters published by Sposito et al. (1979) for sewage sludge-derived fulvic acids (titration #1 in their paper). Both data sets were fit reasonably well by a single-mode Gaussian distribution model (one class of ligands), although minor improvement in the degree of fit was obtained with the bimodal distribution model.

The single-mode results are given in Fig. 20 and Fig. 21 for the Williamson River humus titration ($\mu = 4.15$, $\sigma = 1.20$) and that of

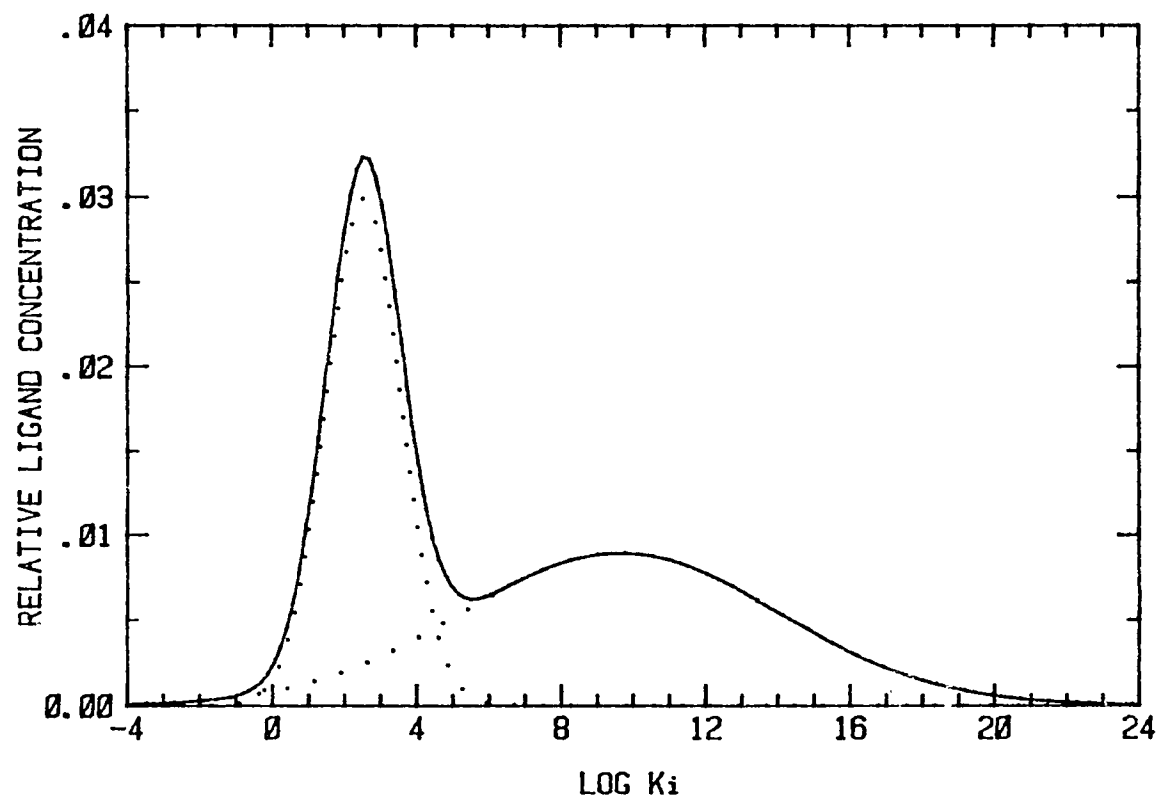


Figure 19. Bimodal Gaussian distribution that yielded the "best" fit for the proton-humus titration.

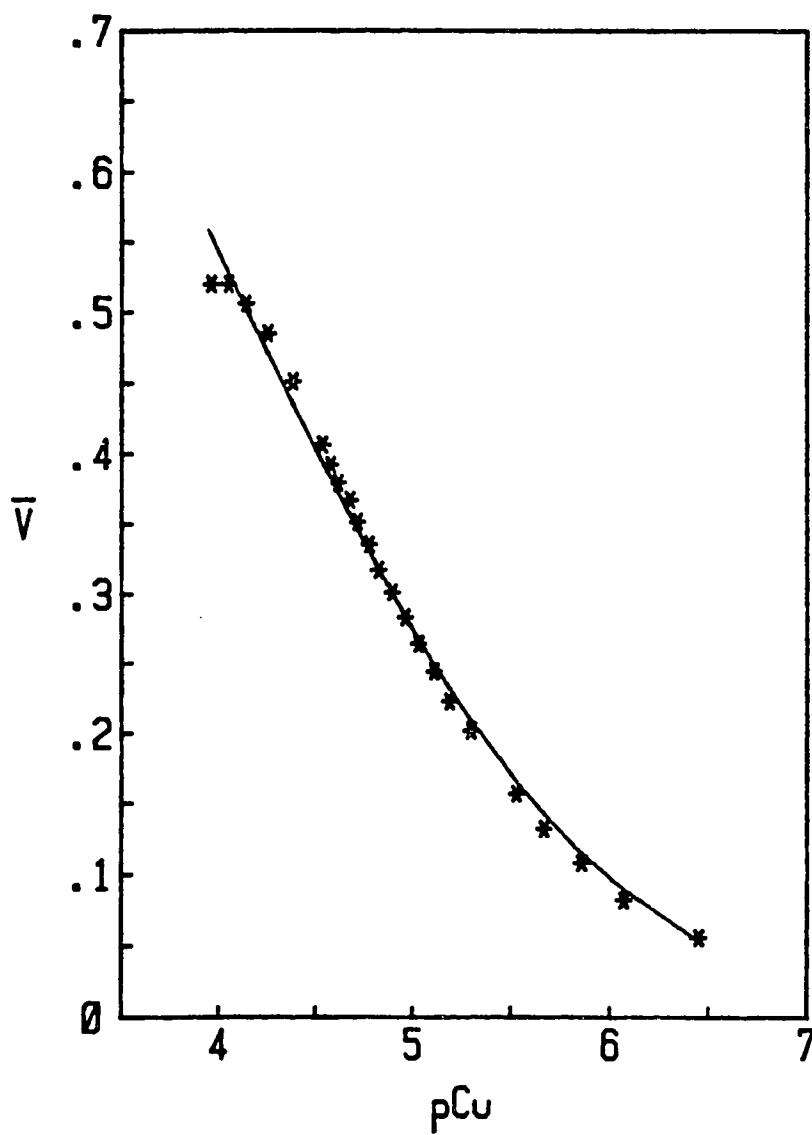


Figure 20. Experimental values (*) and calculated fit (—) using the Gaussian distribution model for a copper titration into Williamson River humus.

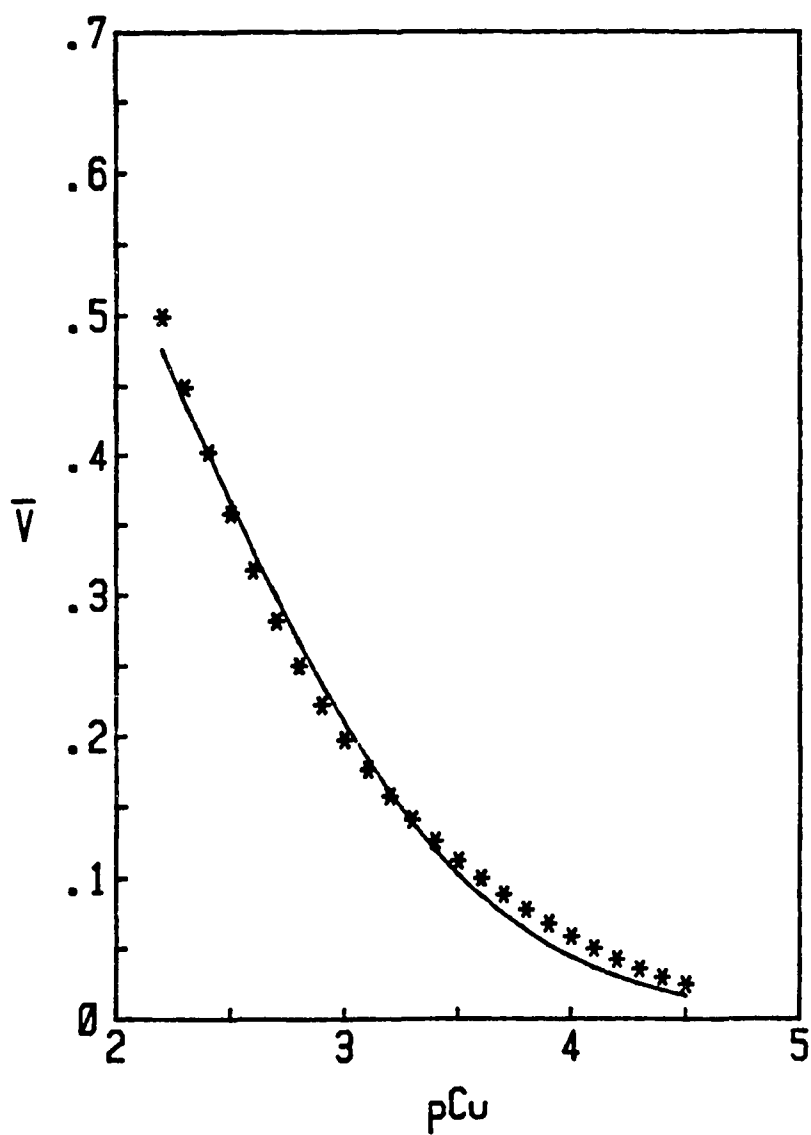


Figure 21. Data constructed from the Scatchard parameters of Sposito *et al.* (1979) (*) and calculated fit (—) using the Gaussian distribution model.

Sposito et al. ($\mu = 2.14$, $\sigma = 0.76$), respectively. In both cases, the agreement between calculated and experimental \bar{v} values is good. It is particularly gratifying to note that the "experimental" points in Fig. 21, which were generated using four fitting parameters in the two-component Scatchard equation, can be described quite well with the two fitting parameters of the Gaussian distribution model. The μ value for aquatic humus is much higher than that for sewage sludge derived fulvic acid. The relatively weaker affinity of the latter material for Cu(II) is also indicated if the Scatchard parameters of Sposito et al. (1979) are compared with results of other workers on soil and water fulvic acids (e.g., Bresnahan et al., 1978). In both cases, the σ values were relatively small, indicating that most Cu(II)-binding ligands are roughly comparable in Cu(II)-binding strength.

The exceedingly complex mixture of ligands that are involved in proton binding and metal binding by aquatic humus cannot be unambiguously described by any type of chemical model that is currently available. It is nevertheless possible to approximate the variation of \bar{v} with $[H^+]$ or $[M]$ using a variety of chemical models, none of which is totally appropriate. Given the complexity of the ligand mixture, there can be no doubt that discrete models that postulate the existence of two or three distinct binding sites with unique equilibrium constants for proton or metal binding are fundamentally incorrect from both a chemical and a mathematical point of view. In contrast, continuous distribution models that postulate the existence of one or two classes of ligands whose individual concentrations are a function of binding strength are at least consistent with the known complexity of the ligand

mixture.

Both proton and metal binding by aquatic humus are efficiently described by the Gaussian distribution model. Even data that were generated from the two-component Scatchard equation (with four fitting parameters) were successfully modeled using a Gaussian distribution with two fitting parameters. In all likelihood, this approach toward modeling the behavior of complex ligand mixtures in homogeneous solutions should also find application in studies of proton and metal binding in complex heterogeneous systems such as aqueous suspensions of particulate humic substances, amorphous metal oxides, etc.

The Complexation Capacity of Williamson River Humus

As in the case for proton binding, there is ambiguity in the definition of total ligand for metal binding to humic substances (Langford et al., 1979). This ambiguity arises from the difficulty in assigning a molecular weight to the complex humic mixture. Researchers generally follow the procedure of Gachter et al. (1973) in which a sample of humus is titrated with metal. The titration is carried out until hopefully all the possible metal binding sites on the humic polymer are saturated. The data are plotted as free metal versus total metal, as shown in Fig. 22A. The resultant curve is nonlinear while the humus is complexing metal, but becomes linear after the humus is saturated with metal. This latter linear portion of the curve is extrapolated back to the X-axis, and the X-intercept is used as the operational definition of total ligand concentration as amount of metal bound per unit weight of humus. This method has been used in direct titrations (Chau et al., 1974; Ernst et al., 1975; Shuman and Woodward, 1977; Baccini and Suter,

1979), dialysis titrations (Truitt and Weber, 1981), and ion-exchange titrations (Crosser and Allen, 1978). The data can also be presented as \bar{v} or ($C_M - [M]$) versus total metal (Gamble *et al.*, 1980), as shown in Fig. 22B. The resultant curve approaches an asymptote as the humus becomes saturated. The asymptote is extrapolated back to the Y-axis, and total ligand concentration is taken either directly as the intercept value or as the intercept divided by the weight of humus used.

Data for copper titrations into Williamson River humus at pH 5.0, 5.5, 6.0, and 6.5 and $I = 0.1$ M NaClO_4 were analyzed using both the above procedures. The results, given in Table X, clearly show that the

TABLE X
COMPARISON OF METHODS FOR ANALYZING COMPLEXATION
CAPACITY DATA

| pH | Humus (mg C/L) | Max. C_M ($\mu\text{M/L}$) | Trials | Complexation Capacity ($\mu\text{moles Cu/mgC}$) | |
|-----|-------------------|-----------------------------------|--------|--|-----------------------|
| | | | | [M] vs. C_M | $C_M - [M]$ vs. C_M |
| 5.0 | 95.5 | 1200 | 1 | 2.10 | 3.49 |
| | 38.2 | 400 | 3 | 1.83 ± 0.19 | 2.11 ± 0.01 |
| 5.5 | 38.2 | 400 | 3 | 2.25 ± 0.10 | 2.96 ± 0.11 |
| 6.0 | 38.2 | 400 | 3 | 2.64 ± 0.02 | 3.45 ± 0.12 |
| 6.5 | 3.82 | 40 | 3 | 2.33 ± 0.40 | 2.45 ± 0.23 |

method of plotting free metal versus total metal gives lower estimates of total ligand than the method of plotting ($C_M - [M]$) versus total metal. This is explained by examining the data from the complexation capacity plots. In general, although the plots of free metal versus total metal appeared linear for high values of total metal (over 13 titrations, slope = 0.874 ± 0.076 and $r = 0.9994 \pm 0.0003$ for the last 10 data

points), $C_M - [M]$ was still increasing over most of these same, high total metal points. This is exemplified in Fig. 22, which shows data from titration #2 at pH 5.0. The line extrapolated to the X-axis in Fig. 22A has a slope of 0.980 and a correlation coefficient of 0.9997 and yields a complexation capacity of $1.69 \mu\text{moles Cu/mg humic carbon}$. The $C_M - [M]$ plot of this same data, shown in Fig. 22B, yields a value of 2.12 when fit on the SMPLX program (Appendix B). For the same range of metal added, the latter method allows a closer estimate of the complexation capacity. For this reason, the results from the former method were not considered.

Examination of the data in Table X shows a dependence of the complexation capacity upon the total amount of humus in solution. The dependence of the degree of association for the general reaction $M^+ + A^- = MA$ is a fundamental concept of equilibrium chemistry, the rule being that the fraction of total M associated decreases as the concentration of MA decreases (Laitinen and Harris, 1975). The effect is illustrated in Fig. 23, which shows \bar{v} (complexation capacity) versus C_M/C_L for a simple copper-oxalic acid binding reaction at three different concentrations of oxalic acid. As C_L decreases, the curves flatten out at lower values of \bar{v} , and any estimate of complexation capacity will decrease.

This fact has been overlooked in the study of aquatic humus. Since C_L is used to determine the overall \bar{K}' of metal humus binding reactions (Eq. 14 and 15), \bar{K}' would be a function of total ligand concentration when C_L is determined in this manner. This problem was found by Bufflé and co-workers, who postulated a number of hypothetical reactions to

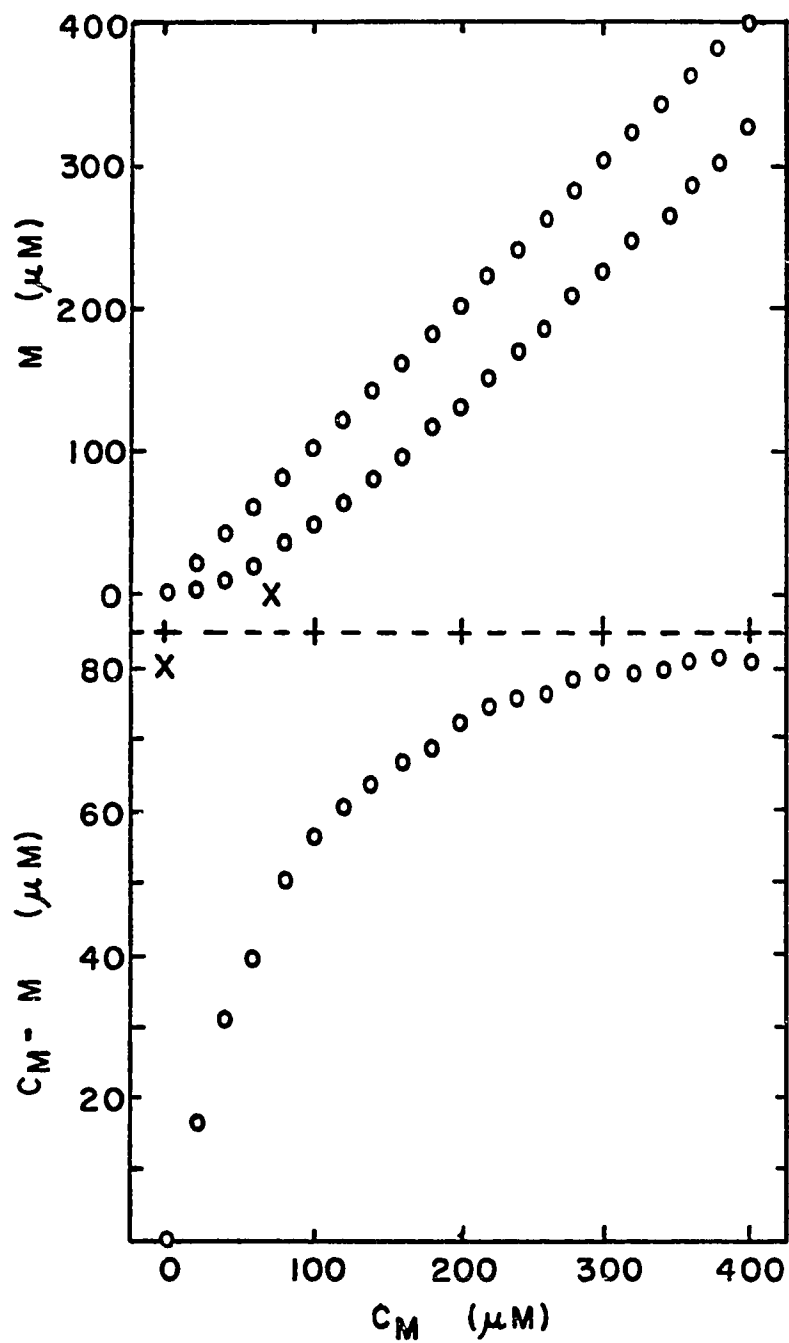


Figure 22A, 22B. Complexation capacity of plots for pH 5 titration #2. Part A (top): free metal versus total metal. Part B (bottom): $C_M - [M]$ versus total metal. X = extrapolated complexation capacities.

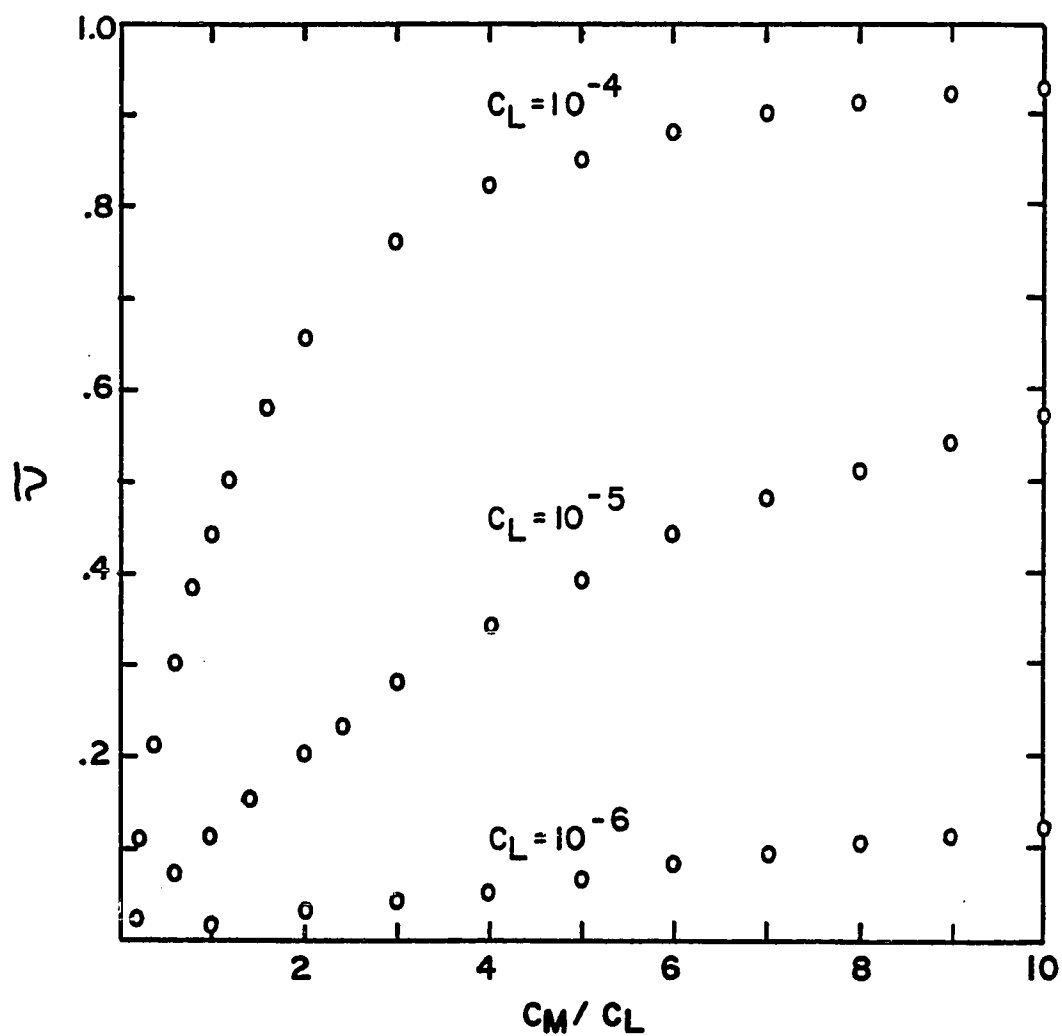


Figure 23. $\bar{\nu}$ versus C_M/C_L at various C_L for copper binding to oxalic acid.

explain variations in \bar{K}' with C_L , none of which proved to be completely satisfactory. (See Chapter III discussion of discrete models and references therein.)

Thus the data in Table X are not unexpected. If $(C_M - [M])$ is normalized by dividing by the amount of humic carbon and plotted against free metal, the C_L dependency is removed. This is shown, for all 13 complexation capacity titrations, in Fig. 24. Now the anomalously high pH 5 titration is grouped with the other pH 5 titrations, which were done at about one-third the value of C_L . Likewise, the pH 6.5 data do not fall between the pH 5.0 and 5.5 groups, as would be expected from the results in Table X. It should be noted that C_L for Fig. 24 is defined as g/L of humic carbon and that C_M is corrected for the presence of hydroxide species by the use of a reagent blank titration. The curves in Fig. 24, which are of the same form of those in Fig. 18, 20, and 21, clearly show that, like the proton binding curve in Fig. 17, total ligand found is in reality the Y-axis value at the last titration point. Even though plots of the same data done as in Fig. 22B show a leveling-off, Fig. 24 clearly shows that this is not the case, and that any values for the complexation capacity obtained from such former plots are in error.

Given the analytical restraints on achieving high concentrations of free metal in solution at pH values close to natural waters conditions, titrations such as those shown in Fig. 24 cannot be continued to the point that the complexation capacity becomes a constant. It is true for a discrete ligand and can be shown for a Gaussian distribution of ligands, that the inflection point in a plot of \bar{v} versus $\log [M]$ occurs

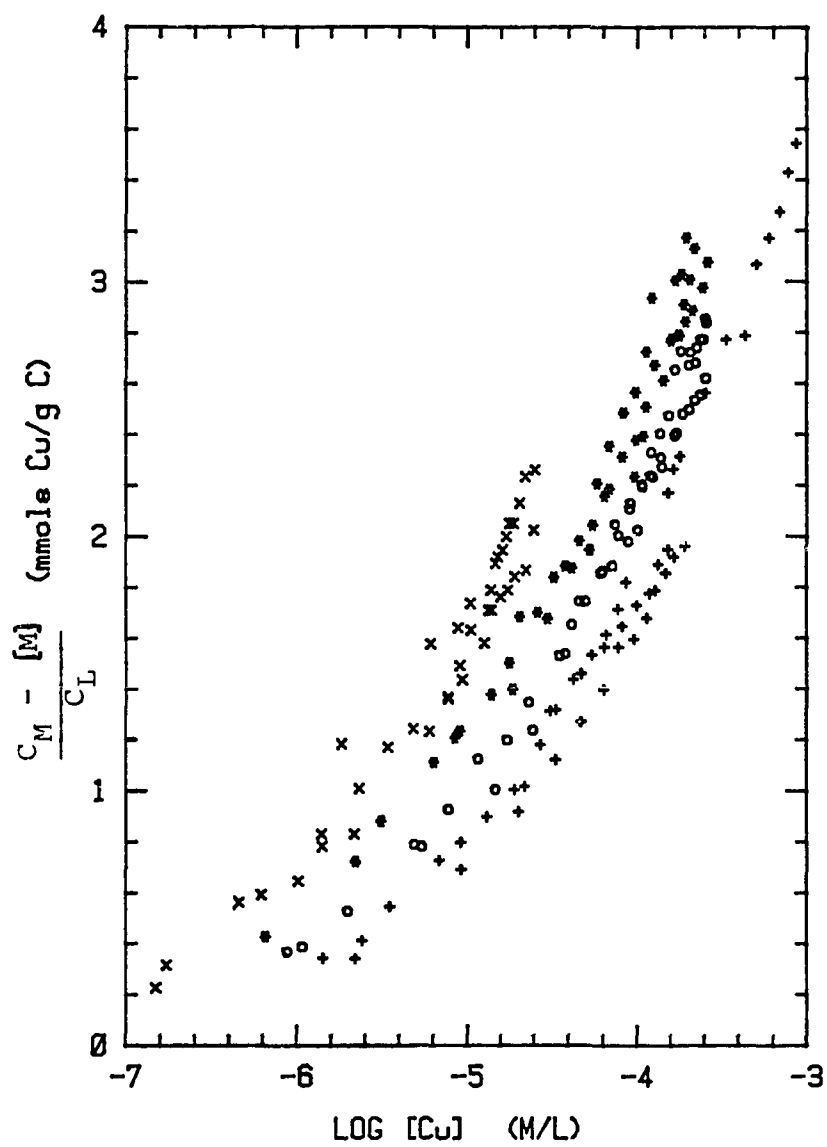


Figure 24. \bar{v} versus pM for copper binding to Williamson River humus at pH 5.0(+), 5.5(o), 6.0(*), 6.5(x).

at $\bar{v} = 0.5$. If the highest value in Fig. 24 is taken as being close to this inflection point, twice this value is an estimate of the lowest possible value for the complexation capacity. This lower estimate is then $7.2 \mu\text{mols Cu/mg humic carbon}$. It was shown in the proton binding discussion that the estimated maximum total acidity for Williamson River humus is about $15.4 \text{ mmols H}^+/\text{g humus}$. Thus the estimated maximum complexation capacity would be $7.7 \mu\text{mols Cu/g humus}$, or $15.4 \mu\text{mols/mg humic carbon}$. Thus the range would be : $7.2 \leq C_L \leq 15.4$, in units of $\mu\text{mols Cu/mg humic carbon}$. For the purposes of this research C_L will be taken as the midpoint of this range, $11.3 \mu\text{mols Cu/mg humic carbon}$. It is important to note that C_L is the concentration of all possible binding sites. The competition between protons and metal for these sites will be a function of pH, but the value of C_L will not be.

It was shown in Table IX that the bimodal fitting parameters are a function of C_L when modeling humus proton binding. Since C_L for copper binding must also be expressed as a range of possible values, the variation of the Gaussian fitting parameters with C_L was investigated using simulated titrations generated from Gaussian fitting parameters obtained from the copper-humus titration shown in Fig. 20. Titrations were simulated assuming a continuous Gaussian ligand distribution of $\mu = 4.15$ and $\sigma = 1.20$ via Eq. 16 and 23. C_L was initially set at 2.0×10^{-4} and increased to 2.0×10^{-3} . Each simulated titration was then fit to a single-mode Gaussian model. The results, shown in Fig. 25, show a significant sensitivity to C_L . For a two-fold increase in C_L (the range expected from the above estimate), μ drops from 4.15 to 1.69 and σ increases from 1.20 to 3.18. Thus the fitting parameters

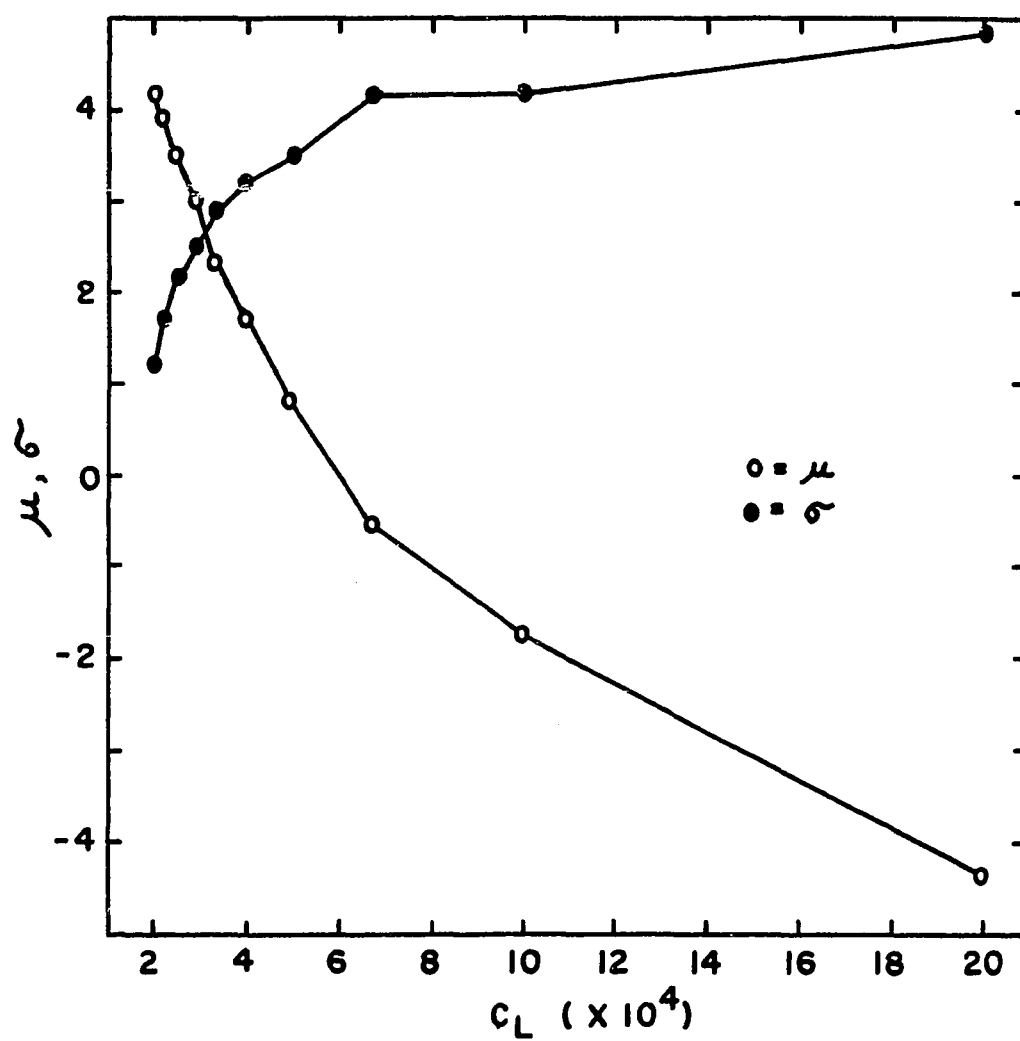


Figure 25. Variability in Gaussian fitting parameters with changes in C_L . Titrations simulated from a continuous Gaussian ligand distribution of $\mu = 4.15$, $\sigma = 1.20$, $C_L = 2 \times 10^{-4}$ M.

resulting from modeling \bar{v} data in which C_L is an estimated value must be interpreted with care.

Copper-Aquatic Humus Stability Function

Determination of Copper-oxalate Binding Constants. The results for the three sets of three copper-oxalate titrations are given in Table XI. Although Student's t-test (Laitinen and Harris, 1975) may be applied to the 10^{-3} M total oxalate data to show that the average result is statistically different from the other two averages, it was felt that the small number of experiments per each total oxalate concentration warranted that all nine determinations be considered as a single group.

TABLE XI
SMPLX DETERMINATIONS OF THE
COPPER-OXALATE BINDING CONSTANTS

| Total Oxalate (M/L) | Log K_1 | Log β_2 | Ave. Log K_1 | Av. Log β_2 |
|------------------------|-----------|---------------|-----------------|-------------------|
| 10^{-3} | 5.43 | 9.83 | | |
| 10^{-3} | 5.43 | 9.82 | 5.42 ± 0.01 | 9.83 ± 0.01 |
| 10^{-3} | 5.41 | 9.83 | | |
| 10^{-4} | 5.78 | 10.25 | | |
| 10^{-4} | 5.74 | 10.34 | 5.76 ± 0.02 | 10.27 ± 0.06 |
| 10^{-4} | 5.77 | 10.23 | | |
| 10^{-5} | 5.88 | 10.98 | | |
| 10^{-5} | 6.07 | 10.53 | 5.99 ± 0.10 | 10.71 ± 0.24 |
| 10^{-5} | 6.01 | 10.63 | | |

The overall averages calculated in this manner ($\log K_1 = 5.72 \pm .25$ and $\log \beta_2 = 10.27 \pm 0.40$) still vary over a much smaller range than values reported by Sillen and Martell (1964): 4.84 - 6.19 for $\log K_1$ and 8.3 - 10.3 for $\log \beta_2$.

Feasibility of the Copper-oxalate Metal Ion Buffer. A series of copper standards at pH 5.0 and 25.01° C in 0.1 M NaClO₄ were run from 10⁻³ M to 10⁻⁸ M to determine a reasonable lower limit of linearity for the copper ISE. The results, shown in Figure 26, indicate a loss of linearity below 10⁻⁷ M. For the line from 10⁻³ M to 10⁻⁷ M, the slope = 29.04, intercept = 271.1, and $r = 0.9999$. (Theoretical slope at 25.01° C is 29.58.) Thus it was felt that a copper, metal ion buffer with total copper equal to 10⁻⁷ M satisfied the requirement that, in order for response to be linear in the buffer system, total metal must be within the linear operating range of ISE in an unbuffered solution (Blaedel and Dinwiddie, 1974).

Since a total copper of 10⁻⁷ M would be at the extreme edge of ISE linear response, it was decided to test the linearity of the buffer system itself by comparing free copper as measured in an oxalate-into-10⁻⁷ M copper titration and as calculated for the same total copper and oxalate concentrations. The results, given in Table XII, show agreements to within about 1.2% for the range of free copper expected.

Aquatic Humus Titrations into Copper Metal Ion Buffers. For the oxalate-copper buffer system, the appropriate mass balances (neglecting charges) are

$$C_M = [Cu] + [CuOX] + [CuHOX] + [Cu(OX)_2] + [CuOH] + [CuL] \quad (27)$$

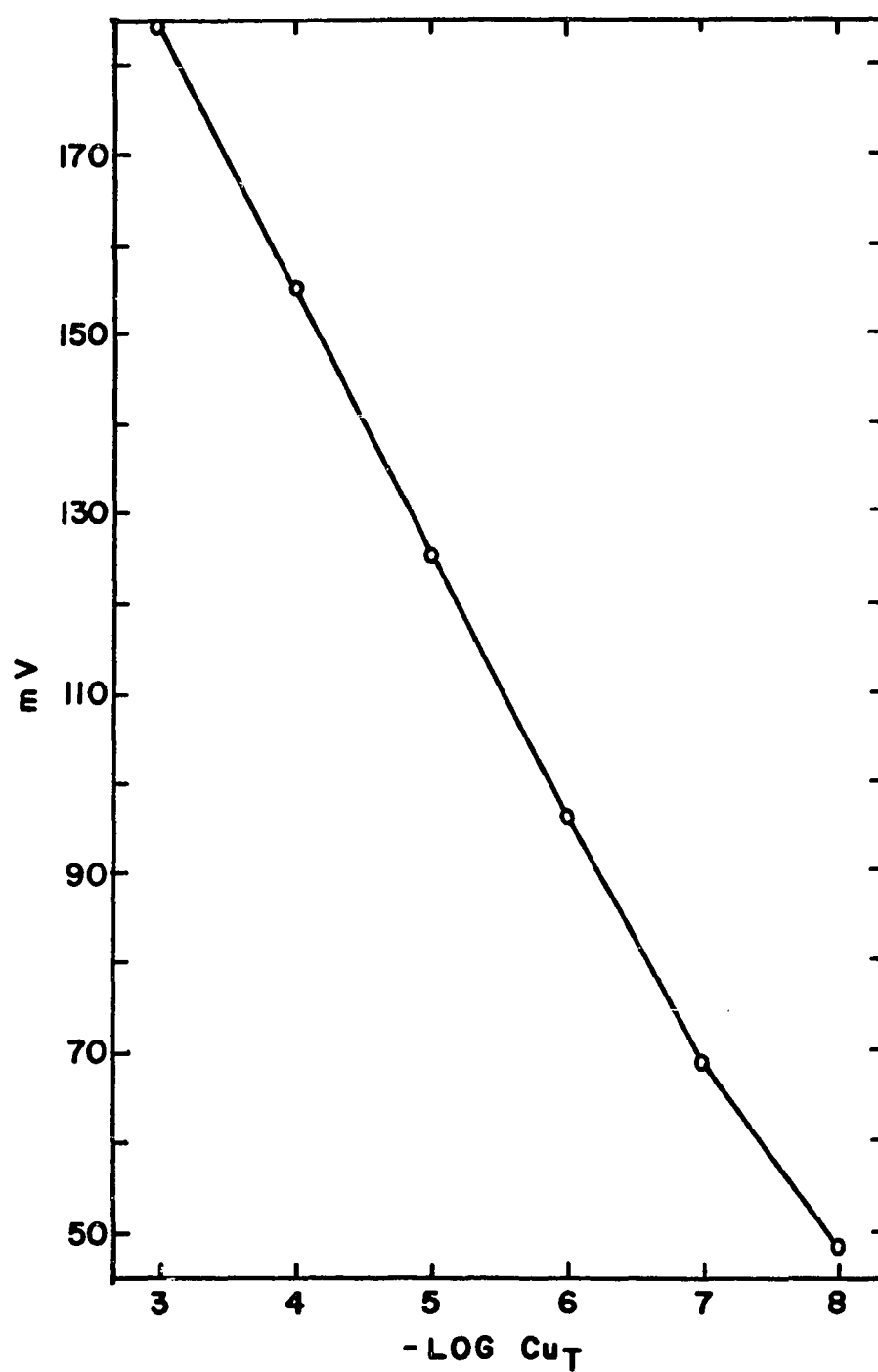


Figure 26. Copper ISE response for $10^{-3} \text{ M} \geq [\text{Cu}] \geq 10^{-8}$, pH 5.0, $I = 0.1 \text{ M NaClO}_4$.

TABLE XII

CALCULATED AND FOUND FREE COPPER CONCENTRATIONS FOR A COPPER-
OXALATE METAL ION BUFFER WITH $C_M = 10^{-7}$ M

| Oxalate Conc. (M/L) | pCu Calc. (M/L) | pCu Expt., #1 (M/L) | pCu Expt., #2 (M/L) |
|------------------------|--------------------|------------------------|------------------------|
| 0 | -- | 6.91 | 6.93 |
| 1×10^{-5} | 7.80 | 7.91 | 7.88 |
| 5×10^{-5} | 8.74 | 8.66 | 8.68 |
| 1×10^{-4} | 9.24 | 9.19 | 9.21 |
| 5×10^{-4} | 10.54 | 10.44 | 10.47 |
| 1×10^{-3} | 11.12 | 11.06 | 11.07 |

$$C_{OX} = [OX] + [HOX] + [H_2OX] + [CuOX] + [CuHOX] + 2[Cu(OX)_2] \quad (28)$$

where CuL is the copper-humus complex and where the mixed ligand complex Cu(OX)L is omitted. The log K values for each of the species formation reactions is given in Table XIII. All values are from Sillen and Martell (1964) except log K1 and log K2, which are from this research. Substituting the appropriate equilibrium expressions into Eq. 28 and rearranging, a quadratic in [OX] is obtained:

$$(2[Cu]K_2)[OX]^2 + (1 + [H]K_{a1} + [H]^2K_{a2} + [Cu]K_1 + [Cu][H]K_3)[OX] - C_{OX} = 0 \quad (29)$$

The resultant value for [OX] is inserted in the copper mass balance equation, which is solved for [CuL]:

$$[CuL] = C_M - (1 + [OX]K_1 + [OX]^2K_2 + [H][OX]K_3 + [OH]K_4)[Cu] \quad (30)$$

TABLE XIII
FORMATION REACTIONS AND CONSTANTS
FOR THE COPPER-OXALATE METAL ION BUFFER

| Reaction | Constant | Log Constant |
|-----------------------|----------|--------------|
| $H + OX = HOX$ | K_{a1} | 3.81 |
| $2H + OX = H_2OX$ | K_{a2} | 5.18 |
| $Cu + OX = CuOX$ | $K1$ | 5.72 |
| $Cu + 2OX = Cu(OX)_2$ | $K2$ | 10.27 |
| $Cu + H + OX = CuHOX$ | $K3$ | 6.30 |
| $Cu + OH = CuOH$ | $K4$ | 5.91 |

where $[OH] = KW/[H]$. Equation 31 is then solved for the overall conditional stability constant at each titration point, where $[Cu]$ is measured experimentally, $[CuL]$ is calculated as above, and C_L is the complexation of capacity of Williamson River humus (1.13×10^{-5} moles Cu/mg humic carbon) times the humus content of the concentrated titrant (4.5 g C/L), corrected for dilution:

$$\bar{K}' = \frac{[CuL]}{[Cu] (C_L - [CuL])} \quad (31)$$

One additional factor is that the freeze-dried humus itself contains copper. The 4.5 g C/L titrant was diluted 1:10 and measured for copper content by graphite furnace AAS. Quadruplicate analyses gave a copper concentration of 21.79 ± 2.22 PPB, or 3.43×10^{-6} M in the original solution. Thus C_M must be corrected for this additional input of

copper at each titration point. At the beginning of the titration, this additional copper is a small correction (1.7×10^{-9} M) but becomes significant in the latter points (3.40×10^{-8} M at point #20 and 6.72×10^{-8} M at point #40).

Plots of $\log \bar{K}'$ versus $\log C_L/C_M$ are given in Figures 27-30 for the duplicate titrations at pH 5.0, 5.5, 6.0, and 6.5, and a composite plot of 4 titrations, one at each pH, is shown in Fig. 31. Clearly, the $\log \bar{K}'$ values do not become constant, even at a ligand:metal ratio of over 5300. (The average ligand metal ratio, corrected for iron, at WR-50, the most humus-rich site on the Williamson River, is about 4300, using a complexation capacity of 1.13×10^{-5} moles Cu/mg humic carbon and the DOM and iron values reported by Perdue et al. (1981)).

All eight of the copper, metal-ion buffer titrations were fitted with the Gaussian model. The results are summarized in Table XIV. Examination of the PHI values shows that the bimodal model gave a small enhancement of fit for the pH 6.0 and 6.5 titrations and no significant improvement of fit to the pH 5.0 and 5.5 titrations. Also, Student's t-test (Laitinen and Harris, 1975) showed that only the means for the bimodal fit at pH 5.0 were significantly different at the 95% confidence level. For these reasons, it was felt that the addition of three more fitting parameters with the bimodal model was not justified and that the single-mode fits were adequate. The average single-mode parameters indicate a small increase in μ and a slightly greater increase in σ with increasing pH. This trend is interpreted as the gradually increasing complexation of copper to weaker binding sites as the competition with hydrogen ion for these sites lessens. The shift in μ is small because

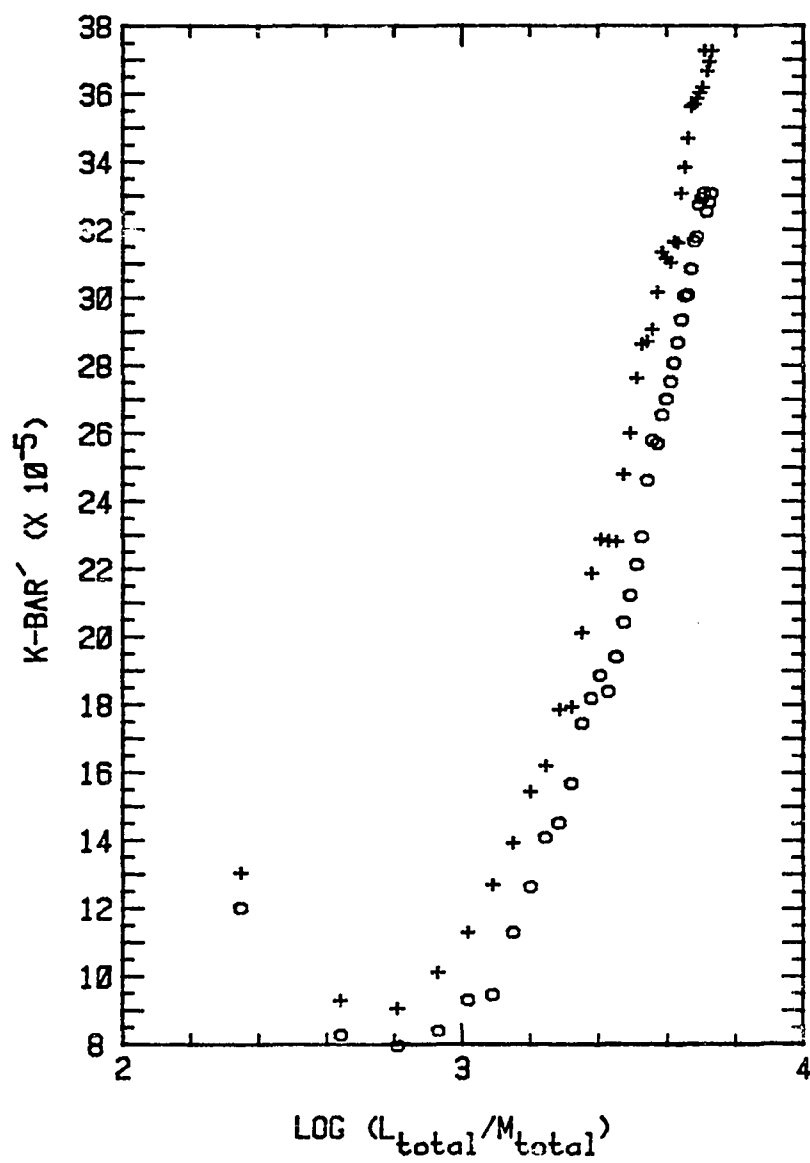


Figure 27. \bar{K}' versus $\log C_L/C_M$ at pH 5.0.

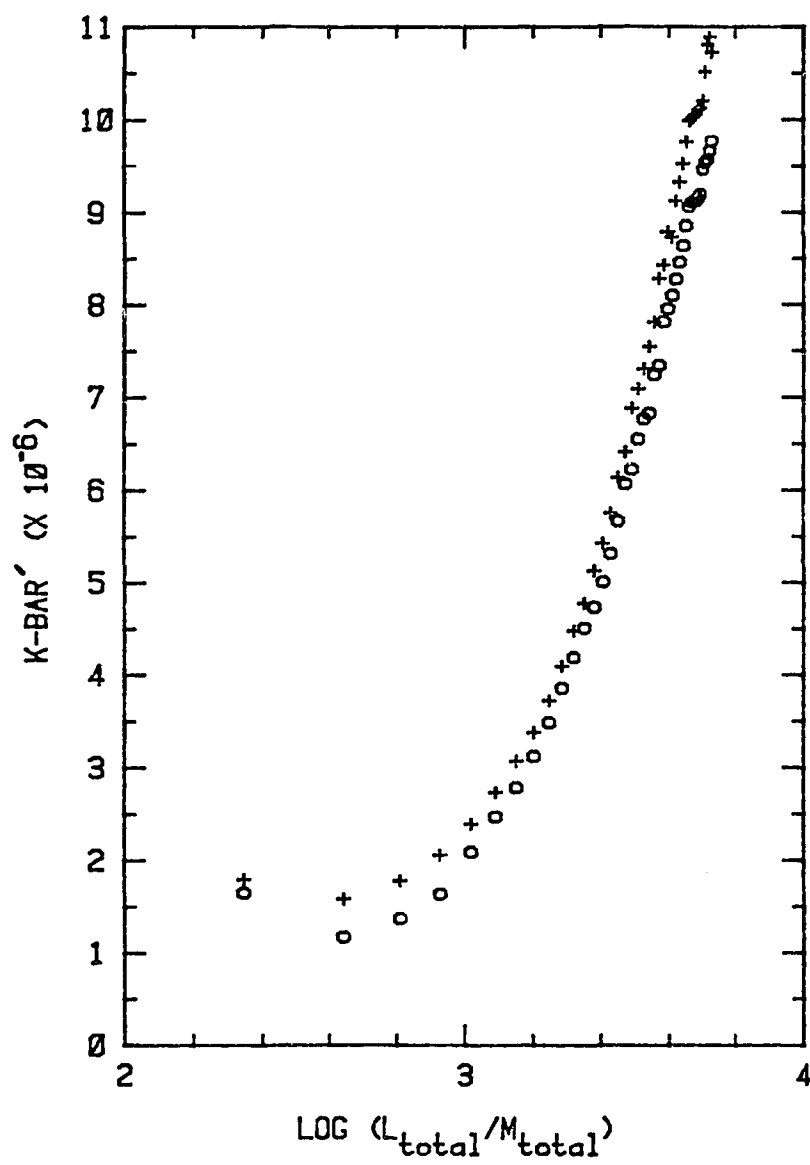


Figure 28. \bar{K}' versus $\log C_L/C_M$ at pH 5.5.

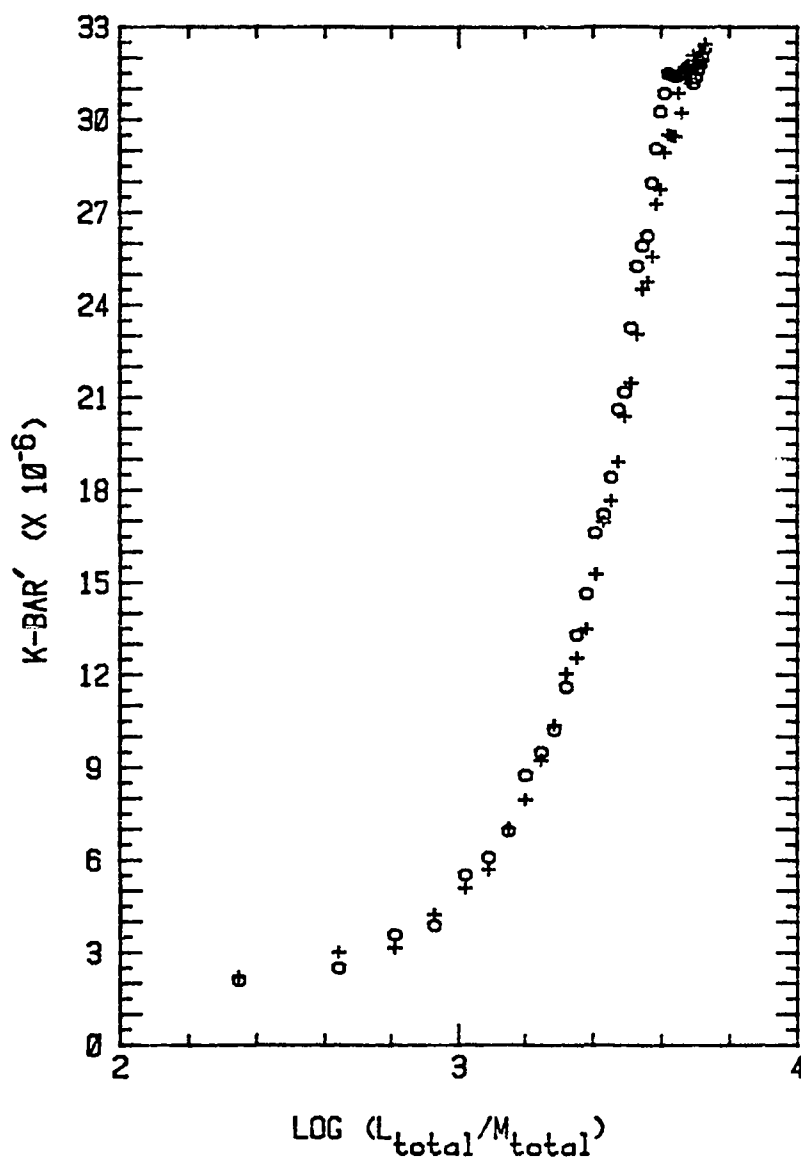


Figure 29. \bar{K}' versus $\log C_L/C_M$ at pH 6.0.

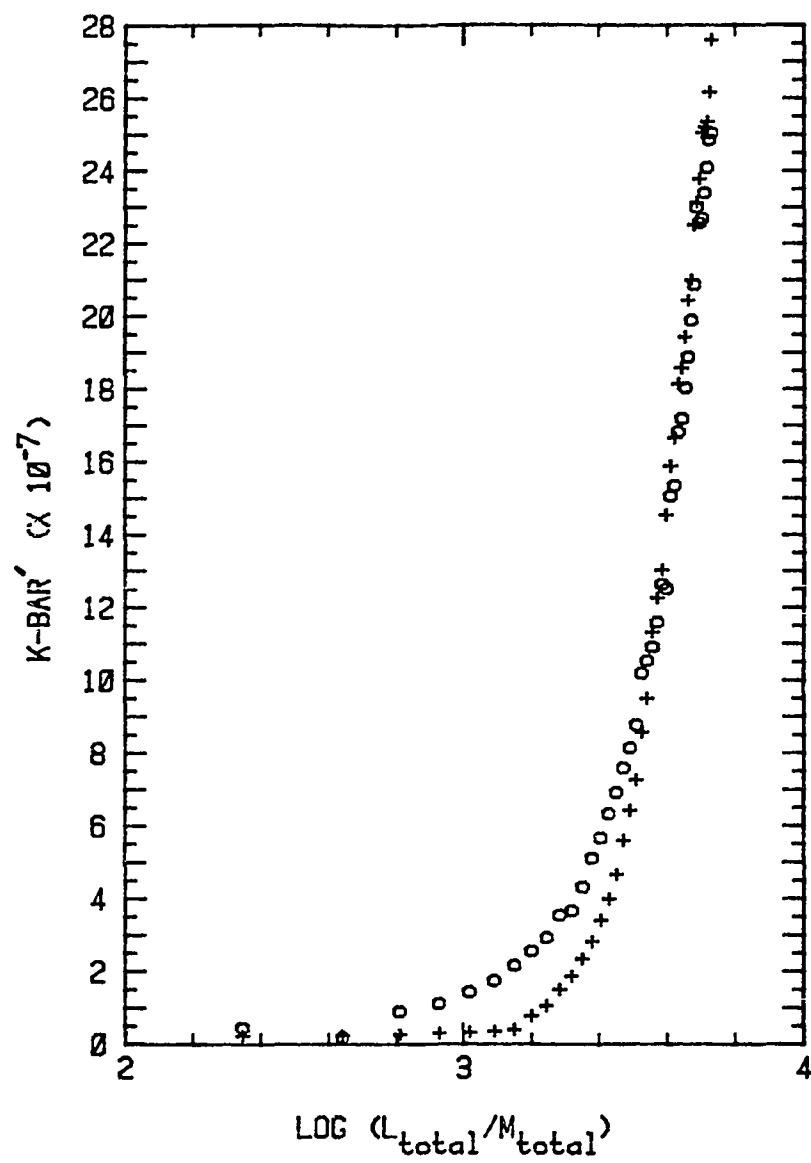


Figure 30. \bar{K}' versus $\log C_L/C_M$ at pH 6.5.

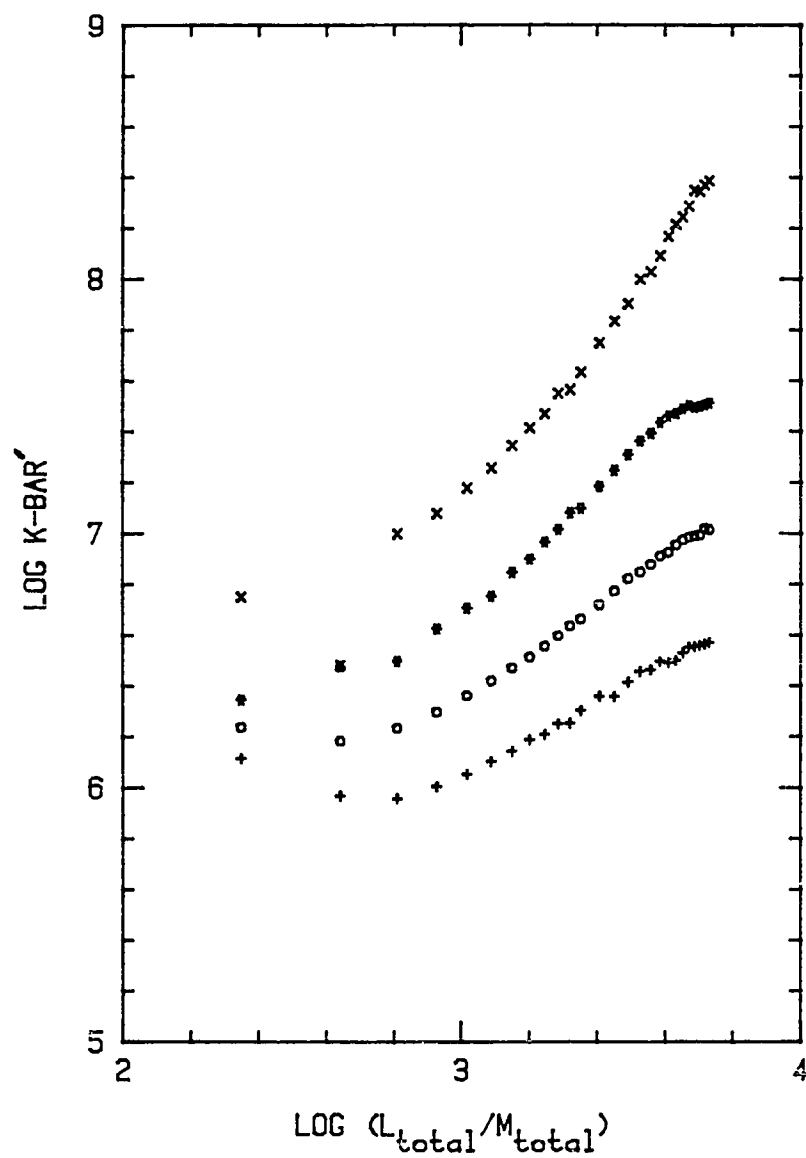


Figure 31. $\text{Log } \bar{K}'$ versus $\text{log } C_L/C_M$. One titration each at pH 5.0(+), 5.5(o), 6.0(*), and 6.5(x).

TABLE XIV

FITTING PARAMETERS FOR THE SINGLE AND BIMODAL GAUSSIAN
MODELING OF THE COPPER, METAL-ION BUFFER TITRATIONS

| pH | Trial | Single-Mode | | | Bimodal | | | | | |
|----------------------|-------|-------------|----------|---------------------------|---------------------------|----------|---------|------------|---------|------------|
| | | μ | σ | PHI* ($\times 10^6$) | PHI* ($\times 10^6$) | θ | μ_1 | σ_1 | μ_2 | σ_2 |
| 5.0 | 1 | 3.81 | 1.52 | 2.2 | 1.9 | 0.06 | 3.11 | 1.56 | 4.68 | 1.16 |
| | 2 | 3.81 | 1.50 | 2.6 | 2.2 | 0.10 | 3.11 | 1.54 | 4.68 | 1.14 |
| 5.5 | 1 | 3.81 | 1.62 | 1.2 | 0.9 | 0.30 | 3.72 | 1.88 | 4.91 | 0.97 |
| | 2 | 3.97 | 1.52 | 2.0 | 1.2 | 0.02 | 3.91 | 2.67 | 5.13 | 0.92 |
| 6.0 | 1 | 3.98 | 1.62 | 1.3 | 0.5 | 0.22 | 3.98 | 2.04 | 4.38 | 1.13 |
| | 2 | 4.01 | 1.62 | 1.5 | 0.6 | 0.28 | 4.00 | 1.98 | 4.01 | 1.12 |
| 6.5 | 1 | 3.99 | 1.59 | 2.5 | 2.2 | 0.22 | 3.96 | 1.95 | 4.08 | 1.34 |
| | 2 | 4.01 | 1.80 | 1.5 | 0.5 | 0.20 | 4.00 | 2.27 | 4.01 | 1.47 |
| ----- Averages ----- | | | | | | | | | | |
| 5.0 | | 3.81 | 1.51 | 2.4 | 2.0 | 0.08 | 3.11 | 1.55 | 4.68 | 1.15 |
| 5.5 | | 3.89 | 1.57 | 1.6 | 1.0 | 0.16 | 3.82 | 2.28 | 5.02 | 0.94 |
| 6.0 | | 4.00 | 1.62 | 1.4 | 0.6 | 0.25 | 3.99 | 2.01 | 4.20 | 1.11 |
| 6.5 | | 4.00 | 1.70 | 2.0 | 0.8 | 0.21 | 3.98 | 2.11 | 4.04 | 1.40 |

* PHI is the weighted sum over all data points of the squares of residuals. See Appendix C.

the strong binding sites still dominate the overall distribution. The shift in σ , then, reflects this increase in weak binding sites by broadening the distribution. This broadening is double-sided because of the symmetry constraint inherent in the Gaussian distribution and is not consistent with a distribution increase to only the high side of μ . This problem can be overcome through the use of a nonsymmetrical distribution model (Parrish, 1982).

Examples of the calculated fits are given in Figures 32-35 for the titrations used in Fig. 31. The model appears to fit the metal ion buffer titration data less well than the data shown in Fig. 20 and 21. However, the Y-axes in Fig. 32-35 are actually magnified 10 times the Y-axes in Fig. 20-21. Thus, similar absolute differences would appear to be 10 times as great in the metal-ion buffer plots. The fits did not improve for a thousand-fold reduction in the convergence criterion. (See Appendix C.)

Comparison of the metal-ion buffer results with the literature is difficult because experimental data are interpreted via discrete models and results are in the form of tabulated constants generated from these models. Since such constants have been shown in this research to be only curve-fitting parameters, they are not directly comparable to the functions shown in Fig. 27-30.

One possible method of comparison is to regenerate raw titration data from tabulated fitting parameters. For Scatchard parameters, Eq. 18 may be used directly. For parameters derived by other methods, such as the multiple-stoichiometry model, similar equations specific to

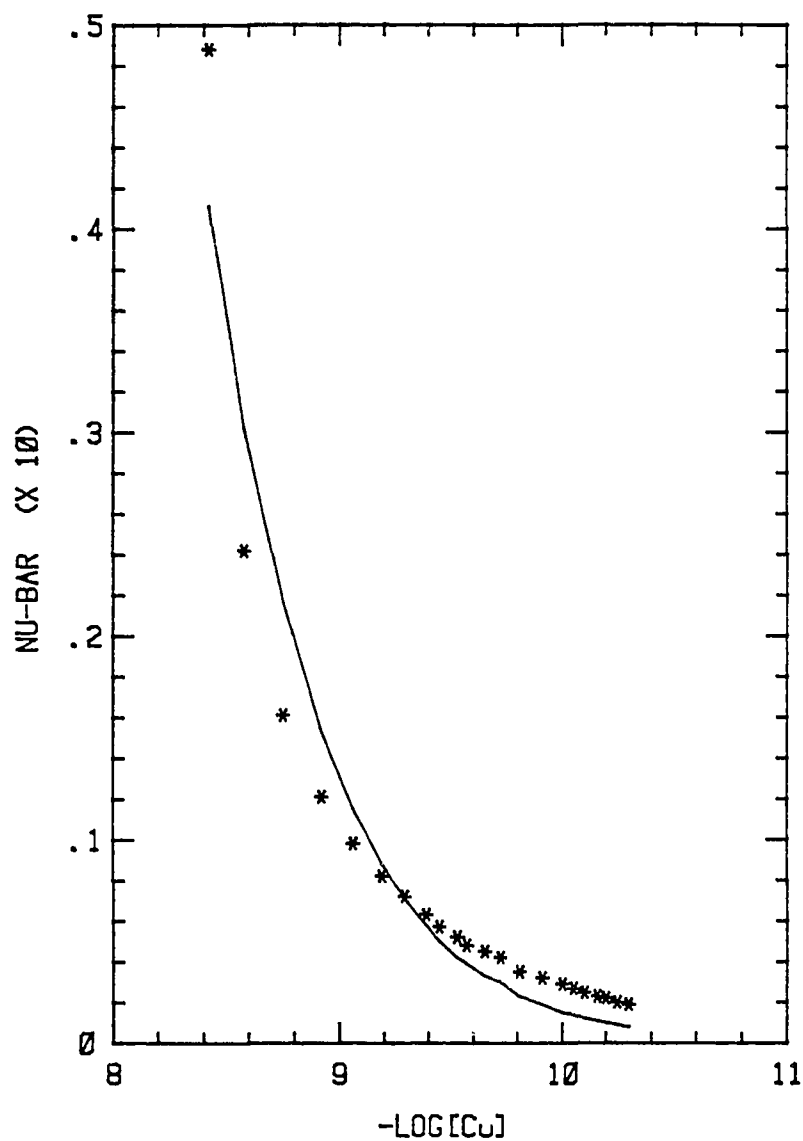


Figure 32. Experimental data (*) and calculated fit (—) for the single mode Gaussian modeling of the pH 5.0 metal ion buffer titration #1. Fitting parameters given in Table XIV.

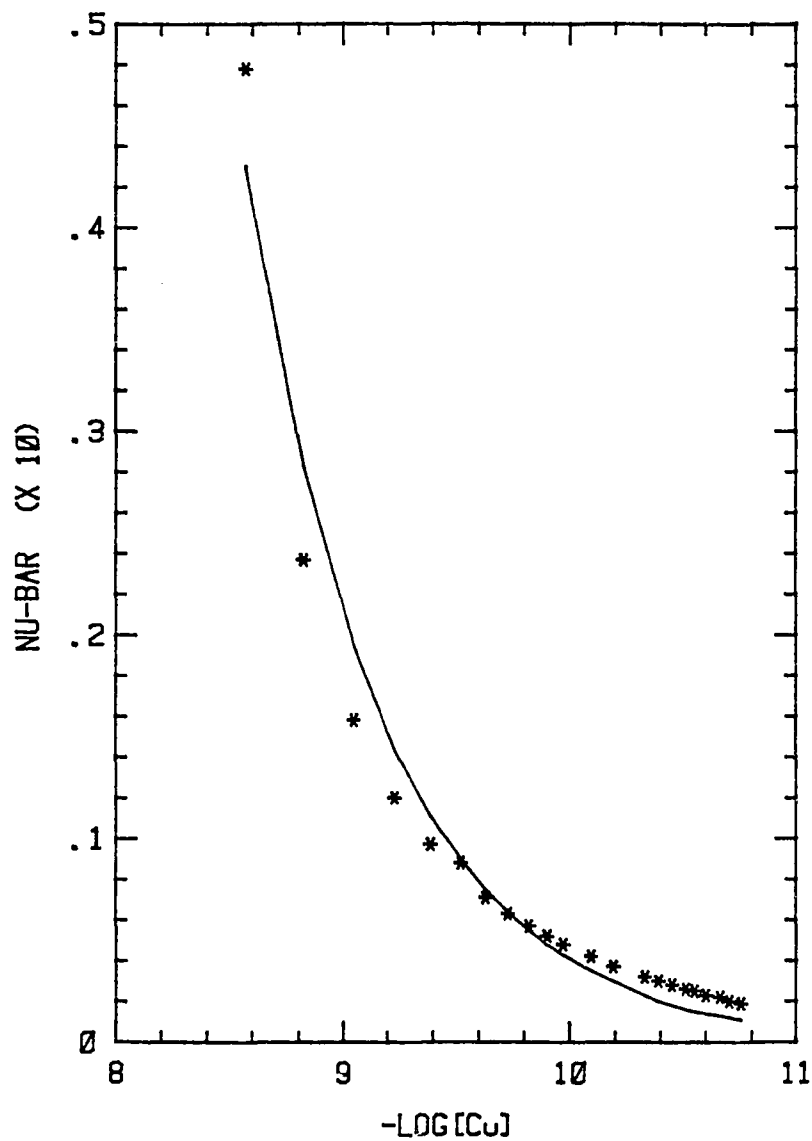


Figure 33. Experimental data (*) and calculated fit (—) for the single mode Gaussian modeling of the pH 5.5 metal ion buffer titration #1. Fitting parameters given in Table XIV.

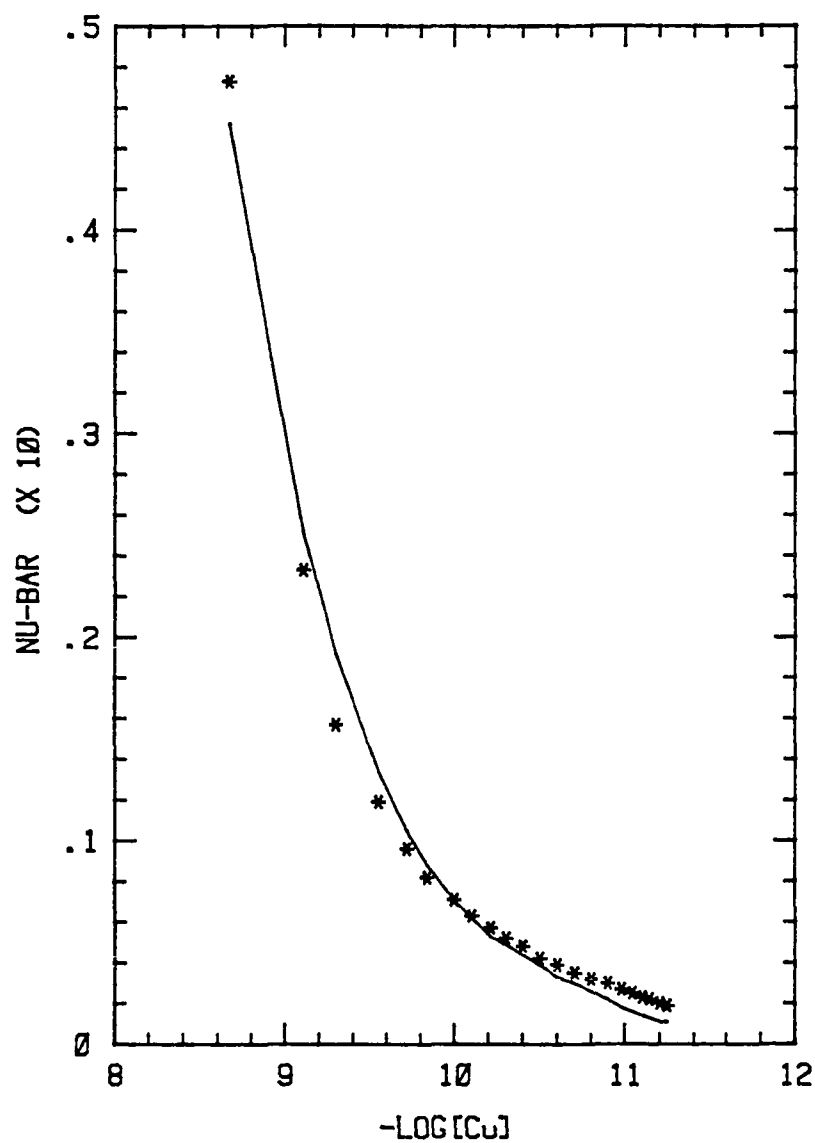


Figure 34. Experimental data (*) and calculated fit (—) for the single mode Gaussian modeling of the pH 6.0 metal ion buffer titration #1. Fitting parameters given in Table XIV.

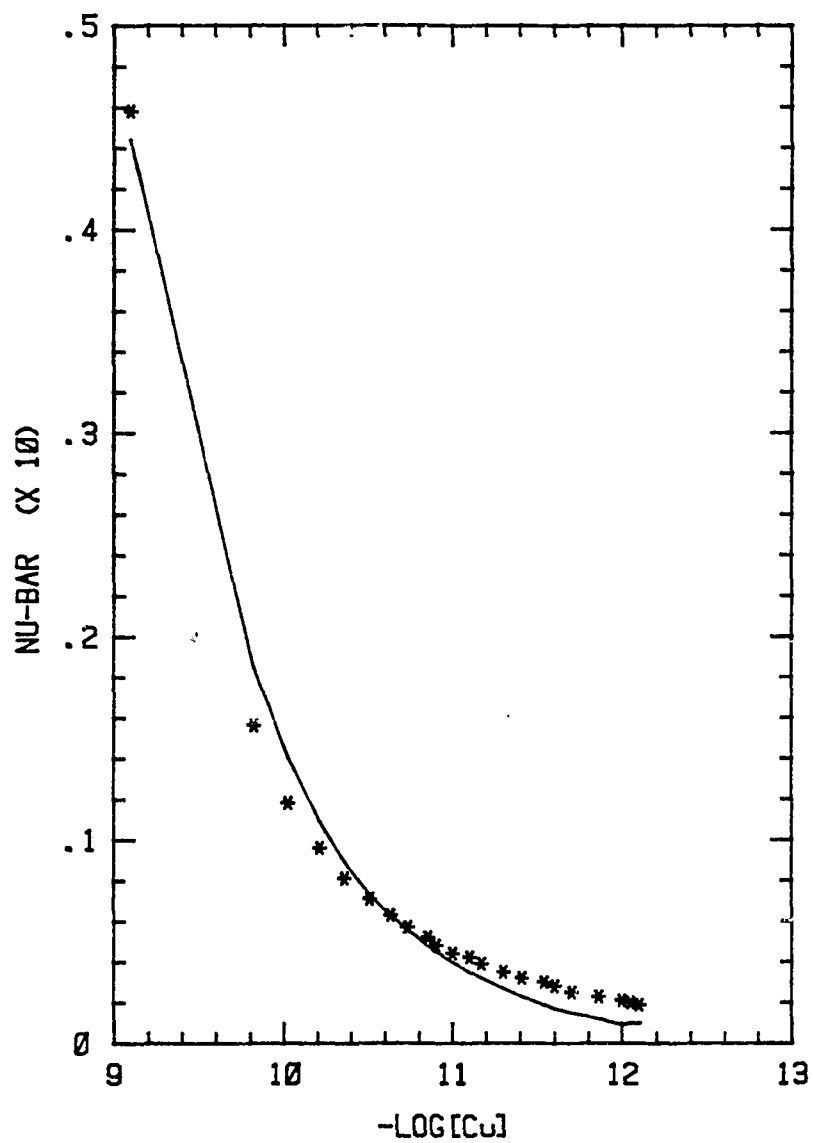


Figure 35. Experimental data (*) and calculated fit (—) for the single mode Gaussian modeling of the pH 6.5 metal ion buffer titration #2. Fitting parameters given in Table XIV.

the particular method must be employed or the data must be estimated from graphs. The calculated "raw" data can then be refitted to the Gaussian model. The underlying assumption is that the original fitting process used in the literature is accurate enough so as to be reversible. Many reports do not show calculated fits along with raw data, and some workers (cf Mantoura and Riley, 1975) use a Scatchard fitting method that does not use all of the raw data points. Another problem is that some literature reports fail to give the range of free metal ion concentrations found. Often, it can be estimated from graphs (Bresnahan et al., 1978) or inferred from C_M , C_L data (McKnight et al., 1982). This is a very important point because the regeneration of the raw data can be done over any free metal concentration range desired. To accurately reflect the experimental conditions in a given literature report, the regenerated data should be calculated only for the free metal concentrations actually measured in that report. At low levels of free metal, \bar{v} changes very rapidly. Any "extra" points added in this region by the regeneration will significantly alter the Gaussian parameters when the recalculated data is refit.

Given these problems and constraints, only three other literature reports contained enough information to allow a raw data regeneration of any reliability: two that used the Scatchard method (Bresnahan et al., 1978; McKnight et al., 1982) and one that used a two-stoichiometry model (Bufflé et al., 1977). The original parameters and the Gaussian fitting parameters are compared in Table XV. Only aquatic humus samples were

TABLE XV

COMPARISON OF FITTING PARAMETERS FROM THIS RESEARCH
AND FROM "CALCULATED" RAW DATA FROM THE LITERATURE

| Reference* | Original Fitting Parameters | | | | Gaussian Parameters | |
|------------|-----------------------------|--------------------|----------------|--------------------|---------------------|----------|
| | n ₁ | Log K ₁ | n ₂ | Log K ₂ | μ | σ |
| A | -- | -- | -- | -- | 4.00 | 1.62 |
| B | -- | 4.8 | -- | 10.1 | 1.36 | 2.38 |
| C | 0.23 | 6.11 | 0.77 | 3.85 | 4.27 | 0.81 |
| D | 0.18 | 7.8 | 0.82 | 5.9 | 6.17 | 0.58 |

* A = this research; B = Bufflé et al. (1977), sample III;
C = Bresnahan et al. (1978), water sample; D = McKnight et al.
(1982), Suwannee River sample.

considered, and all the tabulated work was at pH 6.0. To see if the widely varying discrete and Gaussian parameters could be due to modeling similar distributions over different portions of the distributions, the Gaussian parameters were used via Eq. 15 and 23 to calculate a global $\log \bar{K}'$ for a wide range of C_L/C_M ratios. The results are shown in Fig. 36. Except for the fit for the recalculated data of Bresnahan et al., the values of the fitted $\log \bar{K}'$ are within about 0.4 log units at the ligand:metal ratio of the world average river (indicated by the vertical arrow in Fig. 36). In light of the forementioned problems in generating raw data from literature reports, this similarity is striking

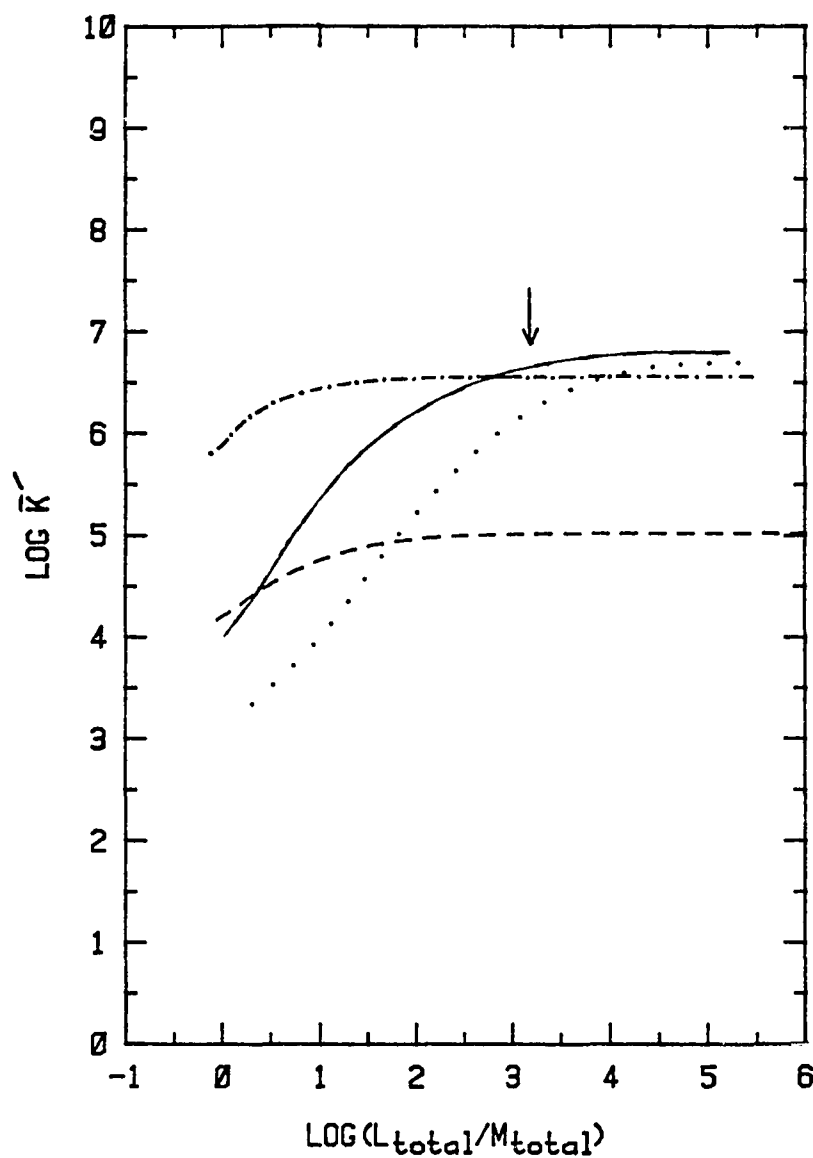


Figure 36. $\text{Log } \bar{K}'$ vs $\text{log } C_L/C_M$ for Gaussian fits of data from this research and data calculated from the literature. (—) = this research; (· · ·) = Buffle et al. (1977); (---) = Bresnahan et al. (1978); (- · -) = McKnight et al. (1982). Arrow marks $\text{log } C_L/C_M$ for the world average river.

and suggests that the different humus samples used may have similar binding properties with copper. Thus it was felt that the widely varying results reported in the literature, as exemplified by the listing of original fitting parameters in Table XV, are not just functions of differences in humus but, more importantly, may also be functions of the range of total ligand and metal concentrations used in the experimental procedures.

This latter point may be the reason for the anomalous fit of the recalculated data of Bresnahan et al. shown in Fig. 36. It was stated earlier that the reason for conducting the copper, metal-ion buffer titrations was to duplicate the ligand:metal ratio found in the Williamson River. This necessitated lowering total copper to 10^{-7} M. The result was a close approximation to the absolute levels in the river. Figure 37 shows the experimental ranges of total copper in molar units and total ligand in mg humic carbon/liter for this research and nine other literature reports. Also shown is the value for the world average river (open circle) and the Williamson River at WR-50 (closed circle). It can be seen that only this research and the work of McKnight et al. match the levels for the world average river. Bresnahan et al. worked at a total copper concentration over three orders of magnitude greater. Thus, if the binding sites in aquatic humus in fact approach a continuum in their binding properties with copper, the work of Bresnahan et al. would represent the binding of copper to a much different portion of this distribution than that of McKnight et al. or of this research. Also, it is admitted that the distribution of binding

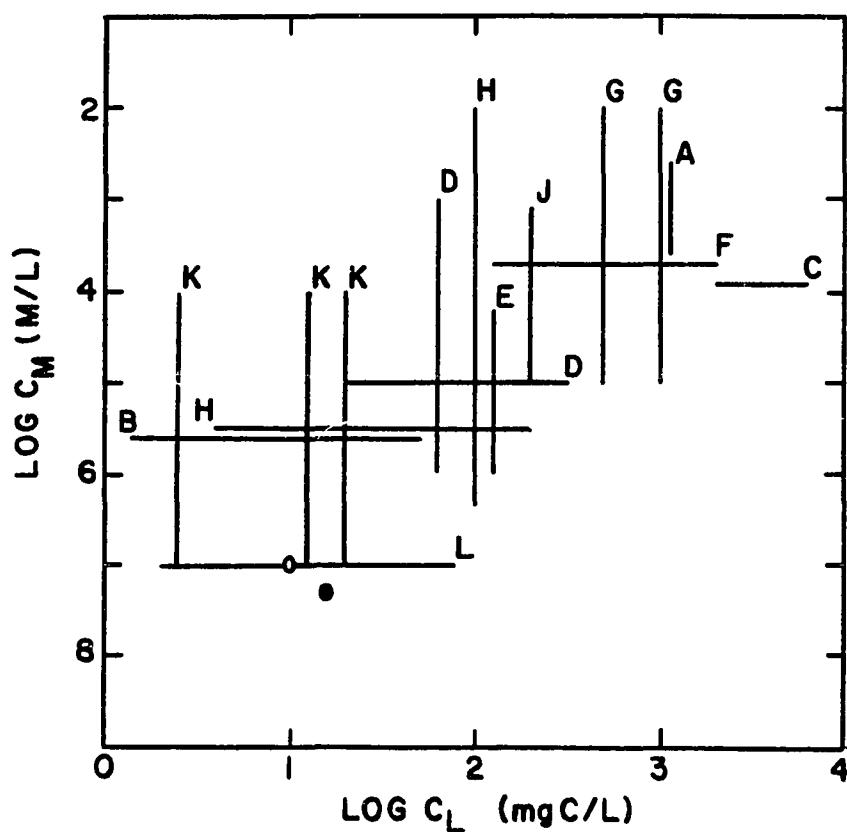


Figure 37. Experimental conditions of total metal (moles/liter) and total ligand (in units of mg humic carbon/liter) for this research and for literature reports. A = Stevenson *et al.* (1973); B = Ernst *et al.* (1975); C = Mantoura and Riley (1975); D = Buffle *et al.* (1977); E = Schuman and Woodward (1977); F = Bresnahan *et al.* (1978); G = Sposito *et al.* (1979); H = Buffle *et al.* (1980); J = Gamble *et al.* (1980); K = McKnight *et al.* (1982); L = this research. (O) = world average river (Livingstone, 1963; Schlesinger and Melack, 1981). (●) = Williamson River at WR-50.

sites may not in fact be symmetrical and that the Gaussian model is only a first approximation. If the degree of asymmetry is significant, the true shape of the distribution at environmental levels of ligand and metal may not be predictable at levels that are orders of magnitude greater. This is a strong possibility in a comparison of the results shown in Fig. 36 for the work of Bresnahan et al. and McKnight et al. because both originally fit their experimental data to the same model.

SUMMARY AND CONCLUSIONS

This research was undertaken to provide a clear understanding of the degree to which dissolved organic matter (DOM) complexes the test heavy metal, copper. While the particular results of this study pertain to DOM isolated from the Williamson River, Oregon (chosen for its high organic matter content), the methods, modeling techniques, and overall results are applicable to DOM in general.

Since DOM is primarily humic substances, carbohydrates, and proteinaceous matter, the first problem was to quantitate these fractions. Since the carbohydrate fraction had been studied by another worker, the proteinaceous fraction was studied in this research. Gas-liquid chromatography was used to examine hydrolyzable amino acids in the Williamson River and its main tributaries at monthly intervals over a two-year period. The relative abundances of amino acids showed little spacial or temporal changes, the order for the five most abundant being Gly > Asp > Ala > Ser > \approx Glu. Total amino acids varied greatly (two-year averages

at twelve sampling sites ranged from about $0.5\mu\text{M}$ to about $8\mu\text{M}$) and correlated strongly with both discharge and humic carbon. A separation technique utilizing a macorecticular resin showed that, for three typical sampling sites, $\geq 96\%$ of the dissolved amino acids were associated with aquatic humus. Since it was found that amino acids contributed less than 1% to humic carbon and since another worker has shown that the carbohydrate portion of DOM contributes less than 2% to humic carbon, this research has provided the necessary data to make the conclusion that the study of copper complexation with DOM is essentially the study of copper complexation with humic substances.

For the examination of the binding of a metal to humus, it is most convenient if humus concentration can be expressed in units of moles/liter. Because of ambiguity in the assigning of a molecular weight to the complex humic mixture, many researchers determine the complexation capacity of humus in terms of moles of metal capable of binding per unit weight of humus. This number, when multiplied by the weight concentration of humus, yields an operational humus concentration in units of moles metal capable of binding per liter. This research has shown that the accepted methods of expressing free metal versus total metal or total metal minus free metal versus total metal for a metal-ion-humus titration, underestimate this parameter. It has also been shown that these data plotting techniques yield complexation capacities that are dependent upon total ligand concentration. However, it was shown that titrimetric data can be combined with acidic functional group analysis to arrive at a probable range for this parameter. For Williamson River humus, this range is $7.2\text{--}15.4\mu\text{mols}$ copper per mg humic

carbon, and for subsequent work the average value of 11.3 was employed.

Once this operational concentration unit was established for the aquatic humus, it was possible to address the problem of quantitating the extent of the copper-humus complexation reaction. Since most analytical techniques are incapable of measuring free metal ion at concentrations typical of natural waters, titration experiments are most often run at levels of metal and ligand concentration many times greater. To avoid this artificiality as much as possible, humus was titrated into a copper-oxalate metal ion buffer. This allowed the measurement of free copper ion with an ISE to a concentration of $< 10^{-11}$ M. The result was the ability to calculate the copper-humus binding constant at the same humus:copper ratio, the same absolute humus concentration, and at almost the same absolute copper concentration as that found in the Williamson River. It was found that the binding "constant" was in fact a variable over the almost two orders of magnitude of humus:copper ratio studied. This variable was also a function of pH. At the average humus:copper ratio for the most humus-rich sampling site on the Williamson River, WR-50, the conditional stability "constants" at pH 5.0, 5.5, 6.0, and 6.5 were: 3.0×10^6 , 8.9×10^6 , 3.0×10^7 , and 1.7×10^8 .

The functional nature of the metal-humus binding "constant" has been reported in the literature for copper and other heavy metals. The dilemma is that there is then no simple constant(s) that can be straightforwardly used to determine the extent of humus-metal binding in any general aquatic system. Researchers have solved this problem by modeling the binding as if humus possessed two or three discrete classes

of binding sites or two or three discrete ligand stoichiometries. Through the application of those models to computer-simulated titration data, this research has shown that these discrete models are inadequate on two counts. First, the derived constants are only curve-fitting parameters and bear no relation to the actual constituents of multiligand mixtures. Second, the models are incapable of modeling the functional nature of the binding "constant" from experimental levels of metal and ligand concentrations to levels that are environmentally significant. The conclusion of this portion of the research is that, because metal-humus titrations yield essentially featureless data plots, goodness-of-fit is not by itself sufficient to validate a model for metal-humus complexation. As an alternative it is proposed in this research that aquatic humus can be modeled as possessing a continuum of binding sites in which concentrations are normally distributed with respect to the average of the microscopic binding constants for each site. This model proved superior in its ability to follow the functional nature of the metal-humus binding "constant". It uses fewer fitting parameters and does not require knowledge of the actual values of the microscopic binding constants. Application of the model to proton binding data showed that the fitting parameters do reflect, both qualitatively and semiquantitatively, that known acid-base attributes of the humic mixture, i.e. that its acid-base properties arise primarily from a combination of carboxylic and phenolic function groups. Application of the model to copper binding data showed that it worked equally well for data collected at conventional levels of free metal concentrations and for data collected in the metal-ion buffer experiments at the extreme

lower limit of free metal concentration. The conclusion of this final portion of the research is that the parameters derived from fitting metal-humus titration data to the proposed Gaussian distribution model can be readily used to supply the quantitative information necessary to integrate the metal binding properties of aquatic humus into the overall matrix of trace metal transport in natural waters.

REFERENCES

- Aiken, G. R., E. M. Thurman, R. L. Malcolm, and H. L. Walton (1979). Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution. Anal. Chem., 51: 1799-1803.
- Alberts, J. J. and J. P. Giesy (1982). Conditional stability constants of trace metals and naturally occurring humic materials: Their application in equilibrium models and verification with field data. In Terrestrial and Aquatic Humic Materials. R. F. Christman and E. Gjessing (eds.) Ann Arbor, Michigan: Ann Arbor Press, in press.
- Allen, H. E., M. L. Crosser, and T. D. Brisbin (1976). Metal speciation in aquatic environments. In Workshop on Toxicity to Biota of Metal Forms in Natural Water. R. W. Andrew, P. V. Hodson, D. E. Konasewich (eds.) Duluth, Minnesota: U.S. Environmental Protection Agency, Environmental Research Laboratory.
- Allen, H. E., R. H. Hall, and T. D. Brisbin (1980). Metal speciation. Effects on aquatic toxicity. Env. Sci. Technol., 14: 441-443.
- Allen, H. E., W. R. Matson, and K. H. Mancy (1970). Trace metal characterization in aquatic environments by anodic stripping voltammetry. J. Water Poll. Contr. Fed., 42: 573-581.
- Andrew, R. W., K. E. Biesinger, and G. E. Glass (1977). Effects of inorganic complexing on the toxicity of copper to Daphnia magna, Water Res., 11: 309-315.
- Andrew, R. W., P. V. Hodson, and D. E. Konasewich (eds.) (1976). Toxicity to Biota of Metal Forms in Natural Waters. Duluth, Minnesota: U.S. Environmental Protection Agency, Environmental Research Laboratory.
- Ardakani, M. S. and F. J. Stevenson (1972). A modified ion-exchange technique for the determination of stability constants of metal-soil organic matter complexes. Soil Sci. Soc. Amer. Proc., 36: 884-890.

- Baccini, P. and U. Suter (1979). MELIMEX, an experimental heavy metal pollution study: Chemical speciation and biological availability of copper in lake water. Schweiz. Zeit. Hydrol., 41: 291-314.
- Balistrieri, L. and J.W. Murray (1979). Surface of goethite in seawater. In Chemical Modeling in Aqueous Systems, E. A. Jenne (ed.) Washington, D.C.: American Chemical Society, Symposium Series 93.
- Barber, R. T. and J. H. Ryther (1969). Organic chelators: Factors affecting primary production in the Cromwell Current upwelling. J. Exp. Mar. Biol. Ecol., 3: 191-199.
- Batley, G.E. and D. Gardner (1977). Sampling and storage of natural waters for trace metal analysis. Water Res., 11: 745-756.
- Batley, G. E. and T. M. Florence (1976). A novel scheme for the classification of heavy metal species in natural waters. Anal. Letters, 9: 379-388.
- Bayer, E., E. Grom, B. Kaltenegger, and R. Uhmman (1976). Separation of amino acids by high performance liquid chromatography. Anal. Chem., 48: 1106-1109.
- Beck, K. C., J. H. Reuter, and E. M. Perdue (1974). Organic and inorganic geochemistry of some coastal plain rivers of the southeastern United States. Geochim. Cosmochim. Acta., 38: 341-364.
- Benjamin, M. M. and J. O. Leckie (1981). Competitive adsorption of Cd, Cu, Zn, and Pb on amorphous iron oxyhydroxide. J. Colloid. Interface Sci., 83: 410-419.
- Black, A. P. and R. F. Christman (1963a). Characteristics of colored surface waters. Am. Water Works Assoc. J., 55: 753-769.
- Black, A. P. and R. F. Christman (1963b). Chemical characteristics of fulvic acids. Am. Water Works Assoc. J., 55: 879-912.
- Blackburn, S. (1968). Amino Acid Determination. New York: Marcel Dekker.
- Blaedel, W. J. and D. E. Dinwiddie (1974). Study of the behavior of copper ion selective electrodes at submicromolar concentration levels. Anal. Chem., 46: 873-877.
- Blunk, D. (1982). Investigation of the contribution of aquatic humus to the transport of DDT in the environment. PhD Thesis. Portland State University, Portland Oregon.
- Bowen, H. J. M., E. Page, I. Valente, and R. J. Wade (1979). Radio-tracer methods for studying speciation in natural waters. J.

Radioanal. Chem., 48: 9-16.

- Boyle, E. A., J. M. Edmond, and E. R. Sholkovitz (1977). The mechanism of iron removal in estuaries. Geochim. Cosmochim. Acta, 41: 1313-1324.
- Bresnahan, W. T., C. L. Grant, and J. H. Weber (1978). Stability constants for the complexation of copper (II) ions with water and soil fulvic acids measured by an ion selective electrode. Anal. Chem., 50: 1675-1679.
- Buffle, J. and A. Cominoli (1981). Voltammetric study of humic and fulvic substances. Part IV. Behavior of fulvic substances at the mercury-water interface. J. Electroanal. Chem., 121: 273-299.
- Buffle, J., P. Deladoey, F.-L. Greter, and W. Haerdi (1980). Study of the complex formation of copper (II) by humic and fulvic substances. Anal. Chim. Acta., 116: 255-274.
- Burch, D. R., C. H. Langford, and D. S. Gamble (1978). Methods for the comparison of fulvic acid samples: the effects of origin and concentration on acidic properties. Can. J. Chem., 56: 1196-1201.
- Burleson, J. L., G. R. Peyton, and W. H. Glaze (1980). Gas-chromatographic/mass-spectrometric analysis of derivatized amino acids in municipal waste water products. Env. Sci. Technol., 14: 1354-1359.
- Campbell, P. G. C., M. Bisson, R. Gagne, and A. Tessier (1977). A critical evaluation of the copper (II) solubilization method for the determination of the complexation capacity of natural waters. Anal. Chem., 49: 2358-2363.
- Carter, P. W. and R. M. Mitterer (1978). Amino acid composition of organic matter associated with carbonate and non-carbonate sediments. Geochim. Cosmochim. Acta., 42: 1231-1238.
- Chau, Y. K., R. Gachter, and K. Lum-Shue-Chan (1973). Determination of the apparent complexing capacity of Lake Waters. J. Fish. Res. Board Can., 31: 1515-1519.
- Cheam, V. (1973). Chelation study of the copper (II): fulvic acid system. Can. J. Soil Sci., 53: 377-382.
- Cheam, V. and D. S. Gamble (1974). Metal-fulvic acid chelation equilibrium in aqueous NaNO_3 solution. Hg(II) , Cd(II) , Cu(II) fulvate complexes. Can. J. Soil Sci., 54: 413-417.
- Christman, R. F. (1970). Chemical structures of color producing organic substances in water. In Organic Matter in Natural Waters, D. W. Hood (ed.) College, Alaska: Institute of Marine Science,

Occasional Publication No. 1.

- Christman, R. F. and M. Ghassemi (1966). Chemical nature of organic color in water. Am. Water Works Assoc. J., 58: 723-741.
- Christman, R. F. and R. A. Minear (1971). Organics in lakes. In Organic Compounds in Aquatic Environments, S. J. Faust and J. V. Hunter (eds.) New York: Marcel Dekker.
- Crosser, M. L. and H. E. Allen (1977). Determination of the complexation capacity of soluble ligands by ion exchange equilibrium. Soil Sci., 123: 176-181.
- Crosser, M. L. and H. E. Allen (1978). Complexation of heavy metals by ligands in industrial waste water - measurement and effect on metals removal. In Proceedings of the 32nd Industrial Water Conference. Ann Arbor: Ann Arbor Science.
- Dahm, C. N. (1980). Oregon State University, personal communication.
- Davey, E. W., M. J. Morgan, and S. J. Erickson (1973). A biological measurement of copper complexation capacity of seawater. Limnol. Oceanogr., 18: 993-997.
- Davis, J. A. and J. O. Leckie (1979). Speciation of adsorbed ions at the oxide/water interface. In Chemical Modeling in Aqueous Systems, E. A. Jenne (ed.). Washington, D.C.: American Chemical Society, Symposium Series 93.
- Erickson, S. J., T. E. Maloney, and J. H. Gentile (1970). Effect of nitrilotriacetic acid on the growth and metabolism of estuarine phytoplankton. J. Water Pollution Cont. Fed., 42: 329-335.
- Ernst, R., H. E. Allen, and K. H. Mancy (1975). Characterization of trace metal species and measurement of trace metal stability constants by electrochemical means. Water Res., 9: 969-979.
- Felker, P. and R. S. Bandurski (1975). Quantitative gas-liquid chromatography and mass spectroscopy of the N(o)-perfluorobutyryl-o-isoamyl derivatives of amino acids. Anal. Biochem., 67: 245-262.
- Florence, T. M. (1977). Trace metal species in fresh waters. Water Res., 11: 681-687.
- Gachter, R., K. Lum-Shue-Chan, and Y. K. Chau (1973). Complexing capacity of the nutrient medium and its relation to inhibition of algal photosynthesis by copper. Schweiz. Zeit. Hydrol., 35: 252-260.
- Gahler, A. R. (1969). Field studies on sediment-water algal nutrient

- interchange processes and water quality of Upper Klamath and Agency Lakes. Corvallis, Oregon: Pacific Northwest Water Laboratory, Working Paper No. 66.
- Gamble, D. S. (1972). Potentiometric titration of fulvic acid: equivalence point calculations and acidic functional groups. Can. J. Chem., 50: 2680-2690.
- Gamble, D. S. (1973). Sodium and potassium binding by fulvic acid. Can. J. Chem., 51: 3217-3222.
- Gamble, D. S., M. Schnitzer, and I. Hoffman (1970). Copper(II) - fulvic acid chelation equilibrium in 0.1 M KCl at 25.0° C. Can. J. Chem., 48: 3197-3204.
- Gamble, D. S., A. W. Underdown, and C. H. Langford (1980). Copper(II) titration of fulvic acid ligand sites with theoretical, potentiometric, and spectrophotometric analysis. Anal. Chem., 52: 1901-1908.
- Gardiner, J. (1974). The chemistry of cadmium in natural water - I. A study of cadmium complex formation using the cadmium ion selective electrode. Water Res., 8: 23-30.
- Gardner, W. S. and G. F. Lee (1973). Gas chromatographic procedure to analyze amino acids in lake waters. Env. Sci. Technol., 7: 719-724.
- Garrels, R. M. and F. T. Mackenzie (1971). Evolution of Sedimentary Rocks. New York: W. W. Norton P. 105.
- Giesy, J. R., L. A. Briesse, and G. J. Leversee (1978). Metal binding capacity of selected Maine surface waters. Env. Geol., 2: 257-268.
- Greter, F.-L., J. Buffle, and W. Haerdi (1979). Voltammetric study of humic and fulvic substances. Part I. Study of the factors influencing the measurement of their complexing properties with lead. J. Electroanal. Chem., 101: 211-229.
- Guy, R. D. and C. L. Chakrabarti (1976). Studies of metal-organic interactions in model systems pertaining to natural waters. Can. J. Chem., 54: 2600-2611.
- Guy, R. D., C. L. Chakrabarti, and L. K. Schramm (1975). The application of a simple chemical model of natural waters to metal fixation in particulate matter. Can. J. Chem., 53: 661-669.
- Hanck, K. W. and J. W. Dillard (1973). Determination of the complexing capacity of natural water. University of North Carolina Water Resources Research Institute, Pub. No. UNC-WRRI-73-85.

- Hedges, J. (1982). University of Washington, personal communication.
- Hirata, S. (1981). Stability constants for the complexes of transition-metal ions with fulvic and humic acids in sediments measured by gel filtration. Talanta, 28: 809-815.
- Hood, D. W. (ed.) (1970). Organic Matter in Natural Waters. College, Alaska: Institute of Marine Science. Occasional Publication No. 1.
- Hullett, D. A. and S. J. Eisenreich (1979). Determination of free and bound fatty acids in river water by high performance liquid chromatography. Anal. Chem., 51: 1953-1960.
- Husek, P. and K. Macek (1975). Gas chromatography of amino acids. J. Chrom., 113: 139-230.
- Hutchinson, G. E. (1957). A Treatise on Limnology. I. Geography, Physics, and Chemistry. New York: Wiley.
- Iliailfar, S. (1982). Portland State University, personal communication.
- Johnston, R. (1964). Sea water, the natural medium of phytoplankton. II. Trace metals and chelation and general discussion. J. Mar. Biol. Assoc. U.K., 44: 87-110.
- Kaiser, K. L. E. (1980). Correlation and prediction of metal toxicity to aquatic biota. Can. J. Fish. Aq. Sci., 37: 211-218.
- Kaiser, F. E., C. W. Gehrke, R. W. Zumwalt, and K. C. Kuo (1974). Amino acid analysis. Hydrolysis, ion-exchange cleanup, derivatization, and quantitation by gas-liquid chromatography. J. Chrom., 94: 113-133.
- Karush, F. and M. Sonenberg (1949). Interaction of homologous alkyl sulfates with bovine serum albumin. J. Am. Chem. Soc., 71: 1369-1376.
- Kennish, J. (1979). University of Alaska, personal communication.
- Kolthoff, I. M., E. B. Sandell, E. J. Meehan, and S. Bruckenstein (1969). Quantitative Chemical Analysis, 4th Ed. New York: MacMillan.
- Kunkel, R. and S. E. Manahan (1973). Atomic absorption analysis of strong heavy metal chelating agents in water and wastewater. Anal. Chem., 45: 1465-1468.
- Laitinen, H. A. and Harris (1975). Chemical Analysis, 2nd Edition. New York: McGraw-Hill.

- Lamar, W. L. (1968). Evaluation of organic color and iron in natural surface waters. U. S. Geol. Survey Prof. Paper, 600-D: D-24 - D-29.
- Langford, C. H., T. R. Khan, and G. B. Skippen (1979). On the nature of the complexing capacity of natural waters: Functional group based fractionation of a sample from the Indian River, Ontario. Inorg. Nucl. Chem. Letters, 15: 291-295.
- Laxen, D. P. H. and R. M. Harrison (1981). Cleaning methods for polythene containers prior to the determination of trace metals in freshwater samples. Anal. Chem., 53: 345-350.
- Lee, C. and J. L. Bada (1977). Dissolved amino acids in the Equatorial Pacific, the Sargasso Sea and Biscayne Bay. Limnol. Oceanogr., 22: 502-510.
- Leenheer, J. A. (1980). Origin and nature of humic substances in the waters of the Amazon River basin. Acta Amazonica., 10: 513-526.
- Leenheer, J. A. and E. W. D. Huffman (1976). Classification of organic solutes in water by using macroreticular resins. J. Res. U. S. Geol. Survey, 4: 737-751.
- Leenheer, J. A. and C. L. Dunham (1973). Preparing high efficiency packed GC columns. Res./Dev., 24: 32-38.
- Leonard, A. R. and A. B. Harris (1974). Ground water in selected areas in the Klamath Basin, Oregon. United States Geological Ground Water Report No. 21.
- Lindroth, P. and K. Mopper (1979). HPLC determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Anal. Chem., 51: 1667-1674.
- Livingstone, D. A. (1963). Chemical composition of rivers and lakes. In Data of Geochemistry, 6th Ed. M. Fleisher (ed.) U. S. Geological Survey, paper 440-G.
- MacCarthy, P. (1974). Fulvic Acid: I. Partial Fractionation. J. Soil Sci., 25: 420-428.
- MacCarthy, P. (1977a). Some comments on the derivation of Schubert's ion-exchange equations. Soil Sci., 123: 207.
- MacCarthy, P. (1977b). An interpretation of stability constants for soil organic matter-metal ion complexes under Schubert's conditions. J. Environ. Sci. Health, A12: 43-59.
- MacCarthy, P. and H. B. Mark (1977). A further examination of the Schubert ion-exchange method as applied to soil organic matter-

- metal interactions. In Biological Implication of Metals in the Environment. H. Drucker and R. E. Wildung (eds.). Springfield, Virginia: National Technical Information Service, ERDA Conference 750929.
- MacCarthy, P. and G. C. Smith (1979). Stability surface concept: A quantitative model for complexation in multiligand mixtures. In Chemical Modeling in Aqueous Systems. E. A. Jenne (ed.). Washington, D. C.: American Chemical Society, Symposium Series 93.
- Macko, S. A. and E. J. Green (1979). The quantification of amino acids in seawater using thin-layer chromatography and fluorimetry, Marine Chem., 8: 181-189.
- Magnuson, V. R., D. K. Harriss, M. S. Sun, D. K. Taylor, and G. E. Glass (1979). Relationships of activities of metal-ligand species to aquatic toxicity, in Chemical Modeling in Aqueous Systems, E. A. Jenne (ed.). Washington, D.C.: American Chemical Society, Symposium Series 93.
- Mantoura, R. F. C., A. Dickson, and J. P. Riley (1978). The complexation of metals with humic materials in natural waters. Estuar. Coastal Marine Sci., 6: 387-408.
- Mantoura, R. F. C. and J. P. Riley (1975). The use of gel filtration in the study of metal binding by humic acids and related compounds. Anal. Chim. Acta., 78: 193-200.
- Mart, L. (1979). Prevention of contamination and other accuracy risks in voltammetric trace metal analysis of natural waters, I: Preparatory steps, filtration, and storage of water samples. Anal. Chemie, 296: 350-357.
- Matson, W. R. (1968). Trace metals, equilibrium, and kinetics of trace metal complexes in natural media. PhD Thesis, Massachusetts Institute of Technology, Cambridge, Massachusetts.
- McDuffie, B., I. El-Barbary, G. L. Hollod, and R. D. Tiberio (1976). Trace metals in rivers-speciation, transport, and role of sediments. In Trace Substances in Environmental Health, D. D. Hemphill (ed.). Columbia, Missouri: Univ. Missouri Press.
- McKnight, D. M., G. L. Feder, E. M. Thurman, R. L. Wershaw, and J. C. Westall (1982). Complexation of Copper by Aquatic Humic Substances from Different Environments. J. Sci. Total Env. (in press).
- Miller, W. E. and J. G. Tash (1967). Interim Report: Upper Kalamath Lake Studies, Oregon. Corvallis, Oregon: Pacific Northwest Water Laboratory, Publication WP-20-8.
- Nordstrom, D. K., L. N. Plummer, T. M. L. Wigley, T. J. Wolery, J. W.

- Ball, E. A. Jenne, R. L. Bassett, D. A. Crerar, T. M. Florence, B. Fritz, M. Hoffman, G. R. Holdren, Jr., G. M. Lafon, S. V. Mattigod, R. E. McDuff, F. Morel, M. M. Reddy, G. Sposito, J. Thrailkill (1979). A comparison of computerized chemical models for equilibrium calculations in aqueous systems. In Chemical Modeling in Aqueous Systems, E. A. Jenne (ed.). Washington, D.C.: American Chemical Society, Symposium Series 93.
- Norris, A. C. (1981). Computational Chemistry. New York: John Wiley and Sons.
- O'Shea, T. A. and K. H. Mancy (1976). Characterization of trace metal-organic interactions by anodic stripping voltammetry. Anal. Chem., 48: 1603-1607.
- Pagenkopf, G. K. and D. Cameron (1979). Deposition of trace metals in stream sediments. Water, Air, Soil Poll., 11: 429-435.
- Pagenkopf, G. K., R. C. Russo, and R. V. Thurston (1974). Effect of complexation on toxicity of copper to fishes. J. Fish. Res. B. Can., 31: 462-465.
- Parrish, R. (1982). U. S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, personal communication.
- Peake, E., B. L. Baker, and G. W. Hodgson (1972). Hydrogeochemistry of the surface waters of the Mackenzie River drainage basin, Canada. II. The contribution of amino acids, hydrocarbons, and chlorins to the Beaufort Sea by the Mackenzie River system. Geochim. Cosmochim. Acta., 36: 867-883.
- Perdue, E. M. (1979). Solution thermochemistry of humic substances. In Chemical Modeling in Aqueous Systems. E. A. Jenne (ed). Washington, D. C.: American Chemical Society, Symposium Series 93.
- Perdue, E. M. (1982). Portland State University, personal communication.
- Perdue, E. M., C. R. Lytle, M. S. Sweet, and J. Sweet (1981). The chemical and biological impact of Klamath Marsh on the Williamson River, Oregon. Corvallis, Oregon: Oregon State University Water Resources Research Institute, Publication No. WRRI-71.
- Perdue, E. M., J. H. Reuter, and M. Ghosal (1980). The operational nature of acidic functional group analyses and its impact on mathematical descriptions of acid-base equilibria in humic substances. Geochim. Cosmochim. Acta, 44: 1841-1851.

- Peterson, N. V. and J. R. McIntyre (1970). The reconnaissance geology and mineral resources of Eastern Klamath County and Western Lake County, Oregon. Portland, Oregon: State of Oregon Department of Geology and Mineral Resources.
- Pocklington, R. (1972). Determination of nanomolar quantities of free amino acids dissolved in North Atlantic Ocean waters. Anal. Biochem., 45: 403-421.
- Posner, A. M. (1964). Titration curves of humic acid. In Proceedings of the 8th International Congress of Soil Science, Part II. Bucharest, Romania.
- Posner, A. M. (1966). The humic acids extracted by various reagents from a soil. Part I. Yield, inorganic components, and titration curves. J. Soil Sci., 17: 65-78.
- Ramamoorthy, S. and D. K. Kushner (1975). Heavy metal binding components of river water. J. Fish. Res. Board Can., 32: 1755-1766.
- Reuter, J. H. and E. M. Perdue (1977). Importance of heavy metal-organic matter interactions in natural waters. Geochim. Cosmochim. Acta., 41: 325-334.
- Roe, D. K. Portland State University, personal communication.
- Rohm and Haas (1971). Summary Bulletin. Amberlite Polymeric Absorbants. Philadelphia: Rohm and Haas Company.
- Ryan, D. K. and J. H. Weber (1982). A fluorescence quenching titration technique for determination of complexing capacities and stability constants of fulvic acid.
- Saar, R. A. and J. H. Weber (1979). Complexation of cadmium (II) with water- and soil-derived fulvic acid: Effect of pH and fulvic acid concentration. Can. J. Chem., 57: 1263-1268.
- Saar, R. A. and J. H. Weber (1980). Lead (II) complexation by fulvic acid: How it differs from fulvic acid complexation of copper (II) and cadmium (II). Geochim. Cosmochim. Acta., 44: 1381-1384.
- Scatchard, G. (1949). The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci., 51: 660-672.
- Scatchard, G., J. S. Coleman, and A. L. Shen (1957). Physical chemistry of protein solutions. VII. The binding of some small anions to serum albumin. J. Am. Chem. Soc., 79: 12-20.
- Schlesinger, W. H. and J. M. Melack (1981). Transport of organic carbon in the world's rivers. Tellus, 33: 172-187.

- Schnitzer, M. and S. U. Khan (1972). Humic Substances in the Environment. New York: Marcel Dekker.
- Schubert, J. (1948). The use of ion exchangers for the determination of physical-chemical properties of substances, particularly radiotracers in solution: I. Theoretical. J. Phys. Coll. Chem, 52: 340-350.
- Semenov, A. D., A. P. Pashanova, T. S. Kishkinova, and L. I. Nemtseva (1967). Content of individual groups of organic substances in the waters of some Soviet rivers. Soviet Hydrology: Selected Papers, 5: 549-553.
- Shapiro, J. (1957). Chemical and biological studies on the yellow organic acids of lake water. Limnol. Oceanogr., 2: 161-169.
- Shaw, T. L. and V. M. Brown (1974). The toxicity of some forms of copper to rainbow trout. Water Res., 8: 377-382.
- Shuman, M. S. and G. P. Woodward (1977). Stability constants of copper-organic chelates in aquatic samples. Env. Sci. Technol., 11: 809-813.
- Sillen, L. G. and A. E. Martell (1964). Stability Constants of Metal-ion Complexes. London: The Chemical Society, Special Publication No. 17.
- Sips, R. (1948). On the structure of a catalyst surface. J. Chem. Physics, 16: 490-495.
- Skoog, D. A. and D. M. West (1963). Fundamentals of Analytical Chemistry. New York: Holt, Rinehart, and Winston.
- Smith, R. G. (1976). Evaluation of combined applications of ultra-filtration and complexation capacity techniques to natural water. Anal. Chem., 48: 74-76.
- Sohn, M. L. and M. C. Hughes (1981). Metal ion complex formation constants of some sedimentary humic acids with Zn(II), Cu(II), and Cd(II). Geochim. Cosmochim. Acta, 45: 2393-2399.
- Spackman, D. H., W. H. Stein, and S. Moore (1958). Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem., 30: 1190-1206.
- Spencer, C. P. (1957). Utilization of trace elements by marine unicellular algae. J. Gen. Microbiol., 16: 282-285.
- Sposito, G. (1981). Trace metals in contaminated waters. Env. Sci. Technol., 15: 396-403.

- Sposito, G. and K. M. Holtzclaw (1979). Copper (II) complexation by fulvic acid extracted from sewage sludge as influenced by nitrate versus perchlorate background ionic media. Soil Sci. Soc. Am. J., 43: 47-51.
- Sposito, G., K. M. Holtzclaw, and C. S. Levesque-Madore (1978). Calcium ion complexation by fulvic acid extracted from sewage sludge-soil mixtures. Soil Sci. Soc. Am. J., 42: 600-606.
- Sposito, K. M. Holtzclaw, and C. S. Levesque-Madore (1979). Cupric ion complexation by fulvic acid extracted from sewage sludge-soil mixtures. Soil Sci. Soc. Am. J., 43: 1148-1155.
- Steemann-Nielsen, E. and S. Wiium-Anderson (1970). Copper ions as poison in the sea and in fresh water. Mar. Biol., 6: 93-97.
- Stella, R. and M. T. Ganzerli-Valentini (1979). Copper ion selective electrode for the determination of inorganic copper species in fresh waters. Anal. Chem., 51: 2148-2151.
- Stevenson, F. J. (1976). Stability constants of copper(II), lead(II), and cadmium(II) complexes with humic acids. Soil Sci. Soc. Am. J., 40: 665-672.
- Stevenson, F. J. and J. H. A. Butler (1969). Chemistry of humic acids and related pigments. In Organic Geochemistry, G. Eglinton and M. T. J. Murphy (eds.). New York: Springer Verlag.
- Stevenson, F. J., S. A. Krastanov, and M. S. Ardakani (1973). Formation constants of copper (II) complexes with humic and fulvic acids. Geoderma, 9: 129-141.
- Stiff, M. J. (1971). Copper-bicarbonate equilibria in solutions of bicarbonate ion at concentrations similar to those found in natural water. Water Res., 5: 171-176.
- Stuermer, D. H. and G. R. Harvey (1978). The isolation of humic substances and alcohol-soluble organic matter from sea water. Marine Chem., 6: 55-70.
- Stumm, W. and H. Bilinski (1977). Trace metals in natural waters; difficulties of interpretation arising from our ignorance on their speciation. In Advances in Water Pollution Research, Sixth International Conference Proceedings, Jerusalem, S. H. Jenkins (ed.). New York: Pergamon Press.
- Stumm, W. and J. J. Morgan (1970). Aquatic Chemistry. New York: Willey-Interscience, p. 490.
- Sturgeon, R. E., S. S. Berman, A. Desaulniers, and D. S. Russell (1980). Preconcentration of trace metals from seawater for determination

- by graphite furnace atomic absorption spectrometry. Talanta, 27: 85-94.
- Subramanian, K. S., C. L. Chakrabari, J. E. Sueiras, and I. S. Maines (1978). Preservation of some trace metals in samples of natural waters. Anal. Chem., 50: 444-448.
- Sunda, W., D. W. Engel, and R. M. Thuotte (1978). Effect of chemical speciation on the toxicity of cadmium to grass shrimp: Importance of free cadmium ion. Env. Sci. Technol., 12: 409-413.
- Sunda, W. and R. R. L. Guillard (1976). The relation between copper ion activity and the toxicity of copper phytoplankton. J. Marine Res., 34: 511-529.
- Sunda, W. G. and J. M. Lewis (1978). Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga. Monochrysis lutheri, Limnol. Oceanogr., 23: 870-876.
- Sweet, M. S. (1979). The concentration and speciation of sugars in natural waters. Master's Thesis, Portland State University, Portland, Oregon.
- Tajima, M. (1978). Comparative study between gas-liquid chromatography and ion exchange chromatography on amino acid analysis of foods. Agri. Biol. Chem., 42: 1949-1953 (1978).
- Tessler, A., P. G. C. Campbell, and M. Bisson (1979). Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem., 51: 844-850.
- Truitt, R. E. and J. H. Weber (1981). Determination of complexing capacity of fulvic acid for copper (II) and cadmium (II) by dialysis titration. Anal. Chem., 53: 337-342.
- Van den Berg, C. M. G. and J. R. Kramer (1979). Determination of complexing capacities of ligands in natural waters and conditional stability constants of the copper complexes by means of manganese dioxide. Anal. Chim. Acta, 106: 113-120.
- Vogel, A. I. (1974). A Textbook of Practical Organic Chemistry. London: Longman Group Limited.
- Vucenta, J. and J. J. Morgan (1978). Chemical modeling of trace metals in fresh waters: role of complexation and adsorption. Env. Sci. Technol., 12: 1302-1309.
- Weber, J. H. and K. H. Cheng (1979). Nonadsorption of fulvic acid from aqueous solutions on glassy carbon or wax sealed graphite electrodes. Anal. Chem., 51: 796-799.

- Weber, J. H. and S. A. Wilson (1975). The isolation and characterization of fulvic acid and humic acid from river waters. Water Res., 9: 1079-1084.
- Westall, J. and H. Hohl (1980). A comparison of electrostatic models for the oxide/solution interface. Adv. Coll. Interface Sci., 12: 265-294.
- Westall, J. C., J. L. Zachary, and F. M. M. Morel (1976). MINEQL, a computer program for the calculation of chemical equilibrium composition of aqueous systems. Cambridge, Massachusetts: Mass. Inst. Technol., Technical Note. No. 18.
- Wetzel, R. G. (1975). Limnology. Philadelphia: W. B. Saunders, p. 540.
- Wilson, A. L. (1959). Determination of fulvic acids in water. J. Appl. Chem., 9: 501-512.
- Zanetta, J. P. and G. Vincendon (1973). Gas-liquid chromatography of the N(o) - Heptafluorobutyrate of the isoamyl esters of amino acids. I. Separation and quantitative determination of the constituent amino acids of proteins. J. Chrom., 76: 91-99.
- Zumwalt, R., K. Kuo, and C. W. Gehrke (1971). Nanogram and picogram method for amino acid analysis in gas-liquid chromatography. J. Chrom., 57: 193-208.

APPENDIX A: HATIT PROGRAM LISTING

This program, written in Basic for the Rockwell Aim 65 microprocessor, was designed to automatically run titrations. The program is in four main parts: data input, standardization, running the titration and data collection, and calculations and printout. Standardization is accomplished by least squares linear regression.

```

20 DIM E(100),D(100),C(100),VT(100),TV(100),CT(100),CS(100)
30 POKE 4,4:POKE 5,144:D=USR(D):POKE 4,32:POKE 5,145
35 POKE 40950,0
40 INPUT "RUN#";R0$
45 INPUT "DATE";D$
50 INPUT "OPERATOR";O$
55 T=USR(14)
60 PRINT "IS THE TITRANT A"
65 IF USR(14)<T+2 THEN 65
70 T=USR(14)
75 PRINT "METAL(M),LIGAND(L),"
80 IF USR(14)<T+2 THEN 80
85 T=USR(14)
90 PRINT "ACID(A), OR BASE(B)?"
95 IF USR(14)<T+2 THEN 95
100 INPUT "ENTER M,L,A, OR B";Q$
110 IF Q$="M" THEN TS="METAL":SS="LIGAND":Q0=0
130 IF Q$="L" THEN TS="LIGAND":SS="METAL":Q0=0
150 IF Q$="A" THEN TS="ACID":SS="BASE":Q0=1
170 IF Q$="B" THEN TS="BASE":SS="ACID":Q0=1
175 PRINT TS;" NAME";
180 INPUT T1$
190 PRINT TS;" CONC.(MOL/L)";
195 INPUT CT
200 PRINT SS;" NAME";
205 INPUT S1$
210 PRINT SS;" CONC(MOL/L)";
220 INPUT CS
230 PRINT SS;" VOL.(ML)";
240 INPUT VS
250 INPUT "IONIC STRENGTH";MU
255 IF Q0=1 THEN 270
260 INPUT "PH";PH
270 INPUT "TEMP(K)";TK
280 REM CALIBRATION CURVE DATA
320 J=0
330 INPUT "READ STD.(Y OR N)";Z$
340 IF Z$="N" THEN 445
350 J=J+1
355 IF Q0=0 THEN INPUT "STD. PM = ";S(J)
360 IF Q0=1 THEN INPUT "STD. PH = ";S(J)
370 INPUT "DELAY TIME(SEC)";TD
380 T=USR(14)
390 IF USR(14)<T+1 THEN 390
400 T=USR(14):TD=TD-1
410 PRINT "DELAY TIME = ";TD
420 IF TD>0 THEN 390
425 GOSUB 2000
430 EC(J)=E(J)
435 DC(J)=D(J)
440 GOTO 330
445 J0=J
450 IF J0=0 THEN 610
455 GOSUB 4000
460 PRINT " CALIBRATION RESULTS"
465 PRINT " "
470 A1$=LEFT$(STR$(A1),6)
475 PRINT " SLOPE = ";A1$
480 A0$=LEFT$(STR$(A0),6)
485 PRINT " INTERCEPT = ";A0$
490 R$=LEFT$(STR$(R),6)
495 PRINT " CORR COEFF = ";R$
500 PRINT " ";PRINT " "
505 IF Q0=0 THEN PRINT " EXP.PM   CALC.PM"
510 IF Q0=1 THEN PRINT " EXP.PH   CALC.PH"
515 PRINT " "
520 FOR I=1 TO J0
525 SC(I)=(EC(I)-A0)/A1
530 P1$=LEFT$(STR$(SC(I)),6)
535 P2$=LEFT$(STR$(SC(I)),6)
540 PRINT TAB(2);P1$;SPC(10-LEN(P1$));P2$
545 NEXT I

```

```

610 REM START TITRATION
625 PRINT! " TITRATION RESULTS"
630 PRINT! " ";PRINT! " "
635 IF Q0=0 THEN PRINT! " VOLUME      CALC.PM"
640 IF Q0=1 THEN PRINT! " VOLUME      CALC.PH"
645 PRINT! " "
650 T=USR(14)
655 PRINT! "HOW MANY INCREMENTS"
660 IF USR(14)<T+2 THEN 660
665 T=USR(14)
670 PRINT! "OF TITRANT WILL BE"
675 IF USR(14)<T+2 THEN 675
680 T=USR(14)
685 PRINT! "ADDED";
687 INPUT J1
688 IF J1=0 THEN 940
690 INPUT! "DELAY TIME(SEC)";TD
695 FOR J=1 TO J1
700 T=USR(14)
705 PRINT! "MICROLITERS OF"
710 IF USR(14)<T+2 THEN 710
715 T=USR(14)
720 PRINT! "TITRANT IN INCR(";J;")";
725 IF USR(14)<T+2 THEN 725
730 INPUT VT(J)
732 IF J=1 THEN VT(J)=VT(J)+40
735 TV(J)=INT(VT(J)*1.2+.5)
740 NEXT J
745 VT=0:TV(0)=0
750 FOR J=0 TO J1
760 TE=TD:T=USR(14):D=USR(10)
790 IF USR(14)<T+TV(J) THEN 790
800 D=USR(9):T=USR(14)
820 IF USR(14)<T+1 THEN 820
830 T=USR(14):TE=TE-1
850 PRINT! "EV#";J;"DELAY=";TE
860 IF TE>0 THEN 820
870 GOSUB 2000
880 C(J)=(E(J)-A0)/A1
900 VT=VT+TV(J)/1.2
910 P1$=LEFT$(STR$(VT),7)
915 P2$=LEFT$(STR$(C(J)),6)
920 PRINT!P1$;SPC(13-LEN(P1$));P2$
930 NEXT J
940 REM COMPUTATIONS
950 REM AXIOM PRINTER ON
955 D=USR(12)
960 P0$="*****"
965 P$=P0$+P0$+P0$+P0$
970 PRINT! " ";PRINT! " ";PRINTP$:PRINT! " ";PRINT! " "
975 PRINTTAB(6)"RUN: ";R0$;SPC(13);"DATE: ";D$;SPC(13);"OPERATOR: ";J$

980 PRINT! " ";PRINT! " "
985 PRINTTAB(26)T1$;"-INTO-";S1$;" TITRATION"
990 PRINT! " "
991 CT$=STR$(CT):CS$=STR$(CS):VS$=STR$(VS)
992 P1$=T1$+" CONC. = "+CT$+" M"
994 P2$=S1$+" CONC. = "+CS$+" M"
996 P3$=S1$+" VOL. = "+VS$+" ML"
998 PRINTP1$;SPC(26-LEN(P1$));P2$;SPC(30-LEN(P2$));P3$
1000 PH$=STR$(PH):MU$=STR$(MU):TK$=STR$(TK)
1002 IF Q0=0 THEN P1$="PH = "+PH$
1004 IF Q0=1 THEN P1$=""
1006 P2$="IONIC STRENGTH = "+MU$
1008 P3$="TEMP. = "+TK$+" K"
1010 PRINTP1$;SPC(26-LEN(P1$));P2$;SPC(30-LEN(P2$));P3$
1030 PRINT! " ";PRINT! " "
1035 IF J0=0 THEN 1175
1040 PRINTTAB(25)"ELECTRODE CALIBRATION RESULTS"
1050 PRINT! " "

```

```

1060 IF Q0=1 THEN 1100
1080 PRINTTAB(10)"PM-EXP";SPC(12);"EMF(MV)";SPC(12);
1085 PRINT"STD.DEV.";SPC(12);"PM-CALC"
1090 GOTO 1110
1100 PRINTTAB(10)"PH-EXP";SPC(12);"EMF(MV)";SPC(12);
1105 PRINT"STD.DEV.";SPC(12);"PH-CALC"
1110 FOR J=1 TO J0
1115 P1$=LEFT$(STR$(S(J)),6)
1120 P2$=LEFT$(STR$(EC(J)),6)
1125 P3$=LEFT$(STR$(DC(J)),4)
1130 P4$=LEFT$(STR$(SC(J)),6)
1135 PRINTTAB(10)P1$;SPC(18-LEN(P1$));P2$;SPC(20-LEN(P2$));
1140 PRINTP3$;SPC(19-LEN(P3$));P4$
1145 NEXT J
1150 PRINT" "
1155 PRINTTAB(10)"SLOPE = ";A1$;SPC(12-LEN(A1$));"INTERCEPT = ";A0$;
1160 PRINTSPC(12-LEN(A0$));"CORR. COEFF. = ";R$
1170 PRINT" ";PRINT" "
1175 IF J1=0 THEN 1550
1180 PRINTTAB(25)T1$;"-INTO-";S1$;" TITRATION RESULTS"
1185 PRINT" "
1190 PRINTTAB(6)"TITRANT";SPC(7);"LOG";SPC(9);"LOG";
1195 PRINTSPC(9);"LOG";SPC(21);"LOG"
1200 IF Q0=1 THEN 1220
1205 PRINTTAB(6)"VOLUME";SPC(6);"M-TOTAL";SPC(5);"L-TOTAL";
1210 PRINTSPC(6);"M-FREE";SPC(6);"NU-BAR";SPC(6);"K-BAR"
1215 GOTO 1235
1220 PRINTTAB(6)"VOLUME";SPC(6);"H-TOTAL";SPC(5);"B-TOTAL";
1225 PRINTSPC(6);"H-FREE";SPC(6);"NU-BAR";SPC(6);"K-BAR"
1235 VT=0;C(0)=-C(0)
1240 P$=LEFT$(STR$(C(0)),6)
1245 PRINTTAB(7)"0";SPC(13);"*";SPC(11);"*";SPC(9);P$;
1250 PRINTSPC(15-LEN(P$));"*";SPC(10);"*"
1300 FOR J=1 TO J1
1305 VT=VT+TV(J)/1.2
1310 CT(J)=CT*(VT/1000)/(VS+VT/1000)
1320 CS(J)=CS*VS/(VS+VT/1000)
1330 IF Q0=1 THEN 1390
1340 IF Q$="M" THEN CM=CT(J);CL=CS(J)
1350 IF Q$="L" THEN CM=CS(J);CL=CT(J)
1355 CJ=10*(-C(J))
1360 VB=(CM-CJ)/CL
1362 IF VB<0 THEN VB=0
1365 KB=VB/((1-VB)*CJ)
1370 CM=LOG(CM)/LOG(10)
1375 CL=LOG(CL)/LOG(10)
1380 GOTO 1450
1390 IF Q$="A" THEN CA=CT(J);CB=CS(J)
1400 IF Q$="B" THEN CA=CS(J);CB=CT(J)
1405 CJ=10*(-C(J))
1410 VB=((CA-CB)-(CJ-(1.0E-14)/CJ))/CA
1415 IF VB<0 THEN VB=0
1420 KB=VB/((1-VB)*CJ)
1430 CA=LOG(CA)/LOG(10)
1440 CB=LOG(CB)/LOG(10)
1450 C(J)=-C(J)
1455 IF KB=0 THEN 1465
1460 KB=LOG(KB)/LOG(10)
1465 IF Q0=1 THEN CM=CA;CL=CB
1470 VT$=LEFT$(STR$(VT),7)
1475 CM$=LEFT$(STR$(CM),6)
1480 CL$=LEFT$(STR$(CL),6)
1485 CJ$=LEFT$(STR$(C(J)),6)

```

```

1490 VBS=LEFT$(STR$(VB),6)
1492 IF VB=0 THEN VBS=" * "
1495 KBS=LEFT$(STR$(KB),6)
1497 IF KB=0 THEN KBS=" * "
1500 PRINTTAB(6)VT$;SPC(12-LEN(VT$));CM$;SPC(13-LEN(CM$));CL$;
1505 PRINTSPC(12-LEN(CL$));CJ$;SPC(12-LEN(CJ$));VBS;
1510 PRINTSPC(12-LEN(VBS));KBS
1520 NEXT J
1540 PRINT" ":PRINT" ":PRINT" "
1550 P$=P0$+P0$+P0$+P0$
1555 PRINTP$
1560 FOR I=1 TO 6:PRINT" ":NEXT I
1565 D=USR(13)
1566 INPUT "ANOTHER RUN";L$
1567 IF L$="Y" THEN 320
1570 END
1950 REM
1960 REM
1970 REM
1980 REM SUBROUTINE TO READ 20 EMF VALUES
1990 REM AND COMPUTE THE AVG. EMF AND STD. DEV.
1992 REM
1994 REM
1996 REM
2000 T=USR(14)
2005 E1=0:E2=0
2010 Q1=.2:NR=10
2012 IF J<3 THEN Q1=1
2015 REM Q1 IS THE ERROR LIMIT ON EMF (1V MV)
2020 REM NR IS THE NUMBER OF READINGS TO BE AVERAGED.
2025 FOR K=1 TO NR
2030 IF USR(14)<T+5 THEN 2030
2040 T=USR(14)
2050 GOSUB 3000
2060 V(K)=V
2070 E1=E1+V(K)
2080 E2=E2+V(K)*V(K)
2090 NEXT K
2100 E(J)=E1/NR
2110 D(J)=SQR((E2-NR*E(J)*E(J))/(NR-1))
2120 IF D(J)<Q1 THEN RETURN
2122 E1=E1-V(1)
2124 E2=E2-V(1)*V(1)
2130 FOR K=1 TO (NR-1)
2140 V(K)=V(K+1)
2150 NEXT K
2160 IF USR(14)<T+5 THEN 2160
2170 T=USR(14)
2180 GOSUB 3000
2190 V(NR)=V
2200 E1=E1+V(NR)
2210 E2=E2+V(NR)*V(NR)
2220 GOTO 2100
2950 REM
2960 REM
2970 REM
2980 REM SUBROUTINE TO SAMPLE AND READ DVM
2985 REM
2990 REM
2995 REM
3000 V=0
3010 POKE 32768,4
3020 D=USR(0)
3030 F1=0
3040 FOR I=0 TO 5
3050 V=V+PEEK(I+32759)*10+I

```

```

3060 NEXT I
3070 V=V*10*(3-PEEK(32765))
3080 ON PEEK(32766)+1 GOTO 3090,3100,3120,3140
3090 RETURN
3100 V=-V:RETURN
3120 F1=1:RETURN
3140 V=-V:F1=1:RETURN
3950 REM
3960 REM
3970 REM
3980 REM SUBROUTINE TO FIT CALIBRATION DATA BY LEAST-SQUARES
3985 REM
3990 REM
3995 REM
4000 X1=0:X2=0:Y1=0:Y2=0:M1=0
4050 FOR I=1 TO J0
4060 X1=X1+S(I)
4070 X2=X2+S(I)*S(I)
4080 Y1=Y1+E(I)
4090 Y2=Y2+E(I)*E(I)
4100 M1=M1+S(I)*E(I)
4110 NEXT I
4120 T1=X2-X1*X1/J0
4130 T2=Y2-Y1*Y1/J0
4140 T3=M1-X1*Y1/J0
4150 A1=T3/T1
4160 A0=Y1/J0-A1*X1/J0
4170 R=SQR(ABS((T3*T3)/(T1*T2)))
4180 RETURN

```

APPENDIX B: SMPLX PROGRAM LISTING

This program, written in Basic for the Rockwell Aim 65 microprocessor, was used to calculate the two copper-oxalate binding constants from titration data. The calculation procedure and equations have been previously described in the Methods section. The iterative procedure minimizes a weighted, residual sum of squares (Norris, 1981).

```

100 POKE 4,4:POKE 5,144:D=USR(D):POKE 4,32:POKE 5,145
102 POKE 40950,0
104 INPUT"# OF DATA PAIRS=";ND
105 Z1=ND
106 INPUT"# OF VARIABLES(1,2,3)";NV
108 N=N1:N1=N1+1
109 INPUT "OX CONC";LT
110 INPUT "PH";H:H=(10*-H)/0.83
111 DIM X(ND),Y(ND),F(ND),GB(ND)
112 DIM V(NV),B0(N1,N1),S0(NV),S1(NV),ES(1),C0(NV,3)
114 FOR I=1 TO ND
115 READ X(I),Y(I):GOTO 120
116 PRINT"X(";I;"),Y(";I;")=";
118 INPUT X(I),Y(I)
120 NEXT I
156 G4=1.0E+30
158 N9=ND
162 G9=0
164 FOR I=1 TO NV:GB(I)=0:NEXT I
166 GOTO 200
168 REM INIT
170 RETURN
172 REM
174 GOSUB 636
176 Y9=SQR(Y9)
178 IF G4<Y9 THEN 186
180 G9=G9+1
182 G4=Y9
184 FOR I=1 TO NV:GB(I)=V(I):NEXT I
186 REM ?!"Y9=";Y9
188 RETURN
200 FOR I=1 TO NV
202 V(I)=0:S0(I)=0:S1(I)=0
204 FOR J=1 TO 3:C0(I,J)=0:NEXT J
206 NEXT I
208 FOR I=1 TO N1
210 FOR J=1 TO N1:B0(I,J)=0:NEXT J
212 NEXT I
224 FOR I1=1 TO N
226 PRINT"GUESS FOR V(";I1;")=";
228 INPUT S0(I1)
230 S1(I1)=0.1*ABS(S0(I1))
232 NEXT I1
250 E1=1.0E-6
252 PRINT"CONV. LIMIT=";
253 INPUT E1
254 PRINT"COMPUTING";
256 Z8=1
258 Z5=5
284 A9=1
286 B9=0.5
288 C9=2
290 Z7=0
292 Z4=0
294 FOR I1=1 TO N
296 B0(I1,N1)=S0(I1)
298 NEXT I1
300 FOR I1=1 TO N
302 S0(I1)=S0(I1)+S1(I1)
304 FOR J1=1 TO N
306 B0(J1,I1)=S0(J1)
308 NEXT J1
310 S0(I1)=S0(I1)-S1(I1)
312 NEXT I1
314 Z0=1
316 FOR I1=1 TO N
318 V(I1)=B0(I1,Z0)
320 NEXT I1

```

```

326 GOSUB 172
328 B0(N1,Z0)=Y9
330 Z0=Z0+1
332 IF Z0<=N1 THEN 316
334 Z9=N1
336 H8=B0(N1,1)
338 H9=1
340 L9=1
342 L8=H8
344 FOR I1=2 TO N1
346 IF H8=>B0(N1,I1) THEN 354
348 H8=B0(N1,I1)
350 H9=I1
352 GOTO 360
354 IF L8<=B0(N1,I1) THEN 360
356 L8=B0(N1,I1)
358 L9=I1
360 NEXT I1
361 FOR I=1 TO NV
362 FOR J=1 TO 3: C0(I,J)=0: NEXT J
363 NEXT I
364 FOR I1=1 TO N1
366 IF I1=H9 THEN 374
368 FOR J1=1 TO N
370 C0(J1,1)=C0(J1,1)+B0(J1,I1)
372 NEXT J1
374 NEXT I1
376 FOR I1=1 TO N
378 C0(I1,1)=C0(I1,1)/N
380 C0(I1,2)=(1+A9)*C0(I1,1)-A9*B0(I1,H9)
382 V(I1)=C0(I1,2)
384 NEXT I1
386 GOSUB 172
388 Z9=Z9+1
390 F1=Y9
392 IF Y9=>L8 THEN 428
394 FOR I1=1 TO N
396 C0(I1,3)=(1-C9)*C0(I1,1)+C9*C0(I1,2)
398 V(I1)=C0(I1,3)
400 NEXT I1
402 GOSUB 172
404 Z9=Z9+1
406 IF Y9=>L8 THEN 418
408 FOR I1=1 TO N
410 B0(I1,H9)=V(I1)
412 NEXT I1
414 B0(N1,H9)=Y9
416 GOTO 510
418 FOR I1=1 TO N
420 B0(I1,H9)=C0(I1,2)
422 NEXT I1
424 B0(N1,H9)=F1
426 GOTO 510
428 J1=0
430 FOR I1=1 TO N1
432 IF I1=H9 THEN 436
433 IF F1<B0(N1,I1) THEN 436
434 J1=J1+1
436 NEXT I1
438 IF J1<N THEN 418
440 IF F1>H8 THEN 452
442 FOR I1=1 TO N
444 B0(I1,H9)=C0(I1,2)
446 NEXT I1
448 B0(N1,H9)=F1
450 H8=F1
452 FOR I1=1 TO N
454 C0(I1,3)=B9*B0(I1,H9)+(1-B9)*C0(I1,1)
456 V(I1)=C0(I1,3)

```

```

460 GOSUB 172
462 Z9=Z9+1
464 F1=Y9
466 IF F1>H8 THEN 478
468 FOR I1=1 TO N
470 B0(I1,H9)=C0(I1,3)
472 NEXT I1
474 B0(N1,H9)=F1
476 GOTO 510
478 FOR I1=1 TO N1
480 IF I1=L9 THEN 488
482 FOR J1=1 TO N
484 B0(J1,I1)=0.5*(B0(J1,I1)+B0(J1,L9))
486 NEXT J1
488 NEXT I1
490 Z0=1
492 IF Z0=L9 THEN 504
494 FOR I1=1 TO N
496 V(I1)=B0(I1,Z0)
498 NEXT I1
500 GOSUB 172
502 B0(N1,Z0)=Y9
504 Z0=Z0+1
506 IF Z0<N1 THEN 492
508 Z9=Z9+N
510 S2=0
512 Z7=Z7+1
514 Z4=Z4+1
515 IF Z7=>Z8 THEN 518
516 IF Z4=>Z5 THEN 518
517 GOTO 336
518 L9=1
520 L8=B0(N1,1)
522 FOR I1=1 TO N1
524 S2=S2+B0(N1,I1)
526 IF L8<=B0(N1,I1) THEN 532
528 L9=I1
530 L8=B0(N1,I1)
532 NEXT I1
534 S2=S2/N1
536 F1=0
538 FOR I1=1 TO N1
540 F1=F1+(B0(N1,I1)-S2)*2
542 NEXT I1
544 F1=F1/N
546 IF F1<=E1 THEN 564
548 IF Z4=Z5 THEN 554
550 Z7=0
552 GOTO 336
554 GOSUB 590
556 IF Z7=>Z8 THEN Z7=0
558 Z4=0
560 GOTO 336
562 GOTO 336
564 GOSUB 590
568 PRINT! " ":PRINT! " ":PRINT!
570 PRINT! "VAR.=";E1;PRINT! " "
572 FOR I=1 TO NV: S0(I)=0:NEXT I
574 FOR I=1 TO N
576 PRINT! "V(";I;")=";G8(I)
578 NEXT I
580 PRINT! "DEG OF FIT=";G4
581 PRINT! Z9;" EVALUATIONS"
582 N=N9
586 END
588 REM RUN 1850
590 H9=1
591 IF L9>1 THEN L8=B0(N1,1)

```

CONVERGED":PRINT!" "

```

592 IF L9=1 THEN L8=B3(N1,2)
593 IF L9<1 THEN L8=0
594 FOR I1=1 TO N1
595 IF I1=L9 THEN 602
596 IF B3(N1,I1)>L8 THEN 602
598 L8=B3(N1,I1)
600 H9=I1
602 NEXT I1
604 PRINT " ";PRINT "      ESTIMATES"
608 FOR I1=1 TO N
616 D4=B3(I1,L9)
618 D5=B3(I1,H9)
620 PRINT "V(";I1;")=";D4
622 NEXT I1
624 PRINT " "
626 REM ? I B3(N1,L9),B3(N1,H9)
628 PRINT "F1=";F1
630 PRINT "Z9=";Z9
632 PRINT " "
634 RETURN
636 Y9=0
638 GO SUB 1000
686 RETURN
1000 REM FUNCTION SUBROUTINE
1010 A=V(1)
1020 B=V(2)
1021 K1=10+3.81;K2=10+5.18;K3=10+5.91;K4=10+6.3
1022 OH=1.59E-14/H
1030 FOR I=1 TO ND
1040 L1=2*B*Y(I)
1041 L2=1+K1*H+K2*H+2+A*Y(I)+K4*Y(I)*H
1042 L=(-L2+SQR(L2+2+4*L1*LT))/(2*L1)
1050 YM=Y(I)*(1+K3*OH+K4*H*L+A*L+B*L+2)-X(I)
1052 F(I)=YM
1055 Y9=Y9+YM+2/X(I)
1060 NEXT I
1070 RETURN
1100 DATA 1E-6,7.58E-8,2.5E-6,1.92E-7,5E-6,6.58E-7
1101 DATA 7.5E-6,1.4E-6,1E-5,2.72E-6,2.5E-5,1.46E-5
1102 DATA 5E-5,3.72E-5,7.5E-5,6.09E-5,1E-4,8.51E-5
11017 .5E-5,1.46E-8,1E-4,2.26E-8,2.5E-4,1.46E-7

```

APPENDIX C: LSTSQR AND GAUSSM3 PROGRAM LISTINGS

LSTSQR was written in Fortran for use on the Honeywell 66/20 computer (Parrish, 1982). The program will accept data entered via the teletype or will generate data assuming a Gaussian distribution of Log K values over a user-selected range of free metal concentrations. Nonlinear regression analysis is used to minimize a weighted residual sum of squares (Norris, 1981).

GAUSSM3 was also written in Fortran for use on the Honeywell 66/20 computer (Parrish, 1982). The program will accept data entered via the teletype, in data statements, or can generate data from Scatchard parameters. This data can be fit to a single or bimodal Gaussian distribution. Numerical integration is by the method of Gaussian quadrature, and fitting is via nonlinear regression and minimization of a weighted residual sum of squares (Norris, 1981).

```

10 IMPLICIT REAL*8(A-H,O-Z)
20 DIMENSION PM(100),DNU(2,100),DKBAR(100),CMTOT(100)
30 COMMON DM(100),DALF(100),N
40 dimension conc(2),dmu(2),dsig(2),bot(2),top(2)
45 dimension x(4),g(4),h(30)
50 DIMENSION DDM(100),DDALF(100)
60 INP=5
70 LOP=6
80 WRITE(LOP,11)
90 11 format(//,"select: 1=gaussian data generation,
100&2=data input via teletype")
110 READ(INP,12)NURD2
120 12 FORMAT(I2)
130 IF(NURD2.EQ.2) GO TO 14
140 WRITE(LOP,10)
150 10 FORMAT(//,"ENTER HI,LO, AND INCREM. pM VALUES")
160 READ(INP,20) PMHI, PMLO, PMINC
170 20 FORMAT(V)
180 PTS=(PMHI-PMLO)/PMINC+1
190 NPTS=IFIX(PTS)
200 WRITE(LOP,30)
210 30 FORMAT(" ENTER NO. OF CLASSES OF LIGANDS. 1 OR 2.")
220 READ(INP,40) NCLASS
230 40 FORMAT(I1)
232 42 WRITE(LOP,43)
234 43 FORMAT(" ",/,/,/,/)
240 CLTOT=0.D0
250 DO 70 I=1,NCLASS
260 WRITE(LOP,50) I
270 50 format(" enter conc., mu, sigma for lingand
275&class ",i1)
280 READ(INP,60) CONC(I),DMU(I),DSIG(I)
290 60 FORMAT(V)
300 CLTOT=CLTOT+CONC(I)
310 70 CONTINUE
320 TEST=5.0D-3
330 DO 150 I=1,NCLASS
340 CPM=PMHI+PMINC
350 BOT(I)=DMU(I)-4.D0*DSIG(I)
360 TOP(I)=DMU(I)+4.D0*DSIG(I)
370 I3=20
380 AAA=DMU(I)
390 BBB=DSIG(I)
400 DO 140 J=1,NPTS
410 CPM=CPM-PMINC
420 PM(J)=CPM
430 DDM(J)=1.D1**(-1.D0*PM(J))
440 V4=DDM(J)/(DSIG(I)*2.5066D0)
450 CCC=DDM(J)
460 W=0.D0
470 I2=4
480 F=1.D0

```

```

490 Q=BOT(I)
500 W=W+SIMPS(Q,AAA,BBB,CCC)
510 Q=TOP(I)
520 W=W+SIMPS(Q,AAA,BBB,CCC)
530 T1=W
540 80 I2=I2+1
550 IF(I2.GT.I3) GO TO 999
560 DI2=FLOAT(I2)
570 DN=2.D0**DI2
580 S=(TOP(I)-BOT(I))/DN
590 Q=S+BOT(I)
600 W=W+4.D0*(SIMPS(Q,AAA,BBB,CCC))
610 D=BOT(I)
620 90 D=D+2.D0*S
630 IF(D.LT.TOP(I)) GO TO 120
640 W=W*S/3.D0
650 IF(F.EQ.0.D0) GO TO 100
660 F=0.D0
670 GO TO 110
680 100 IF(DABS((W2-W)/W).LT.TEST) GO TO 130
690 110 W2=W
700 W=T1
710 GO TO 80
720 120 Q=D
730 W=W+2.D0*(SIMPS(Q,AAA,BBB,CCC))
740 Q=D+S
750 W=W+4.D0*(SIMPS(Q,AAA,BBB,CCC))
760 GO TO 90
770 130 DNU(I,J)=V4*W
780 140 CONTINUE
790 150 CONTINUE
800 DO 170 J=1,NPTS
810 Y=0.D0
820 DO 160 I=1,NCLASS
830 Y=Y+DNU(I,J)*CONC(I)/CLTOT
840 160 CONTINUE
850 DDALF(J)=Y
860 DKBAR(J)=DDALF(J)/(DDM(J)*(1.D0-DDALF(J)))
870 DKBAR(J)=DLOG10(DKBAR(J))
880 CMTOT(J)=DDM(J)+DDALF(J)*CLTOT
885 CMTOT(J)=CMTOT(J)/CLTOT
890 170 CONTINUE
900 GO TO 13
910 14 WRITE(LOP,15)
920 15 format("//,"enter total ligand conc. and total
925&no. data pts.")
930 READ(INP,16)CLTOT,NPTS
940 16 FORMAT(V)
950 WRITE(LOP,17)
960 17 format(" select: 1=m and cmtot, 2=ph and
965&alpha")
970 READ(INP,18)NURD3

```

```

980 18 FORMAT(I2)
990 IF(NURD3.EQ.2) GO TO 23
1000 WRITE(LOP,19)
1010 19 FORMAT(" ENTER M AND CMTOT AS DATA PAIRS")
1020 DO 21 I=1,NPTS
1030 READ(INP,22)DDM(I),CMTOT(I)
1040 22 FORMAT(V)
1050 DDALF(I)=(CMTOT(I)-DDM(I))/CLTOT
1060 DKBAR(I)=DLOG10(DDALF(I)/DDM(I)*(1.D0-DDALF(I)))
1065 21 CONTINUE
1070 GO TO 13
1080 23 WRITE(LOP,24)
1090 24 FORMAT(" ENTER pH (pM) AND ALPHA AS DATA PAIRS")
1100 DO 25 I=1,NPTS
1110 READ(INP,26)PM(I),DDALF(I)
1120 26 FORMAT(V)
1125 DDM(I)=1.D1**(-PM(I))
1130 DKBAR(I)=DLOG10(DDALF(I)/DDM(I)*(1.D0-DDALF(I)))
1140 CMTOT(I)=DDM(I)+DDALF(I)*CLTOT
1145 25 CONTINUE
1150 13 GO TO 7215
1160 501 FORMAT(" PRINT TABLES?  1 = YES.  0 = NO.")
1170 READ(INP,502) O1
1180 502 FORMAT(I1)
1190 IF(O1.EQ.0.0) GO TO 211
1200 WRITE(LOP,720)
1210 720 FORMAT(" ENTER 1 FOR LIST OF NU-BAR TO 8 PLACES")
1220 READ(INP,721)NURD
1230 721 FORMAT(V)
1240 IF(NURD.NE.1)GO TO 724
1245 7215 CONTINUE
1250 DO 722 I=1,NPTS
1260 WRITE(LOP,723)DDM(I),DDALF(I),CMTOT(I)
1270 723 FORMAT(" ",3(5X,1PE10.3))
1280 722 CONTINUE
1285 GO TO 42
1290 724 WRITE(LOP,180)
1300 180 FORMAT(" DATA      LOG FREE      TOTAL      NUBAR      LOG")
1310 WRITE(LOP,190)
1320 190 format(" point      metal      metal
1325&kbar",/)
1330 DO 210 J=1,NPTS
1340 WRITE(LOP,200)J,PM(J),CMTOT(J),DDALF(J),DKBAR(J)
1350 200 FORMAT(" ",I3,5X,F5.1,3X,1PD9.2,2X,0PF6.3,2X,F6.2)
1360 210 CONTINUE
1370 211 CONTINUE
1380 WRITE(LOP,715)
1390 715 FORMAT("//,"NONLINEAR, LEAST-SQUARES ANALYSIS.")
1400 WRITE(LOP,716)
1410 716 format("use n's and k's that generated data as
1415&first guesses.")
1420 IMPLICIT REAL*8(A-H,O-Z)

```

```

1430 EXTERNAL FUN
1440 DATA M/4/
1450 WRITE(LOP,400)
1460 400 FORMAT(//,"ENTER n1,K1,n2,K2")
1470 READ(INP,700)X
1480 700 FORMAT(V)
1490 712 WRITE(LOP,707)
1500 707 format(//,"enter starting data point index for
1505&scatchard analysis")
1510 READ(INP,708) NSTART
1520 708 FORMAT(I2)
1530 J=0
1540 DO 709 I=NSTART,NPTS
1550 J=J+1
1560 DM(J)=DDM(I)
1570 DALF(J)=DDALF(I)
1580 709 CONTINUE
1590 N=J
1600 EST=0.D0
1610 DEPS=1.D-4
1620 2 WRITE(LOP,402)
1630 402 format("enter no. iterations. -1=values,0=stop,
1635&l=another analysis.")
1650 READ(INP,403)ITER
1660 403 FORMAT(I3)
1670 IF(ITER.EQ.0) GO TO 99
1680 IF(ITER.EQ.-1) GO TO 98
1690 IF(ITER.EQ.1) GO TO 211
1700 DO 4 I=1,M
1710 4 X(I)=DSQRT(X(I))
1720 CALL FMFP(FUN,M,X,SSE,G,EST,DEPS,ITER,IER,H)
1730 DO 3 I=1,M
1740 3 X(I)=X(I)**2
1750 WRITE(LOP,404)IER,SSE,(X(I),I=1,M),(G(I),I=1,M)
1760 404 format(/x,10herror code,i3/17h sum of squares
1765& =,1p13.5/3h x=,1p4e15.5/3h g=,1p4e15.5/)
1780 GO TO 2
1790 98 WRITE(LOP,92)X
1800 92 FORMAT(5H1PRED,1P4E15.5//)
1810 DO 198 I=1,N
1820 PRED=(X(1)*X(2)*DM(I)/(1.D0+(X(2)*DM(I))))+
1830&(X(3)*X(4)*DM(I)/(1.D0+(X(4)*DM(I))))
1840 198 WRITE(LOP,91)DM(I),DALF(I),PRED
1850 91 FORMAT(4X,1P3E13.2)
1860 GO TO 2
1870 999 WRITE(LOP,900)
1880 900 format("no conv. in 16 iter. incr. i3 in
1890& LINE 0280 OR INCREASE TEST IN LINE 0230")
1900 99 STOP
1910 END
1920 SUBROUTINE FUN(M,XX,F,G)
1930 IMPLICIT REAL*8(A-H,O-Z)

```

```

1940 COMMON DM(100),DALF(100),N
1950 DIMENSION X(4),G(1),XX(1)
1960 DATA DZ,DE/0.D0,1.D0/
1970 DO 100 I=1,M
1980 100 X(I)=XX(I)**2
1990 F=DZ
2000 DO 1 I=1,M
2010 1 G(I)=DZ
2020 DO 2 I=1,N
2030 WT=DE/DALF(I)
2040 D1=DE/(DM(I)+DE/X(2))
2050 D2=DE/(DM(I)+DE/X(4))
2060 PRED=DM(I)*(X(1)*D1+X(3)*D2)
2070 ERROR=(DALF(I)-PRED)
2080 F=F+WT*(ERROR**2)
2090 CONS=-2.D0*ERROR*WT
2100 G(1)=G(1)+DM(I)*D1*CONS*2.D0*XX(1)
2110 G(2)=G(2)+X(1)*DM(I)*D1**2/X(2)**2*CONS*2.D0*XX(2)
2120 G(3)=G(3)+DM(I)*D2*CONS*2.D0*XX(3)
2130 G(4)=G(4)+X(3)*DM(I)*D2**2/X(4)**2*CONS*2.D0*XX(4)
2140 2 CONTINUE
2150 RETURN
2160 END
2170 FUNCTION SIMPS(Q,AAA,BBB,CCC)
2180 V5=1.D1**Q/(1.D0+CCC*1.D1**Q)
2190 V6=DEXP(-5.D-1*((AAA-Q)/BBB)**2)
2200 SIMPS=V5*V6
2210 RETURN; END
2220 SUBROUTINE FMFP(FUNCT,N,X,F,G,EST,EPS,LIMIT,IER,H)
2230 IMPLICIT REAL*8(A-H,O-Z)
2240 COMMON DM(100),DALF(100)
2250 DIMENSION X(1),G(1),H(1)
2260 KOUNT=0
2270 CALL FUNCT(N,X,F,G)
2280 IER=0
2290 N2=N+N
2300 N3=N2+N
2310 N31=N3+1
2320 1 K=N31
2330 DO 4 J=1,N
2340 H(K)=1.D0
2350 NJ=N-J
2360 IF(NJ)5,5,2
2370 2 DO 3 L=1,NJ
2380 KL=K+L
2390 3 H(KL)=0.D0
2400 4 K=KL+1
2410 5 KOUNT=KOUNT+1
2420 KNT=KOUNT
2430 OLDF=F
2440 DO 9 J=1,N
2450 K=N+J

```

```

2460 H(K)=G(J)
2470 K=K+N
2480 H(K)=X(J)
2490 K=J+N3
2500 T=0.D0
2510 DO 8 L=1,N
2520 T=T-G(L)*H(K)
2530 IF(L-J)6,7,7
2540 6 K=K+N-L
2550 GO TO 8
2560 7 K=K+1
2570 8 CONTINUE
2580 9 H(J)=T
2590 DY=0.D0
2600 HNRM=0.D0
2610 GNRM=0.D0
2620 DO 10 J=1,N
2630 HNRM=HNRM+DABS(H(J))
2640 GNRM=GNRM+DABS(G(J))
2650 10 DY=DY+H(J)*G(J)
2660 IF(DY)11,51,51
2670 11 IF(HNRM/GNRM-EPS)51,51,12
2680 12 FY=F
2690 ALFA=2.D0*(EST-F)/DY
2700 AMBDA=1.D0
2710 IF(ALFA)15,15,13
2720 13 IF(ALFA-AMBDA)14,15,15
2730 14 AMBDA=ALFA
2740 15 ALFA=0.D0
2750 16 FX=FY
2760 DX=DY
2770 217 DO 17 I=1,N
2780 17 X(I)=X(I)+AMBDA*H(I)
2790 CALL FUNCT(N,X,F,G)
2800 218 FY=F
2810 DY=0.D0
2820 DO 18 I=1,N
2830 18 DY=DY+G(I)*H(I)
2840 IF(DY)19,36,22
2850 19 IF(FY-FX)20,22,22
2860 20 AMBDA=AMBDA+ALFA
2870 ALFA=AMBDA
2880 IF(HNRM*AMBDA-1.D10)16,16,21
2890 21 IER=2
2900 RETURN
2910 22 T=0.D0
2920 23 IF(AMBDA)24,36,24
2930 24 Z=3.D0*(FX-FY)/AMBDA+DX+DY
2940 ALFA=DMAX1(DABS(Z),DABS(DX),DABS(DY))
2950 DALFA=Z/ALFA
2960 DALFA=DALFA**2-DX/ALFA*DY/ALFA
2970 IF(DALFA)51,25,25

```

```

2980 25 W=ALFA*DSQRT(DALFA)
2990 ALFA=DY-DX+W+W
3000 IF(ALFA)250,251,250
3010 250 ALFA=(DY-Z+W)/ALFA
3020 GO TO 252
3030 251 ALFA=(Z+DY-W)/(Z+DX+Z+DY)
3040 252 ALFA=ALFA*AMBDA
3050 DO 26 I=1,N
3060 26 X(I)=X(I)+(T-ALFA)*H(I)
3070 CALL FUNCT(N,X,F,G)
3080 IF(F-FX)27,27,28
3090 27 IF(F-FY)36,36,28
3100 28 DALFA=0.D0
3110 DO 29 I=1,N
3120 29 DALFA=DALFA+G(I)*H(I)
3130 IF(DALFA)30,33,33
3140 30 IF(F-FX)32,31,33
3150 31 IF(DX-DALFA)32,36,32
3160 32 FX=F
3170 DX=DALFA
3180 T=ALFA
3190 AMBDA=ALFA
3200 GO TO 23
3210 33 IF(FY-F)35,34,35
3220 34 IF(DY-DALFA)35,36,35
3230 35 FY=F
3240 DY=DALFA
3250 AMBDA=AMBDA-ALFA
3260 GO TO 22
3270 36 IF(OLDF-F+EPS)51,38,38
3280 38 DO 37 J=1,N
3290 K=N+J
3300 H(K)=G(J)-H(K)
3310 K=N+K
3320 H(K)=X(J)-H(K)
3330 37 CONTINUE
3340 IER=0
3350 39 T=0.D0
3360 Z=0.D0
3370 DO 40 J=1,N
3380 K=N+J
3390 W=H(K)
3400 K=K+N
3410 T=T+DABS(H(K))
3420 40 Z=Z+W*H(K)
3430 IF(HNRM-EPS)41,41,42
3440 41 IF(T-EPS)56,56,42
3450 42 IF(KOUNT-LIMIT)43,50,50
3460 43 ALFA=0.D0
3470 DO 47 J=1,N
3480 K=J+N3
3490 W=0.D0

```

```
3500 DO 46 L=1,N
3510 KL=N+L
3520 W=W+H(KL)*H(K)
3530 IF(L-J)44,45,45
3540 44 K=K+N-L
3550 GO TO 46
3560 45 K=K+1
3570 46 CONTINUE
3580 K=N+J
3590 ALFA=ALFA+W*H(K)
3600 47 H(J)=W
3610 IF(Z*ALFA)48,1,48
3620 48 K=N31
3630 DO 49 L=1,N
3640 KL=N2+L
3650 DO 49 J=L,N
3660 NJ=N2+J
3670 H(K)=H(K)+H(KL)*H(NJ)/Z-H(L)*H(J)/ALFA
3680 49 K=K+1
3690 GO TO 5
3700 50 IER=1
3710 KOUNT=KNT
3720 RETURN
3730 51 DO 52 J=1,N
3740 K=N2+J
3750 52 X(J)=H(K)
3760 CALL FUNCT(N,X,F,G)
3770 IF(GNRM-EPS)55,55,53
3780 53 IF(IER)56,54,54
3790 54 IER=-1
3800 GO TO 1
3810 55 IER=0
3820 56 RETURN
3830 END
```

DRIVER FOR MIXED NORMALS

1 DEC 1981 - CHANGES TO: (1) ADD CAPABILITY TO MINIMIZE ON "ALPHA",
 (2) EVALUATE INTEGRALS FOR SELECTED PH RANGES, AND
 (3) ADD NEW INPUT SUBROUTINE FOR DATA MODIFICATIONS AND
 INPUT FORMAT VARIATIONS

```

IMPLICIT REAL*8(A-H,O-Z)
INTEGER OPT,OPTHC
COMMON /INDX/IND(5),IPRHT,IPHLO,IPHHI,IPHINC,IFUNC
COMMON /IO/INP,OPT,OPTHC
EXTERNAL FUN
DIMENSION X(5),G(5),H(30),XX(5),GG(5)

```

LOGICAL UNIT NUMBER ASSIGNMENTS:

```

INP=5
OPT=6
OPTHC=6

```

BEGIN INPUT FROM USER

```

X = VECTOR OF PARAMETERS: (THETA, MU1, SIGMA1, MU2, SIGMA2)
400 WRITE(OPT,500)
500 FORMAT("ENTER X VECTORS: THETA,MU1,SIGMA1,MU2,SIGMA2")
READ(INP,501)X
501 FORMAT(V)
WRITE(OPT,502)
502 FORMAT("ENTER MINIMUM ESTIMATE AND EPSILON")
READ(INP,503)EST,EPS
503 FORMAT(V)

```

THE "SEQUENCE OF FIVE INDICES" BELOW REFERS TO A PERMUTATION OF THE INTEGERS 1,2,3,4,5 WHICH DENOTES THE ORDER IN WHICH THE PARAMETERS ARE REARRANGED PRIOR TO FITTING. IF THE NUMBER OF VARIABLES TO BE FITTED IS LESS THAN FIVE, THEN THE PARAMETERS LISTED FIRST WILL BE VARIED WHILE THE REMAINING ONES ARE HELD FIXED. FOR EXAMPLE, IF THE INPUT CORRESPONDS TO: NVAR=3 AND SEQUENCE=1 4 5 2 3, THEN THETA, MU2, AND SIGMA2 WILL BE VARIED WITHIN THE MINIMIZATION ROUTINE, AND MU1 AND SIGMA1 WILL BE HELD FIXED AT THEIR CURRENT VALUES. THE PARAMETERS ARE RESTORED TO THEIR ORIGINAL ORDERING PRIOR TO LISTING.

```

200 WRITE(OPT,504)
504 FORMAT("ENTER NBR OF VARIABLES AND SEQUENCE OF FIVE INDICES")
READ(INP,505)NVAR,IND
505 FORMAT(V)

```

THE "LO,HI,INC" CORRESPONDS TO DATA SUBSET SELECTION, AS BEFORE.

```

WRITE(OPT,506)
506 FORMAT("ENTER LO,HI,INC FOR FITTING(SELECT OBSERVATIONS)")
READ(INP,507)IPHLO,IPHHI,IPHINC
507 FORMAT(V)

```

THE NUMBER OF ITERATIONS, IF POSITIVE, ENGAGES THE MINIMIZATION ROUTINE FOR THAT NUMBER OF "CYCLES". SUGGEST USING SMALL NUMBERS, SUCH AS 2 OR 3, REPEATING AS NECESSARY.

IF A CODE IS ENTERED, CONTROL TRANSFERS EITHER TO END THE PROGRAM OR TO PROMPT FOR AND ACCEPT NEW INPUT INFORMATION. OPTION -1 ALLOWS A "RESTART" WITH A NEW SEQUENCE. OPTION -2 CAN BE USED PRIOR TO ENTERING OPTION -3; THIS EFFECTS A CALL TO THE SUBROUTINE "FUN" WHICH PERFORMS INITIAL CALCULATIONS, BUT IS NECESSARY ONLY WHEN OPTION -3 IS TO BE SELECTED WITHOUT UTILIZING THE MINIMIZATION PROCEDURE. OPTION -3 WILL EVALUATE THE INTEGRALS AND PRINT

```

        INFORMATION FOR A SEQUENCE OF "PH" VALUES; PRIOR TO USING THIS
        OPTION, "FUN" MUST HAVE BEEN CALLED VIA OPTION -2 OR VIA INVOKING
        THE MINIMIZATION ROUTINE (I.E., ENTERING A POSITIVE NUMBER OF,
        ITERATIONS). OPTION -4 "RESTARTS" WITH A NEW X VECTOR.
100 WRITE(OPT,508)
508 FORMAT("ENTER NBR OF ITERATIONS OR CODE:")
    WRITE(OPT,509)
509 FORMAT(" 0=STOP, -1=NEW VRRL SEQ, -2=INITIALIZE, -3=EVAL,
        -4=NEW X GUESSES")
    READ(INP,511)LIMIT
511 FORMAT(V)
    IPRNT=0
    IF(LIMIT.EQ.-1)GO TO 200
    IF(LIMIT.EQ.-4)GO TO 400
    IF(LIMIT.EQ.0)GO TO 99
    DELTA=1.D-20
    X(1)=DASIN(DSQRT(X(1)))
    X(3)=DSQRT(X(3)-DELTA)
    X(5)=DSQRT(X(5)-DELTA)

RE-ARRANGE PARAMETERS FOR INPUT TO FMFP
DO 12 I=1,5
12 XX(I)=X(IND(I))

ACT ON SELECTED OPTION
    IF(LIMIT.EQ.-3)GO TO 300
    IF(LIMIT.EQ.-2)CALL FUN(S,XX,PHI,GG)
    IF(LIMIT.GT.0)CALL FMFP(FUN,NVBL,XX,PHI,GG,EST,EPS,LIMIT,IER,H)

DE-ARRANGE PARAMETERS
10 DO 20 I=1,5
    U(IND(I))=GG(I)
20 X(IND(I))=XX(I)
    EPSQ=1.D-35
    IF(DABS(X(1)).GT.EPSQ)G(1)=G(1)/DSIN(2.DU*X(1))
    IF(DABS(X(3)).GT.EPSQ)G(3)=G(3)*0.500/X(3)
    IF(DABS(X(5)).GT.EPSQ)G(5)=G(5)*0.500/X(5)
    X(1)=(DSIN(X(1)))**2
    X(3)=X(3)**2+DELTA
    X(5)=X(5)**2+DELTA

OUTPUT RESULTS OF MINIMIZATION ATTEMPT
    AN ERROR CODE OF 0 INDICATES CONVERGENCE, -1 AN ERROR, AND 1 MEANS
    THAT FINAL CONVERGENCE HAS NOT YET OCCURRED TO THE DEGREE REQUESTED.
    WRITE(OPT,512)IER
512 FORMAT(11H ERROR CODE,I2)
    WRITE(OPT,82)X
    82 FORMAT(9H X VECTOR/(4E18,10))
    WRITE(OPT,83)G
    83 FORMAT(16H GRADIENT VECTOR/(4E18,10))
    WRITE(OPT,84)PHI
    84 FORMAT(6H PHI =,G18,10)
    WRITE(OPT,513)
513 FORMAT("ENTER 1=PRINT OBSERVED, PREDICTED VALUES")
    READ(INP,514)IPRNT
514 FORMAT(V)
    IF(IPRNT.EQ.1)WRITE(OPTH,80)IFUNC
    80 FORMAT(17H1PH,OBS,PRED,DIFF/10H PHI CODE=,I2/)
    IF(IPRNT.EQ.1)CALL FUN(S,XX,PHI,G)
    GO TO 100

OPTION CODE -3: FOR CONSTRUCTION OF TABLES OF PREDICTED VALUES
300 WRITE(OPT,4080)

```

```

4080 FORMAT(37H ENTER PHLO,PHHI,PHINC FOR EVALUATION)
      HEAD(INP,S15)PHLO,PHHI,PHINC
515  FORMAT(V)
      WRITE(OPTHC,4081)X
4081  FORMAT(1H1,5E18.10//17H PREDICTED VALUES/20H PH,NUM,DEN,K,PK,VGL/)
      NTODD=(PHHI+1.D-6-PHLO)/PHINC+1
      DO 4001 I=1,NTODD
        PHO=PHLO+PHINC*FLOAT(I-1)
        CALL FUNE(2,X,PHO,G)
        DKBAR=G(1)/G(2)
        PKBAR=-DLOG10(DKBAR)
        MU=1.D1*(-PHO)
        MUH=HO-1.D-14/HO

      THE VOLUME CALCULATION MIGHT NOT BE APPROPRIATELY CODED.....
      VOL=(.652100+DKBAR/(HO+DKBAR)-1.D2*HOH)/(.499700+MUH)
4001  WRITE(OPTHC,4082)PHO,G(1),G(2),DKBAR,PKBAR,VOL
4082  FORMAT(1X,F5.2,2E12.4,2E14.6,F10.4)
      GO TO 100
99  STOP
      END
SUBROUTINE FUN - THIS VERSION CORRESPONDS TO FITTING A MIXTURE OF TWO
                  TRUNCATED NORMALS.
1 DEC 1981      - IMPLEMENTED CHANGES
7 DEC 1981      - CONVERTED TO NONTRUNCATED CASE

      SUBROUTINE FUN(M,XX,PHI,GG)
      IMPLICIT REAL*8(A-H,O-Z)
      REAL*8 K(50)
      INTEGER OPT,OPTHC
      COMMON /INDX/IND(5),IPRNT,IPHL0,IPHHI,IPHINC,IFUNC
      COMMON /IO/INP,OPT,OPTHC

      DATA ARRAYS HAVE BEEN RESERVED FOR A MAXIMUM OF 50 DATA POINTS.
      TO CHANGE, ALTER DIMENSION STATEMENTS BELOW.
      DIMENSION X(5),G(5),DM(30,2),W(20),Z(20),DNUM(2),DDEN(2),
      &DNDMU(2),DND SIG(2),XX(5),DDDSIG(2),DDDMU(2),GG(5),WW(10),ZZ(10)
      DIMENSION H(25),V(25),HOH(25),PK(25),PH(25),ALPHA(25)

      DMAGL IS A CONSTANT THAT INDICATES THE MAXIMUM PERMISSIBLE EXPONENT
      FOR THE MACHINE.
      IPRNTI = CONTROL SWITCH FOR PRINTING INTERMEDIATE RESULTS DURING
      THE FITTING PROCESS (1=PRINT, 0=NO+PRINT)
      DATA NO,DE,DZ/O,1.D0,C.D0/,DMAGL/35.D0/
      DATA IPRNTI/O/
      DATA NPT/20/
      DATA DELTA/1.D-20/
      DATA ZZ/-5.387480890011200,-4.003682449550700,-3.944764040115600,
      &-5.347854567383200,-2.788806059428100,-2.254974002089300,
      &-1.738537712116600,-1.234076215395300,-0.737473726545400,
      &-0.245340708300900/
      DATA WW/2.2293936455340-13,4.3993409922730-10,1.0860693707690-07,
      &7.8025564785320-06,2.2833863601630-04,3.2437733422380-03,
      &2.4810520887460-02,1.0901720602000-01,2.8667550536200-01,
      &4.6224366960060-01/
      DATA PH/4.600,4.900,5.600,5.0900,5.200,5.500,5.4900,5.600,
      &5.700,5.7800,5.8400,5.900,5.9500,6.00,6.0800,6.1500,6.2900,
      &6.2600,6.300,6.3400,6.3800,6.4200,6.4500,6.4800,6.500/
      DATA ALPHA/9.630-2,7.840-2,7.520-2,6.950-2,6.450-2,5.780-2,
      &4.990-2,4.530-2,4.160-2,3.890-2,3.660-2,3.430-2,3.350-2,
      &3.230-2,3.050-2,2.890-2,2.720-2,2.590-2,2.460-2,2.330-2,
      &2.210-2,2.130-2,2.050-2,1.960-2,1.870-2/

```

```

DE-ARRANGE PARAMETERS
  DO 20 I=1,5
20  X(IND(I))=XX(I)
    X(1)=(DSIN(X(1)))**2
    X(3)=X(3)**2+DELTA
    X(5)=X(5)**2+DELTA
INITIALIZE
  IF(NC.NE.0)GO TO 100
  NU=1
  DL=DLOG(1.01)
  DRTP=DE/(DSQRT(3.1415926535897*32384600))
  DRT2=DSQRT(2.00)
  DO 1099 I=1,20
    J=(I+1)/2
    W(I)=WW(J)
1099  Z(I)=ZZ(J)+DRT2
    DO 1098 I=2,20,2
1098  Z(I)=-Z(I)

INPUT DATA ARRAYS VIA SUBROUTINE
  CALL DATAIN(N,PH,H,HOH,PK,K,ALPHA,V)

PHI(.) REPRESENTS THE MINIMIZATION CRITERION
  WRITE(OUT,600)
600  FORMAT("SELECT 1=PHI(PK), 2=PHI(VCL), 3=PHI(ALPHA)")
  READ(INP,601)IFUNC
601  FORMAT(V)

100  PHI=DZ
    JLO=1
    JHI=2
    IF(X(1).EQ.DE)JHI=1
    IF(X(1).EQ.DZ)JLO=2

    DO 101 I=1,5
101  G(I)=DZ

PH LOOP
  DO 200 IPH=IPHLO,IPHHI,IPHINC
    UPH=PH(IPH)
    TPH=1.01**(-DPH)

    DO 221 J=1,2
      DNUM(J)=DZ
      DNDMU(J)=DZ
221  DNDSIG(J)=DZ

DISTRIBUTION LOOP
  DO 201 J=JLO,JHI

LOOP OVER GAUSSIAN POINTS
  DO 202 I=1,NPT
    DX=X(2*J)+X(2*J+1)*Z(I)
    IF(DX.GT.DMAGL) GO TO 202
    IF(DX.LT.-DMAGL) GO TO 1200
    TDX=1.01**(-DX)
    TDXL=-DX*DL
    TDXPHL=DLOG(TDX+TPH)
    HZ1L=TDXL-TDXPHL
    DLW=DLOG(W(I))
    DLWWW=DLW+HZ1L
    IF(DLWWW.LT.-DMAGL)DLWWW=-DMAGL
    NNIM(I)=NNIM(J)+DEXP(DLWWW)

```

```

CONL=TDXL-TDXPHL-TDXPHL
DLWWW=DLW+CONL
IF(DLWWW.LT.-DMAGL)DLWWW=-DMAGL
DNDMU(J)=DNDMU(J)-DEXP(DLWWW)
DND SIG(J)=DND SIG(J)-DEXP(DLWWW)*Z(1)
GO TO 202
1200 DNUM(J)=DNUM(J)+W(1)
202 CONTINUE

DNUM(J)=D RTP*DNUM(J)
DNDMU(J)=DL*TPH*DNDMU(J)*D RTP
DND SIG(J)=DL*TPH*DND SIG(J)*D RTP
DDEN(J)=DE-DNUM(J)
DDDMU(J)=-DNDMU(J)
DDDSIG(J)=-DND SIG(J)
201 CONTINUE

CORRECT FOR 10*(-PH)
GO 313 J=JLO,JHI
DDEN(J)=DDEN(J)/TPH
DDDMU(J)=DDDMU(J)/TPH
313 DDDSIG(J)=DDDSIG(J)/TPH

DEX=DE-X(1)
DN=X(1)*DNUM(1)+DEX*DNUM(2)
DD=X(1)*DDEN(1)+DEX*DDEN(2)
WT=1.00

IF(IFUNC.EQ.2)GO TO 2222
IF(IFUNC.EQ.3)GO TO 3333

PERTAINS TO PHI(PK)
IF(DN/DD.LE.DZ)PKHAT=DPH
IF(DN/DD.GT.DZ)PKHAT=-DLOG10(DN/DD)
ERROR=PK(IPH)-PKHAT

IF(IPRNT.EQ.1)WRITE(OPTHC,80)DPH,PK(IPH),PKHAT,ERROR
80 FORMAT(1X,F7.4,1X,3E12.4)

G(1)=G(1)+((DNUM(1)-DNUM(2))/DN-(DDEN(1)-DDEN(2))/DD)*ERROR
GO 300 J=JLO,JHI
IF(J.EQ.1)DEX=X(1)
IF(J.EQ.2)DEX=DE-X(1)
G(2+J)=G(2+J)+(DEX*DNDMU(J)/DN-DEX*DDDMU(J)/DD)*ERROR*2.00
300 G(2+J+1)=G(2+J+1)+(DEX*DND SIG(J)/DN-DEX*DDDSIG(J)/DD)*ERROR
    *2.00
GO TO 200

PERTAINS TO PHI(VOL) --- NOTE VOLUME CONSTANTS
2222 C=.652100/(.499700+HOH(IPH))
FI=DN/DD
IF(FI.LT.DZ)FI=DZ
VOLHAT=C*FI/(H(IPH)+FI)-1.DZ*HOH(IPH)/(.499700+HOH(IPH))
ERROR=VOLHAT-V(IPH)
IF(IPRNT.EQ.1)WRITE(OPTHC,81)DPH,V(IPH),VOLHAT,ERROR
81 FORMAT(1X,F6.1,F8.3,2F9.4)
C=(H(IPH)/(H(IPH)+FI)*.2)*C*ERROR/(DE-DN)*.2*TPH*2.D0
G(1)=G(1)+C*(DNUM(1)-DNUM(2))
G(2)=G(2)+C*X(1)*DNDMU(1)
G(3)=G(3)+C*X(1)*DND SIG(1)
G(4)=G(4)+C*(DE-X(1))*DNDMU(2)
G(5)=G(5)+C*(DE-X(1))*DND SIG(2)
WRITE(1,*)C,DN,DD,H(IPH),HOH(IPH),DE,G,DNUM,DDEN,DNDMU,

```

```

      *UNDSIG,X
      GU TO 200

      PENTAINS TO PHI(ALPHA)
3333 AOUS=DE-ALPHA(IPH)
      AHAT=DD*TPH
      WT=DE/ALPHA(IPH)
      ERROR=AOUS-AHAT
      IF(IPRNT.EQ.1)WRITE(UPTHC,62)DPH,AORS,AHAT,ERROR
      62 FORMAT(1X,F7.4,1X,3E12.4)
      C=-2.00*ERROR*TPH*WT
      G(1)=G(1)+(DDEN(1)-DDEN(2))*C
      G(2)=G(2)+X(1)*DDDMU(1)*C
      G(3)=G(3)+X(1)*DDDSIG(1)*C
      G(4)=G(4)+DEX*DDDMU(2)*C
      G(5)=G(5)+DEX*DDDSIG(2)*C
      200 PHI=PHI+WT*ERROR**2

      PRINT INTERMEDIATE RESULTS
      IF(IPRNT1.EQ.1)WRITE(UPTHC,3309)PHI,X,G
3309 FORMAT(1X,E12.4/1X,5E10.3/1X,5E10.3)

      RE-ARRANGE GRADIENTS
      G(1)=G(1)*DSIN(2.00*XX(1))
      G(3)=G(3)*2.00*XX(3)
      G(5)=G(5)*2.00*XX(5)
      DO 2001 I=1,5
2001 GG(I)=G(IND(I))
      RETURN

*****
      EVALUATE INTEGRALS - MAINLINE OPTION -3
      ENTRY TO "FUN" MUST HAVE BEEN MADE PRIOR TO ENTERING "FUNE"
      ENTRY FUNE(M,XX,PHI,GG)
      DPH=PHI
      TPH=1.01*(-DPH)
      DNUM(1)=DZ
      DNUM(2)=DZ
      DO 4201 J=JLO,JHI
      DO 4202 I=1,NPT
      XX=X(2+J)+X(2+J+1)*Z(I)
      IF(DX.GT.DMAGL) GO TO 4202
      IF(DX.LT.-DMAGL) GO TO 4200
      TDX=1.01*(-DX)
      TDXL=-DX*DL
      TDXPFL=DLOG(TDX+TPH)
      HZ1L=TDXL-TDXPFL
      DLW=DLOG(W(I))
      DNUM(J)=DNUM(J)+DEXP(DLW+HZ1L)
      GO TO 4202
4200 DNUM(J)=DNUM(J)+W(I)
4202 CONTINUE
      DNUM(J)=DNUM(J)*DRTP
      DDEN(J)=DE-DNUM(J)
4201 DNUM(J)=DNUM(J)*TPH
      DEX=DE-X(1)
      GG(1)=X(1)*DNUM(1)+DEX*DNUM(2)
      GG(2)=X(1)*DDEN(1)+DLX*DDEN(2)
      RETURN
      END
      SUBROUTINE FOR DATA CONSTRUCTION
      (CALLED FROM FUN)
      SUBROUTINE DATAIN(N,PHI,H,HOH,PK,K,ALPHA,V)

```

```

      IMPLICIT REAL*8(A-H,O-Z)
      REAL*8 K(1)
      DIMENSION PH(1),H(1),HOH(1),PK(1),ALPHA(1),V(1)

      WRITE(6,603)
603  FORMAT("ENTER NBR OF DATA POINTS")
      READ(5,604)N
604  FORMAT(V)

6000 WRITE(6,605)
605  FORMAT("ENTER TYPE OF INPUT:")
      WRITE(6,606)
606  FORMAT("      1 = PH (OR PM) AND ALPHA")
      WRITE(6,607)
607  FORMAT("      2 = PH (OR PM) AND PK")
      WRITE(6,608)
608  FORMAT("      3 = M (OR H) AND ALPHA")
      WRITE(6,609)
609  FORMAT("      4 = M (OR H) AND PK")
      WRITE(6,610)
610  FORMAT("      5 = PH AND VOLUME")
      READ(5,611)INPTYP
611  FORMAT(V)
      GO TO (7001,7002,7003,7004,7005),INPTYP

      FOR OTHER CASES, INSERT YOUR CODE HERE.

      NOTE THAT NO VOLUME CALCULATIONS ARE INCLUDED HERE. IF YOU WANT THEM
      YOU MUST CODE THEM BELOW.

      THE APPROPRIATE TRANSFORMATIONS OF THE DATA ARE EFFECTED IN THE
      SECTIONS BELOW. THESE TRANSFORMATIONS ARE NECESSARY IN ORDER TO
      ADAPT THE PROGRAM TO THE "NEW" NOTATION. CHANGE ONLY THE READ
      STATEMENTS TO INPUT AS YOU WISH. DATA STATEMENTS, IF USED, MUST
      BE INCLUDED IN THE CALLING PROGRAM, FUN.

7001 WRITE(6,619)
619  FORMAT("      1=HARD-WIRED, 2=TELETYPE, 3=
      SCATCHARD PARAMETERS")
      READ(5,620)NOOP
620  FORMAT(V)
      GO TO(9000,9001,9002),NOOP
9000 DO 9003 I=1,N
      H(I)=1.D1*(-PH(I))
      ALPHA(I)=1.D0-ALPHA(I)
      HOH(I)=H(I)-1.D-14/H(I)
      K(I)=H(I)*ALPHA(I)/(1.D0-ALPHA(I))
9003 PK(I)=-DLOG10(K(I))
      GO TO 7000
9001 WRITE(6,9004)
9004 FORMAT(" ENTER PH (PM) AND ALPHA AS DATA PAIRS")
      DO 9005 I=1,N
      READ(5,9006)PH(I),ALPHA(I)
9006  FORMAT(V)
      H(I)=1.D1*(-PH(I))
      ALPHA(I)=1.D0-ALPHA(I)
      HOH(I)=H(I)-1.D-14/H(I)
      K(I)=H(I)*ALPHA(I)/(1.D0-ALPHA(I))
9005 PK(I)=-DLOG10(K(I))
      GO TO 7000
9002 WRITE(6,9007)
9007 FORMAT(" ENTER N1,K1,N2,K2 FROM SCATCHARD ANALYSIS")

```

```

      READ(5,9008)PARA1,PARA2,PARA3,PARA4
7008 FORMAT(V)
      WRITE(6,9012)
7012 FORMAT(" ENTER BEGINNING AND INCRMENT PH (PM) VALUES")
      READ(5,9013)GARB1,GARU2
7013 FORMAT(V)
      GARH3=C.D0
      DO 9009 I=1,N
      PH(I)=GARH1+GARU3
      GARB3=GARB2
      GARH1=PH(I)
      H(I)=1.D1*(-PH(I))
      ALPHA(I)=1.D0-(((PARA1+PARA2+H(I))/(1.D0+PARA2+H(I)))+
      &((PARA3+PARA4+H(I))/(1.D0+PARA4+H(I))))
      HUH(I)=H(I)-1.D-14/H(I)
      K(I)=H(I)+ALPHA(I)/(1.D0-ALPHA(I))
7009 PK(I)=-DLOG10(K(I))
      GO TO 7000

7002 DO 7012 I=1,N

      READ(1,613)PH(I),PK(I)
613 FORMAT(V)
      PK(I)=-PK(I)

      K(I)=1.D1*(-PK(I))
      H(I)=1.D1*(-PH(I))
      HUH(I)=H(I)-1.D-14/H(I)
7012 ALPHA(I)=K(I)/(H(I)+K(I))
      GO TO 7000

7003 DO 7013 I=1,N

      READ(1,614)DM,ALPHA(I)
614 FORMAT(V)
      H(I)=DM
      ALPHA(I)=1.D0-ALPHA(I)

      PH(I)=-DLOG10(H(I))
      HUH(I)=H(I)-1.D-14/H(I)
      K(I)=H(I)+ALPHA(I)/(1.D0-ALPHA(I))
7013 PK(I)=-DLOG10(K(I))
      GO TO 7000

7004 DO 7014 I=1,N

      READ(1,615)DM,PK(I)
615 FORMAT(V)
      PK(I)=-PK(I)
      H(I)=DM

      PH(I)=-DLOG10(H(I))
      K(I)=1.D1*(-PK(I))
      HUH(I)=H(I)-1.D-14/H(I)
7014 ALPHA(I)=K(I)/(H(I)+K(I))
      GO TO 7000

7005 DO 7015 I=1,N

```

```

      READ(1,616)PH(I),V(I)
616  FORMAT(V)

      H(I)=1.01*(-PH(I))
      H0H(I)=H(I)-1.0-14/H(I)
      K(I)=H(I)/(-1.00+.652100/(V(I)*(.499700+H0H(I))+1.02*H0H(I)))
      PK(I)=-DLOG10(K(I))
7015 ALPHA(I)=K(I)/(H(I)+K(I))

7000 WRITE(6,617)
617  FORMAT("ENTER 1=TO PRINT DATA")
      READ(5,618)ICK
618  FORMAT(V)
      IF(ICK.NE.1)GO TO 99
      WRITE(6,81)
81  FORMAT(20H1DATA: PH,K,PK,ALPHA)
      DO 7700 I=1,N
      AE=1.00-ALPHA(I)
7700 WRITE(6,80)PH(I),K(I),PK(I),AE
80  FORMAT(1X,F5.2,3(4X,D17.9))

99  RETURN
END
SUBROUTINE FMFP(FUNCT,N,X,F,G,EST,EPS,LIMIT,IER,H)
IMPLICIT REAL*8(A-H,C-Z)
DIMENSION X(1),G(1),H(1)
KOUNT=0
CALL FUNCT(N,X,F,G)
IER=0
N2=N+N
N3=N2+N
N31=N3+1
1  K=N31
  DO 4 J=1,N
    H(K)=1.00
    NJ=N-J
    IF(NJ)5,5,2
2  DO 3 L=1,NJ
    KL=K+L
3  H(KL)=0.00
4  K=KL+1
5  KOUNT=KOUNT+1
  KNT=KOUNT
  OLDF=F
  DO 9 J=1,N
    K=N+J
    H(K)=G(J)
    K=K+N
    H(K)=X(J)
    K=J+N3
    T=0.00
    DO 8 L=1,N
      T=T-G(L)*H(K)
      IF(L-J)6,7,7
6  K=K+N-L
    GO TO 8
7  K=K+1
8  CONTINUE
9  H(J)=T
  DY=0.00
  HARM=0.00
  GARM=0.00

```

```

      DO 10 J=1,N
      HNRM=HNRM+DABS(H(J))
      GNRM=GNRM+DABS(G(J))
10  DY=DY+H(J)*G(J)
      IF(DY)11,51,51
11  IF(HNRM/GNRM-EPS)51,51,12
12  FY=F
      ALFA=2.00*(EST-F)/DY
      AMBDA=1.00
      IF(ALFA)15,15,13
13  IF(ALFA-AMBDA)14,15,15
14  AMBDA=ALFA
15  ALFA=0.00
16  FX=FY
      DX=DY
217 DO 17 I=1,N
17  X(I)=X(I)+AMBDA*H(I)
      CALL FUNCT(N,X,F,G)
218 FY=F
      DY=0.00
      DO 18 I=1,N
18  DY=DY+G(I)*H(I)
      IF(DY)19,36,22
19  IF(FY-FX)20,22,22
20  AMBDA=AMBDA+ALFA
      ALFA=AMBDA
      IF(HNRM-AMBDA-1.010)**6,16,21
21  IER=2
      RETURN
22  T=0.00
23  IF(AMBDA)24,36,24
24  Z=3.00*(FX-FY)/AMBDA+DX+DY
      ALFA=DMAX1(DABS(Z),DAHS(DX),DABS(DY))
      DALFA=Z/ALFA
      DALFA=DALFA**2-DX/ALFA*DY/ALFA
      IF(DALFA)51,25,25
25  W=ALFA*DSQRT(DALFA)
      ALFA=DY-DX+W+W
      IF(ALFA)250,251,250
250 ALFA=(DY-Z+W)/ALFA
      GO TO 252
251 ALFA=(Z+DY-W)/(Z+DX+Z+DY)
252 ALFA=ALFA+AMBDA
      DO 26 I=1,N
26  X(I)=X(I)+(T-ALFA)*H(I)
      CALL FUNCT(N,X,F,G)
      IF(F-FX)27,27,28
27  IF(F-FY)36,36,28
28  DALFA=0.00
      DO 29 I=1,N
29  DALFA=DALFA+G(I)*H(I)
      IF(DALFA)30,33,33
30  IF(F-FX)32,31,33
31  IF(DX-DALFA)32,36,32
32  FX=F
      DX=DALFA
      T=ALFA
      AMBDA=ALFA
      GO TO 23
33  IF(FY-F)35,34,35
34  IF(DY-DALFA)35,36,35
35  FY=F
      DY=DALFA

```

```

      AMBDA=AMBDA-ALFA
      GO TO 22
36  IF(OLDF-F+EPS)51,38,36
38  DO 37 J=1,N
      K=N+J
      H(K)=G(J)-H(K)
      K=N+K
      H(K)=X(J)-H(K)
37  CONTINUE
      IER=0
39  T=0.00
      Z=0.00
      DO 40 J=1,N
      K=N+J
      W=H(K)
      K=K+N
      T=T+DABS(H(K))
40  Z=Z+W*H(K)
      IF(HNRM-EPS)41,41,42
41  IF(T-EPS)56,56,42
42  IF(KOUNT-LIMIT)43,50,50
43  ALFA=0.00
      DO 47 J=1,N
      K=J+N3
      W=0.00
      DO 46 L=1,N
      KL=N+L
      W=W+H(KL)*H(K)
      IF(L-J)44,45,45
44  K=K+N-L
      GO TO 46
45  K=K+1
46  CONTINUE
      K=N+J
      ALFA=ALFA+W*H(K)
47  H(J)=W
      IF(Z*ALFA)48,1,48
48  K=N31
      DO 49 L=1,N
      KL=N2+L
      DO 49 J=L,N
      NJ=N2+J
      H(K)=H(K)+H(KL)*H(NJ)/Z-H(L)*H(J)/ALFA
49  K=K+1
      GO TO 5
50  IER=1
      KOUNT=KNT
      RETURN
51  DO 52 J=1,N
      K=N2+J
52  X(J)=H(K)
      CALL FUNCT(N,X,F,G)
      IF(GNRM-EPS)55,55,53
53  IF(IER)56,54,54
54  IER=-1
      GO TO 1
55  IER=0
56  RETURN
      END

```

APPENDIX D: AMINO ACID DATA

The following tables give the total amino acids in μM for unfiltered samples. Missing data are indicated by an asterisk.

170

[illegible]

[illegible]

172

[illegible]

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-21-77 | * | * | * | * | * | * | * | * |
| 01-15-78 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| 02-18-78 | 0.18 | 0.00 | 0.07 | 0.07 | 0.00 | 0.07 | 0.00 | 0.07 |
| 03-19-78 | 0.12 | 0.06 | 0.13 | 0.16 | 0.04 | 0.00 | 0.11 | 0.06 |
| 04-15-78 | 0.08 | 0.01 | 0.24 | 0.08 | 0.00 | 0.24 | 0.00 | 0.00 |
| 05-13-78 | 0.00 | 0.00 | 0.33 | 0.15 | 0.00 | 0.41 | 0.00 | 0.00 |
| 06-12-78 | 0.00 | 0.00 | 0.10 | 0.07 | 0.00 | 0.15 | 0.00 | 0.00 |
| 07-14-78 | 0.00 | 0.03 | 0.10 | 0.02 | 0.00 | 0.07 | 0.00 | 0.00 |
| 08-19-78 | 0.00 | 0.00 | 0.12 | 0.06 | 0.03 | 0.07 | 0.04 | 0.00 |
| 09-18-78 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 |
| 10-14-78 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.03 | 0.01 | 0.00 | 0.05 | 0.04 | 0.00 |
| 12-17-78 | * | * | * | * | * | * | * | * |
| 01-13-79 | 0.08 | 0.02 | 0.28 | 0.06 | 0.00 | 0.42 | 0.02 | 0.00 |
| 02-17-79 | 0.07 | 0.03 | 0.63 | 0.14 | 0.00 | 0.42 | 0.06 | 0.00 |
| 03-15-79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.03 | 0.06 | 0.07 | 0.02 | 0.00 | 0.07 | 0.01 | 0.00 |
| 05-19-79 | 0.09 | 0.04 | 0.25 | 0.01 | 0.00 | 0.14 | 0.00 | 0.00 |
| 06-17-79 | 0.00 | 0.00 | 0.35 | 0.25 | 0.00 | 0.21 | 0.00 | 0.00 |
| 07-14-79 | 0.00 | 0.06 | 0.29 | 0.04 | 0.00 | 0.13 | 0.00 | 0.00 |
| 08-11-79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 09-08-79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-22-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 01-15-78 | 0.40 | 0.70 | 0.00 | 0.20 | 0.20 | 0.30 | 0.00 | 0.20 |
| 02-18-78 | 0.28 | 0.54 | 0.09 | 0.10 | 0.25 | 0.05 | 0.06 | 0.05 |
| 03-19-78 | 0.24 | 0.33 | 0.06 | 0.06 | 0.08 | 0.06 | 0.04 | 0.07 |
| 04-15-78 | 0.09 | 0.13 | 0.03 | 0.03 | 0.07 | 0.00 | 0.00 | 0.04 |
| 05-13-78 | 0.64 | 1.26 | 0.22 | 0.24 | 1.11 | 0.12 | 0.12 | 0.33 |
| 06-12-78 | 0.38 | 0.30 | 0.10 | 0.15 | 0.10 | 0.03 | 0.00 | 0.10 |
| 07-14-78 | 0.14 | 0.23 | 0.01 | 0.01 | 0.03 | 0.02 | 0.00 | 0.00 |
| 08-19-78 | 0.16 | 0.22 | 0.12 | 0.09 | 0.08 | 0.03 | 0.00 | 0.00 |
| 09-18-78 | 0.08 | 0.14 | 0.07 | 0.04 | 0.06 | 0.00 | 0.00 | 0.04 |
| 10-14-78 | 0.05 | 0.06 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| 11-18-78 | 0.06 | 0.12 | 0.01 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 |
| 12-17-78 | 0.15 | 0.25 | 0.04 | 0.04 | 0.10 | 0.02 | 0.00 | 0.04 |
| 01-13-79 | 0.77 | 0.99 | 0.24 | 0.36 | 0.59 | 0.00 | 0.07 | 0.22 |
| 02-17-79 | 0.23 | 0.33 | 0.06 | 0.09 | 0.16 | 0.03 | 0.05 | 0.03 |
| 03-15-79 | 0.06 | 0.10 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.16 | 0.26 | 0.06 | 0.09 | 0.23 | 0.06 | 0.00 | 0.03 |
| 05-19-79 | 0.15 | 0.31 | 0.06 | 0.04 | 0.02 | 0.03 | 0.00 | 0.01 |
| 06-17-79 | 0.05 | 0.10 | 0.01 | 0.01 | 0.04 | 0.05 | 0.00 | 0.00 |
| 07-14-79 | 0.24 | 0.27 | 0.07 | 0.07 | 0.15 | 0.03 | 0.08 | 0.10 |
| 08-11-79 | 0.20 | 0.43 | 0.05 | 0.02 | 0.08 | 0.06 | 0.00 | 0.00 |
| 09-08-79 | 0.14 | 0.12 | 0.02 | 0.08 | 0.12 | 0.09 | 0.00 | 0.02 |

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-22-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 01-15-78 | 0.00 | 0.00 | 0.50 | 0.10 | 0.00 | 0.20 | 0.00 | 0.00 |
| 02-18-78 | 0.05 | 0.02 | 0.22 | 0.04 | 0.00 | 0.18 | 0.00 | 0.06 |
| 03-19-78 | 0.00 | 0.04 | 0.16 | 0.03 | 0.00 | 0.09 | 0.00 | 0.06 |
| 04-15-78 | 0.06 | 0.00 | 0.12 | 0.02 | 0.00 | 0.09 | 0.00 | 0.21 |
| 05-13-78 | 0.00 | 0.18 | 0.53 | 0.13 | 0.00 | 1.05 | 0.12 | 0.00 |
| 06-12-78 | 0.17 | 0.00 | 0.57 | 0.04 | 0.00 | 0.13 | 0.06 | 0.11 |
| 07-14-78 | 0.11 | 0.02 | 0.04 | 0.02 | 0.00 | 0.05 | 0.00 | 0.00 |
| 08-19-78 | 0.07 | 0.00 | 0.12 | 0.02 | 0.00 | 0.09 | 0.06 | 0.00 |
| 09-18-78 | 0.00 | 0.00 | 0.08 | 0.04 | 0.00 | 0.06 | 0.03 | 0.00 |
| 10-14-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.04 | 0.01 | 0.00 | 0.04 | 0.04 | 0.14 |
| 12-17-78 | 0.06 | 0.00 | 0.14 | 0.04 | 0.00 | 0.12 | 0.00 | 0.12 |
| 01-13-79 | 0.11 | 0.09 | 0.80 | 0.23 | 0.00 | 0.72 | 0.06 | 0.23 |
| 02-17-79 | 0.05 | 0.01 | 0.33 | 0.11 | 0.00 | 0.25 | 0.03 | 0.21 |
| 03-15-79 | 0.00 | 0.00 | 0.15 | 0.00 | 0.00 | 0.05 | 0.00 | 0.03 |
| 04-14-79 | 0.00 | 0.05 | 0.33 | 0.07 | 0.00 | 0.26 | 0.00 | 0.23 |
| 05-19-79 | 0.00 | 0.02 | 0.32 | 0.02 | 0.00 | 0.12 | 0.00 | 0.00 |
| 06-17-79 | 0.00 | 0.00 | 0.09 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 |
| 07-14-79 | 0.00 | 0.11 | 0.50 | 0.09 | 0.00 | 0.21 | 0.00 | 0.00 |
| 08-11-79 | 0.00 | 0.02 | 0.17 | 0.02 | 0.00 | 0.12 | 0.00 | 0.00 |
| 09-08-79 | 0.00 | 0.00 | 0.23 | 0.03 | 0.00 | 0.20 | 0.00 | 0.00 |

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.80 | 0.70 | 1.30 | 0.50 | 1.10 | 0.50 | 0.20 | 0.20 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.50 | 1.10 | 0.20 | 0.20 | 0.60 | 0.20 | 0.10 | 0.00 |
| 12-22-77 | 1.20 | 2.10 | 0.30 | 0.40 | 1.00 | 0.50 | 0.30 | 0.20 |
| 01-15-78 | 1.30 | 2.10 | 0.50 | 0.60 | 0.80 | 0.60 | 0.20 | 0.30 |
| 02-18-78 | 0.80 | 1.53 | 0.47 | 0.46 | 0.67 | 0.47 | 0.27 | 0.21 |
| 03-19-78 | 0.78 | 1.42 | 0.20 | 0.27 | 0.36 | 0.18 | 0.12 | 0.19 |
| 04-15-78 | 0.63 | 0.86 | 0.03 | 0.56 | 0.41 | 0.24 | 0.13 | 0.38 |
| 05-13-78 | 1.16 | 1.63 | 0.65 | 0.93 | 1.07 | 0.52 | 0.34 | 0.53 |
| 06-12-78 | 0.28 | 0.44 | 0.09 | 0.17 | 0.21 | 0.12 | 0.05 | 0.13 |
| 07-14-78 | 0.29 | 0.35 | 0.28 | 0.34 | 0.38 | 0.01 | 0.00 | 0.08 |
| 08-19-78 | 0.32 | 0.46 | 0.17 | 0.21 | 0.28 | 0.05 | 0.03 | 0.06 |
| 09-18-78 | 0.17 | 0.32 | 0.10 | 0.12 | 0.12 | 0.05 | 0.01 | 0.09 |
| 10-14-78 | 0.33 | 0.66 | 0.17 | 0.24 | 0.26 | 0.12 | 0.08 | 0.12 |
| 11-18-78 | 0.09 | 0.19 | 0.04 | 0.03 | 0.13 | 0.02 | 0.00 | 0.02 |
| 12-17-78 | 0.47 | 0.74 | 0.18 | 0.24 | 0.33 | 0.15 | 0.10 | 0.16 |
| 01-13-79 | 0.73 | 0.84 | 0.26 | 0.36 | 0.50 | 0.20 | 0.12 | 0.27 |
| 02-17-79 | 0.44 | 0.66 | 0.16 | 0.29 | 0.40 | 0.17 | 0.08 | 0.21 |
| 03-15-79 | 0.17 | 0.30 | 0.08 | 0.07 | 0.14 | 0.09 | 0.00 | 0.01 |
| 04-14-79 | 0.58 | 0.95 | 0.22 | 0.37 | 0.78 | 0.23 | 0.27 | 0.23 |
| 05-19-79 | 0.95 | 1.36 | 0.32 | 0.51 | 0.62 | 0.34 | 0.17 | 0.39 |
| 06-17-79 | 0.21 | 0.32 | 0.10 | 0.12 | 0.17 | 0.06 | 0.00 | 0.08 |
| 07-14-79 | 0.27 | 0.29 | 0.12 | 0.17 | 0.24 | 0.07 | 0.01 | 0.09 |
| 08-11-79 | 1.05 | 1.35 | 0.38 | 0.61 | 0.84 | 0.57 | 0.20 | 0.37 |
| 09-08-79 | 0.29 | 0.43 | 0.10 | 0.24 | 0.35 | 0.13 | 0.10 | 0.10 |

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.70 | 1.40 | 0.30 | 0.00 | 0.60 | 0.00 | 0.00 |
| 10-08-77 | 0.90 | 0.30 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.20 | 0.50 | 0.10 | 0.00 | 0.40 | 0.00 | 0.00 |
| 12-22-77 | 0.50 | 0.40 | 1.00 | 0.20 | 0.00 | 0.50 | 0.00 | 0.00 |
| 01-15-78 | 0.00 | 0.50 | 1.50 | 0.30 | 0.00 | 0.70 | 0.00 | 0.00 |
| 02-18-78 | 0.11 | 0.51 | 0.88 | 0.18 | 0.00 | 0.47 | 0.00 | 0.08 |
| 03-19-78 | 0.18 | 0.35 | 0.57 | 0.09 | 0.00 | 0.32 | 0.00 | 0.07 |
| 04-15-78 | 0.32 | 0.17 | 0.59 | 0.14 | 0.00 | 0.47 | 0.11 | 0.36 |
| 05-13-78 | 0.27 | 0.33 | 1.12 | 0.24 | 0.00 | 1.01 | 0.27 | 0.16 |
| 06-12-78 | 0.08 | 0.03 | 0.35 | 0.07 | 0.00 | 0.31 | 0.00 | 0.00 |
| 07-14-78 | 0.22 | 0.01 | 0.19 | 0.04 | 0.00 | 0.15 | 0.03 | 0.00 |
| 08-19-78 | 0.08 | 0.00 | 0.28 | 0.10 | 0.00 | 0.20 | 0.02 | 0.12 |
| 09-18-78 | 0.06 | 0.00 | 0.29 | 0.04 | 0.00 | 0.14 | 0.00 | 0.15 |
| 10-14-78 | 0.11 | 0.12 | 0.39 | 0.07 | 0.02 | 0.27 | 0.06 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.14 | 0.03 | 0.00 | 0.19 | 0.00 | 0.17 |
| 12-17-78 | 0.11 | 0.08 | 0.52 | 0.11 | 0.00 | 0.35 | 0.00 | 0.11 |
| 01-13-79 | 0.12 | 0.17 | 0.96 | 0.23 | 0.00 | 0.86 | 0.10 | 0.21 |
| 02-17-79 | 0.18 | 0.39 | 1.12 | 0.25 | 0.04 | 1.00 | 0.68 | 0.72 |
| 03-15-79 | 0.06 | 0.06 | 0.25 | 0.03 | 0.00 | 0.19 | 0.00 | 0.00 |
| 04-14-79 | 0.19 | 0.55 | 1.08 | 0.17 | 0.00 | 0.84 | 0.08 | 0.18 |
| 05-19-79 | 0.07 | 0.46 | 1.09 | 0.16 | 0.00 | 0.84 | 0.04 | 0.00 |
| 06-17-79 | 0.00 | 0.26 | 0.49 | 0.14 | 0.00 | 0.38 | 0.02 | 0.00 |
| 07-14-79 | 0.00 | 0.11 | 0.47 | 0.08 | 0.00 | 0.33 | 0.00 | 0.00 |
| 08-11-79 | 0.69 | 0.39 | 1.73 | 0.33 | 0.00 | 1.38 | 0.06 | 0.35 |
| 09-08-79 | 0.32 | 0.18 | 0.51 | 0.06 | 0.00 | 0.41 | 0.00 | 0.09 |

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | * | * | * | * | * | * | * | * |
| 10-08-77 | * | * | * | * | * | * | * | * |
| 11-12-77 | 1.80 | 4.60 | 0.80 | 0.90 | 2.10 | 0.70 | 0.40 | 0.30 |
| 12-21-77 | 1.40 | 3.20 | 0.40 | 0.60 | 1.90 | 0.50 | 0.30 | 0.30 |
| 01-15-78 | 0.60 | 1.20 | 0.30 | 0.20 | 0.30 | 0.20 | 0.10 | 0.20 |
| 02-18-78 | 0.55 | 1.36 | 0.25 | 0.30 | 0.42 | 0.26 | 0.15 | 0.14 |
| 03-19-78 | 0.81 | 1.54 | 0.43 | 0.50 | 0.63 | 0.44 | 0.25 | 0.28 |
| 04-15-78 | 0.51 | 0.75 | 0.31 | 0.41 | 0.40 | 0.24 | 0.14 | 0.27 |
| 05-13-78 | 0.30 | 0.51 | 0.19 | 0.22 | 0.26 | 0.18 | 0.03 | 0.09 |
| 06-12-78 | 0.77 | 1.36 | 0.52 | 0.59 | 0.69 | 0.45 | 0.24 | 0.36 |
| 07-14-78 | 0.77 | 1.34 | 0.52 | 0.58 | 0.59 | 0.46 | 0.27 | 0.35 |
| 08-19-78 | * | * | * | * | * | * | * | * |
| 09-18-78 | * | * | * | * | * | * | * | * |
| 10-14-78 | * | * | * | * | * | * | * | * |
| 11-18-78 | * | * | * | * | * | * | * | * |
| 12-17-78 | 1.52 | 2.40 | 0.65 | 1.02 | 1.14 | 0.64 | 0.34 | 0.62 |
| 01-13-79 | 1.00 | 1.15 | 0.33 | 0.53 | 0.69 | 0.39 | 0.15 | 0.37 |
| 02-17-79 | 0.31 | 0.62 | 0.13 | 0.29 | 0.38 | 0.17 | 0.08 | 0.20 |
| 03-15-79 | 0.18 | 0.30 | 0.08 | 0.08 | 0.15 | 0.07 | 0.00 | 0.00 |
| 04-14-79 | 0.88 | 1.25 | 0.38 | 0.56 | 0.96 | 0.38 | 0.19 | 0.25 |
| 05-19-79 | 0.96 | 1.33 | 0.34 | 0.41 | 0.71 | 0.37 | 0.19 | 0.33 |
| 06-17-79 | 0.95 | 1.52 | 0.34 | 0.55 | 0.72 | 0.38 | 0.19 | 0.33 |
| 07-14-79 | * | * | * | * | * | * | * | * |
| 08-11-79 | * | * | * | * | * | * | * | * |
| 09-08-79 | * | * | * | * | * | * | * | * |

179

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | * | * | * | * | * | * | * | * |
| 10-08-77 | * | * | * | * | * | * | * | * |
| 11-12-77 | 0.30 | 0.60 | 1.90 | 0.30 | 0.00 | 1.20 | 0.00 | 0.00 |
| 12-21-77 | 0.40 | 0.60 | 1.20 | 0.20 | 0.00 | 0.90 | 0.00 | 0.00 |
| 01-15-78 | 0.10 | 0.10 | 0.70 | 0.10 | 0.00 | 0.30 | 0.00 | 0.00 |
| 02-18-78 | 0.09 | 0.17 | 0.62 | 0.08 | 0.00 | 0.30 | 0.00 | 0.06 |
| 03-19-78 | 0.24 | 0.56 | 0.86 | 0.20 | 0.00 | 0.42 | 0.00 | 0.06 |
| 04-15-78 | 0.20 | 0.12 | 0.52 | 0.15 | 0.00 | 0.40 | 0.14 | 0.21 |
| 05-13-78 | 0.09 | 0.00 | 0.20 | 0.06 | 0.07 | 0.28 | 0.53 | 0.14 |
| 06-12-78 | 0.96 | 0.12 | 0.71 | 0.21 | 0.00 | 0.53 | 0.30 | 0.00 |
| 07-14-78 | 0.22 | 0.38 | 0.67 | 0.19 | 0.00 | 0.44 | 0.26 | 0.12 |
| 08-19-78 | * | * | * | * | * | * | * | * |
| 09-18-78 | * | * | * | * | * | * | * | * |
| 10-14-78 | * | * | * | * | * | * | * | * |
| 11-18-78 | * | * | * | * | * | * | * | * |
| 12-17-78 | 1.52 | 2.40 | 0.65 | 1.02 | 1.14 | 0.64 | 0.34 | 0.62 |
| 01-13-79 | 0.15 | 0.34 | 1.28 | 0.26 | 0.00 | 1.02 | 0.03 | 0.21 |
| 02-17-79 | 0.07 | 0.17 | 0.88 | 0.08 | 0.02 | 0.60 | 0.13 | 0.02 |
| 03-15-79 | 0.03 | 0.05 | 0.33 | 0.03 | 0.00 | 0.19 | 0.03 | 0.03 |
| 04-14-79 | 0.28 | 0.82 | 1.23 | 0.31 | 0.00 | 1.31 | 0.16 | 0.19 |
| 05-19-79 | 0.23 | 0.70 | 1.18 | 0.23 | 0.00 | 1.06 | 0.02 | 0.00 |
| 06-17-79 | 0.32 | 0.77 | 1.30 | 0.26 | 0.08 | 0.92 | 0.17 | 0.14 |
| 07-14-79 | * | * | * | * | * | * | * | * |
| 08-11-79 | * | * | * | * | * | * | * | * |
| 09-08-79 | * | * | * | * | * | * | * | * |

[illegible]

181

[illegible]

[illegible]

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.40 | 0.30 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 |
| 12-22-77 | 0.40 | 0.40 | 0.60 | 0.20 | 0.00 | 0.60 | 0.00 | 0.00 |
| 01-15-78 | 0.20 | 0.20 | 0.40 | 0.10 | 0.00 | 0.10 | 0.00 | 0.20 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.09 | 0.02 | 0.26 | 0.06 | 0.00 | 0.24 | 0.00 | 0.15 |
| 04-15-78 | 0.09 | 0.00 | 0.22 | 0.05 | 0.00 | 0.20 | 0.04 | 0.10 |
| 05-13-78 | 0.00 | 0.06 | 0.13 | 0.04 | 0.00 | 0.12 | 0.00 | 0.00 |
| 06-12-78 | 0.00 | 0.00 | 0.22 | 0.06 | 0.00 | 0.15 | 0.03 | 0.00 |
| 07-14-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 08-19-78 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.04 | 0.06 | 0.15 |
| 09-18-78 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-14-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.07 | 0.00 | 0.00 | 0.18 | 0.00 | 0.14 |
| 12-17-78 | 0.09 | 0.06 | 0.47 | 0.08 | 0.02 | 0.28 | 0.00 | 1.27 |
| 01-13-79 | 0.07 | 0.11 | 0.32 | 0.07 | 0.01 | 0.22 | 0.02 | 0.17 |
| 02-17-79 | 0.00 | 0.00 | 0.27 | 0.06 | 0.00 | 0.26 | 0.00 | 0.24 |
| 03-15-79 | 0.03 | 0.00 | 0.17 | 0.04 | 0.00 | 0.00 | 0.06 | 0.05 |
| 04-14-79 | 0.00 | 0.00 | 0.21 | 0.00 | 0.00 | 0.37 | 0.00 | 0.00 |
| 05-19-79 | 0.06 | 0.16 | 0.38 | 0.05 | 0.00 | 0.26 | 0.00 | 0.00 |
| 06-17-79 | 0.00 | 0.00 | 0.07 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 |
| 07-14-79 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 |
| 08-11-79 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 |
| 09-08-79 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 |

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-11-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-21-77 | 0.40 | 0.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 |
| 01-15-78 | 0.20 | 0.20 | 0.20 | 0.10 | 0.20 | 0.10 | 0.00 | 0.10 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.12 | 0.21 | 0.05 | 0.06 | 0.08 | 0.01 | 0.00 | 0.06 |
| 04-15-78 | 0.26 | 0.31 | 0.11 | 0.19 | 0.17 | 0.07 | 0.07 | 0.17 |
| 05-13-78 | 0.19 | 0.32 | 0.09 | 0.11 | 0.15 | 0.02 | 0.00 | 0.06 |
| 06-12-78 | 0.24 | 0.32 | 0.04 | 0.08 | 0.13 | 0.03 | 0.00 | 0.04 |
| 07-14-78 | 0.12 | 0.18 | 0.04 | 0.05 | 0.08 | 0.00 | 0.00 | 0.00 |
| 08-19-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 09-18-78 | 0.18 | 0.31 | 0.13 | 0.03 | 0.16 | 0.00 | 0.00 | 0.08 |
| 10-14-78 | 0.07 | 0.11 | 0.02 | 0.01 | 0.06 | 0.00 | 0.00 | 0.00 |
| 11-18-78 | 0.05 | 0.09 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 |
| 12-17-78 | 0.13 | 0.26 | 0.05 | 0.06 | 0.10 | 0.03 | 0.00 | 0.02 |
| 01-13-79 | 0.27 | 0.39 | 0.07 | 0.12 | 0.20 | 0.03 | 0.04 | 0.07 |
| 02-17-79 | 0.61 | 0.88 | 0.19 | 0.24 | 0.54 | 0.07 | 0.07 | 0.14 |
| 03-15-79 | 0.05 | 0.07 | 0.02 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.09 | 0.19 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.34 |
| 05-19-79 | 0.29 | 0.39 | 0.13 | 0.13 | 0.16 | 0.12 | 0.05 | 0.08 |
| 06-17-79 | 0.12 | 0.21 | 0.05 | 0.04 | 0.08 | 0.01 | 0.00 | 0.00 |
| 07-14-79 | 0.04 | 0.04 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| 08-11-79 | 0.14 | 0.21 | 0.03 | 0.07 | 0.14 | 0.05 | 0.00 | 0.01 |
| 09-08-79 | 0.08 | 0.01 | 0.00 | 0.09 | 0.07 | 0.06 | 0.00 | 0.00 |

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.60 | 0.10 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-11-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-21-77 | 0.50 | 0.20 | 0.40 | 0.10 | 0.10 | 0.10 | 0.00 | 0.00 |
| 01-15-78 | 0.10 | 0.10 | 0.30 | 0.10 | 0.00 | 0.10 | 0.00 | 0.00 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.04 | 0.00 | 0.12 | 0.02 | 0.00 | 0.08 | 0.00 | 0.00 |
| 04-15-78 | 0.08 | 0.03 | 0.25 | 0.07 | 0.00 | 0.20 | 0.04 | 0.11 |
| 05-13-78 | 0.13 | 0.01 | 0.15 | 0.02 | 0.00 | 0.16 | 0.03 | 0.09 |
| 06-12-78 | 0.10 | 0.02 | 0.20 | 0.04 | 0.00 | 0.13 | 0.01 | 0.00 |
| 07-14-78 | 0.06 | 0.00 | 0.09 | 0.03 | 0.00 | 0.07 | 0.00 | 0.00 |
| 08-19-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 09-18-78 | 0.00 | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10-14-78 | 0.00 | 0.00 | 0.03 | 0.01 | 0.00 | 0.04 | 0.00 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.13 | 0.00 | 0.00 |
| 12-17-78 | 0.05 | 0.00 | 0.20 | 0.04 | 0.00 | 0.12 | 0.00 | 0.00 |
| 01-13-79 | 0.07 | 0.05 | 0.34 | 0.08 | 0.00 | 0.24 | 0.04 | 0.11 |
| 02-17-79 | 0.04 | 0.00 | 0.46 | 0.11 | 0.00 | 0.52 | 0.00 | 0.32 |
| 03-15-79 | 0.03 | 0.01 | 0.09 | 0.06 | 0.00 | 0.06 | 0.00 | 0.00 |
| 04-14-79 | 0.00 | 0.05 | 0.08 | 0.05 | 0.00 | 0.16 | 0.01 | 0.00 |
| 05-19-79 | 0.07 | 0.11 | 0.33 | 0.09 | 0.00 | 0.29 | 0.03 | 0.08 |
| 06-17-79 | 0.00 | 0.07 | 0.21 | 0.07 | 0.00 | 0.16 | 0.00 | 0.00 |
| 07-14-79 | 0.00 | 0.01 | 0.04 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 |
| 08-11-79 | 0.00 | 0.00 | 0.23 | 0.02 | 0.00 | 0.14 | 0.00 | 0.00 |
| 09-08-79 | 0.00 | 0.01 | 0.18 | 0.02 | 0.00 | 0.12 | 0.00 | 0.00 |

[illegible]

[illegible]

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 1.00 | 1.90 | 0.50 | 0.60 | 1.00 | 0.70 | 0.50 | 0.20 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-11-77 | 7.40 | 9.80 | 3.30 | 3.50 | 4.00 | 5.40 | 2.90 | 1.50 |
| 12-21-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 01-15-78 | 0.10 | 0.20 | 0.10 | 0.10 | 0.00 | 0.20 | 0.00 | 0.10 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.45 | 0.60 | 0.31 | 0.36 | 0.38 | 0.27 | 0.16 | 0.22 |
| 04-15-78 | 1.03 | 1.24 | 0.77 | 0.65 | 0.72 | 0.64 | 0.44 | 0.44 |
| 05-13-78 | 0.33 | 0.44 | 0.15 | 0.18 | 0.20 | 0.07 | 0.04 | 0.09 |
| 06-12-78 | 0.55 | 0.73 | 0.28 | 0.37 | 0.42 | 0.22 | 0.15 | 0.22 |
| 07-14-78 | 0.45 | 0.73 | 0.19 | 0.30 | 0.31 | 0.09 | 0.06 | 0.10 |
| 08-19-78 | 0.55 | 0.73 | 0.34 | 0.49 | 0.40 | 0.27 | 0.15 | 0.18 |
| 09-18-78 | 1.09 | 1.16 | 0.51 | 0.73 | 0.81 | 0.43 | 0.34 | 0.41 |
| 10-14-78 | 0.51 | 0.66 | 0.23 | 0.29 | 0.33 | 0.12 | 0.08 | 0.13 |
| 11-18-78 | 0.33 | 0.45 | 0.13 | 0.18 | 0.22 | 0.06 | 0.00 | 0.08 |
| 12-17-78 | 1.08 | 1.18 | 0.43 | 0.73 | 0.74 | 0.26 | 0.14 | 0.35 |
| 01-13-79 | 0.68 | 0.81 | 0.25 | 0.37 | 0.47 | 0.08 | 0.06 | 0.15 |
| 02-17-79 | 0.29 | 0.46 | 0.09 | 0.11 | 0.38 | 0.04 | 0.00 | 0.06 |
| 03-15-79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.61 | 0.77 | 0.22 | 0.33 | 0.64 | 0.30 | 0.18 | 0.00 |
| 05-19-79 | 0.61 | 0.67 | 0.26 | 0.31 | 0.30 | 0.31 | 0.12 | 0.11 |
| 06-17-79 | 0.48 | 0.57 | 0.17 | 0.25 | 0.32 | 0.18 | 0.15 | 0.16 |
| 07-14-79 | 0.23 | 0.36 | 0.10 | 0.16 | 0.21 | 0.11 | 0.01 | 0.05 |
| 08-11-79 | 1.44 | 1.71 | 0.50 | 0.97 | 1.26 | 0.91 | 0.28 | 0.53 |
| 09-08-79 | 3.38 | 2.99 | 1.19 | 1.78 | 2.10 | 2.11 | 1.04 | 1.16 |

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 1.10 | 1.40 | 0.20 | 0.00 | 0.80 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-11-77 | 0.80 | 2.60 | 9.30 | 1.70 | 0.00 | 3.40 | 0.00 | 1.30 |
| 12-21-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 01-15-78 | 0.10 | 0.00 | 0.10 | 0.10 | 0.00 | 0.10 | 0.00 | 0.10 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.11 | 0.06 | 0.49 | 0.16 | 0.05 | 0.38 | 0.02 | 0.20 |
| 04-15-78 | 0.23 | 0.24 | 0.95 | 0.41 | 0.00 | 0.80 | 0.41 | 0.00 |
| 05-13-78 | 0.07 | 0.04 | 0.26 | 0.05 | 0.00 | 0.18 | 0.04 | 0.12 |
| 06-12-78 | 0.09 | 0.14 | 0.56 | 0.15 | 0.04 | 0.40 | 0.06 | 0.15 |
| 07-14-78 | 0.06 | 0.04 | 0.40 | 0.09 | 0.00 | 0.24 | 0.06 | 0.15 |
| 08-19-78 | 0.08 | 0.36 | 0.65 | 0.27 | 0.03 | 0.38 | 0.08 | 0.16 |
| 09-18-78 | 0.11 | 0.23 | 1.03 | 0.20 | 0.04 | 0.65 | 0.18 | 0.24 |
| 10-14-78 | 0.08 | 0.08 | 0.46 | 0.10 | 0.00 | 0.31 | 0.04 | 0.14 |
| 11-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 12-17-78 | 0.10 | 0.41 | 1.11 | 0.24 | 0.06 | 0.74 | 0.03 | 0.18 |
| 01-13-79 | 0.08 | 0.14 | 0.60 | 0.12 | 0.04 | 0.48 | 0.12 | 0.20 |
| 02-17-79 | 0.00 | 0.00 | 0.34 | 0.07 | 0.01 | 0.47 | 0.00 | 0.15 |
| 03-15-79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.13 | 0.31 | 0.88 | 0.20 | 0.01 | 0.70 | 0.07 | 0.16 |
| 05-19-79 | 0.07 | 0.12 | 0.60 | 0.17 | 0.00 | 0.59 | 0.07 | 0.00 |
| 06-17-79 | 0.00 | 0.10 | 0.57 | 0.45 | 0.00 | 0.48 | 0.00 | 0.00 |
| 07-14-79 | 0.18 | 0.26 | 0.44 | 0.07 | 0.00 | 0.31 | 0.00 | 0.12 |
| 08-11-79 | 0.22 | 0.59 | 2.69 | 0.52 | 0.00 | 2.07 | 0.03 | 0.49 |
| 09-08-79 | 0.21 | 0.67 | 3.87 | 0.80 | 0.00 | 3.47 | 0.74 | 0.97 |

[illegible]

[illegible]

| DATE | ALA | GLY | VAL | THR | SER | LEU | ILE | PRO |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.30 | 0.50 | 0.20 | 0.10 | 0.20 | 0.00 | 0.00 | 0.00 |
| 10-08-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-22-77 | 0.30 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 01-15-78 | 0.50 | 1.20 | 0.10 | 0.10 | 0.20 | 0.20 | 0.10 | 0.00 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.16 | 0.26 | 0.08 | 0.11 | 0.12 | 0.04 | 0.00 | 0.05 |
| 04-15-78 | 0.25 | 0.36 | 0.12 | 0.16 | 0.18 | 0.04 | 0.07 | 0.19 |
| 05-13-78 | 0.20 | 0.28 | 0.06 | 0.06 | 0.10 | 0.02 | 0.00 | 0.05 |
| 06-12-78 | 0.17 | 0.23 | 0.09 | 0.15 | 0.11 | 0.02 | 0.00 | 0.01 |
| 07-14-78 | 0.16 | 0.23 | 0.05 | 0.06 | 0.10 | 0.02 | 0.00 | 0.00 |
| 08-19-78 | 0.12 | 0.14 | 0.05 | 0.07 | 0.04 | 0.03 | 0.00 | 0.01 |
| 09-18-78 | 0.13 | 0.23 | 0.06 | 0.08 | 0.11 | 0.02 | 0.00 | 0.03 |
| 10-14-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-18-78 | 0.05 | 0.05 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 |
| 12-17-78 | 0.08 | 0.09 | 0.00 | 0.02 | 0.05 | 0.00 | 0.00 | 0.04 |
| 01-13-79 | 0.38 | 0.52 | 0.09 | 0.13 | 0.25 | 0.05 | 0.01 | 0.12 |
| 02-17-79 | 0.26 | 0.39 | 0.09 | 0.11 | 0.18 | 0.03 | 0.00 | 0.05 |
| 03-15-79 | 0.02 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.22 | 0.43 | 0.09 | 0.10 | 0.55 | 0.07 | 0.02 | 0.03 |
| 05-19-79 | 0.24 | 0.35 | 0.12 | 0.09 | 0.14 | 0.12 | 0.03 | 0.06 |
| 06-17-79 | * | * | * | * | * | * | * | * |
| 07-14-79 | 0.13 | 0.24 | 0.06 | 0.09 | 0.13 | 0.05 | 0.00 | 0.03 |
| 08-11-79 | 0.16 | 0.22 | 0.03 | 0.14 | 0.19 | 0.12 | 0.00 | 0.09 |
| 09-08-79 | 0.11 | 0.10 | 0.01 | 0.08 | 0.09 | 0.05 | 0.00 | 0.07 |

| DATE | MET | PHE | ASP | LYS | TYR | GLU | ARG | HIS |
|----------|------|------|------|------|------|------|------|------|
| 09-10-77 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| 10-08-77 | 0.30 | 1.20 | 0.40 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| 11-12-77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12-22-77 | 0.30 | 0.00 | 0.30 | 0.20 | 0.00 | 0.20 | 0.00 | 0.00 |
| 01-15-78 | 0.10 | 0.20 | 1.00 | 0.10 | 0.00 | 0.40 | 0.00 | 0.00 |
| 02-18-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 03-19-78 | 0.00 | 0.00 | 0.19 | 0.04 | 0.00 | 0.14 | 0.00 | 0.00 |
| 04-15-78 | 0.16 | 0.05 | 0.30 | 0.06 | 0.00 | 0.25 | 0.08 | 0.12 |
| 05-13-78 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 |
| 06-12-78 | 0.00 | 0.00 | 0.17 | 0.03 | 0.00 | 0.11 | 0.00 | 0.00 |
| 07-14-78 | 0.00 | 0.00 | 0.10 | 0.03 | 0.00 | 0.07 | 0.00 | 0.00 |
| 08-19-78 | 0.06 | 0.00 | 0.10 | 0.00 | 0.00 | 0.07 | 0.04 | 0.17 |
| 09-18-78 | 0.05 | 0.00 | 0.12 | 0.02 | 0.02 | 0.08 | 0.04 | 0.14 |
| 10-14-78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 11-18-78 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.07 | 0.00 | 0.18 |
| 12-17-78 | 0.00 | 0.00 | 0.07 | 0.03 | 0.00 | 0.07 | 0.00 | 0.00 |
| 01-13-79 | 0.09 | 0.03 | 0.40 | 0.10 | 0.00 | 0.36 | 0.00 | 0.15 |
| 02-17-79 | 0.00 | 0.00 | 0.28 | 0.03 | 0.00 | 0.21 | 0.00 | 0.30 |
| 03-15-79 | 0.07 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 04-14-79 | 0.11 | 0.06 | 0.48 | 0.13 | 0.00 | 0.50 | 0.10 | 0.20 |
| 05-19-79 | 0.03 | 0.05 | 0.28 | 0.12 | 0.00 | 0.28 | 0.02 | 0.00 |
| 06-17-79 | * | * | * | * | * | * | * | * |
| 07-14-79 | 0.09 | 0.16 | 0.33 | 0.06 | 0.00 | 0.21 | 0.00 | 0.12 |
| 08-11-79 | 0.12 | 0.14 | 0.41 | 0.07 | 0.00 | 0.26 | 0.00 | 0.00 |
| 09-08-79 | 0.04 | 0.07 | 0.21 | 0.03 | 0.00 | 0.12 | 0.00 | 0.00 |