COMPLEXATION OF COPPER IN NATURAL WATERS

DETERMINATION OF COMPLEXING CAPACITIES AND CONDITIONAL STABILITY
CONSTANTS IN NATURAL WATERS BY MnO₂, AND THE IMPLICATIONS FOR
PHYTOPLANKTON TOXICITY

By

C.M.G. VAN DEN BERG VAN SAPAROEA
Landbouwkundig Ingenieur

A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

April, 1979
COMPLEXATION OF COPPER IN NATURAL WATERS

\[ \text{MnO}_2 \rightarrow \text{H}^+ + \text{Cu}^{2+} \]

\[ \text{Cu}^{2+} + \text{OH}^- \rightarrow \text{Cu}^{2+} \text{OH}^- \]

\[ \text{Cu}^{2+} + \text{FA}^- \rightarrow \text{CuFA} \]

\[ \text{Cu}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CuCO}_3 \]
Complexation of copper in natural waters. Determination of complexing capacities and conditional stability constants in natural waters by MnO₂, and the implications for phytoplankton toxicity

C.M.G. van den Berg van Saparoea, Landbouwkundig Ingenieur

Professor James R. Kramer

xv; 260.
ABSTRACT

After a recapitulation of complexation theory, a theory is developed for the determination of copper complexation for mono- and bidentate complexes and for 2:1 complexes \((\text{CuL}_2)\) by ion exchange. This theory can be applied in principle to other ion exchange methods that make use of weak ion exchange media with an adsorption behavior of metal ions similar to Langmuir adsorption. From this theory it has been deduced that titration with \(\text{Cu}^{2+}\) of a single ligand, in presence of a weak ion exchange medium, can be graphically represented by a straight line; slope and intercept with the Y-axis provide information on the conditional stability constant and the ligand concentration. In certain conditions similar data may be obtained for a second ligand, from a single titration of the sample with \(\text{Cu}^{2+}\) at fixed pH.

An aged dispersion of \(\text{MnO}_2\) is used as an ion exchange medium to determine complexing capacities of natural waters and conditional stability constants for complexes with copper ions. The method is sufficiently sensitive to apply to original water samples: no pre-concentration is necessary. Limits of detection are a minimum of 0.15 \(\mu\text{M}\) ligands and log conditional stability constants (log \(K'\)) between 6 and 10 at pH 8; analyses may be performed at any pH between 6 and 9.
For fulvic acid (FA) and for ligands in dystrophic rivers $\log K' = \approx 7.8$ at pH 7.6, the ligand concentration is 5-30 $\mu$M; for ligands in lakes log $K'$ ranges from 7.6 to 8.5 at pH 7.6, the ligand concentration is 0.2-3 $\mu$M. Generally only one ligand is dominantly present. From pH 5 to 9 the state of copper in natural waters is regulated by organic complexing ligands.

Literature survey shows that conditional stability constants for complexes of humic acids (HAs) and FAs with $Cu^{2+}$ have a constant relationship with the pH: $\log K' = pH - 0.5$, when $5 < pH < 8.5$; for the same pH limits, the affinity of HAs and FAs for protons is about 3x larger than for $Cu^{2+}$ ions. $K'$ values for natural ligands analysed with the MnO$_2$ method also vary with the pH on a 1:1 basis of $\log K'$ vs. pH. Apparently a proton is exchanged for each $Cu^{2+}$ ion bound.

Three algal species, Anabaena cylindrica, Navicula pelliculosa and Scenedesmus quadricauda excreted 6.73, 2.86, and 0.66 $\mu$M of complexing ligands, respectively, with values for $\log K'$ of 7.7, 8.1, and 8.6, respectively, for complexes with copper. A free copper ion concentration of $10^{-10.3}$ M is calculated for each exudate, while in lakes a concentration of $10^{-9.8}$ to $10^{-10.2}$ M is found. Algal exudates are similar
in complexing ability to some amino acids and to ligands in lakes, and ameliorate toxicity of copper ions to the primary production of *Chlorella vulgaris*. From model calculation it is apparent that in presence of dissolved organic ligands toxicity or availability of Cu\(^{2+}\) to phytoplankton does not depend on pH, for 5.5 < pH < 8 and low p\(_{CO_2}\).
ACKNOWLEDGEMENTS

Many people have contributed to the experiments and ideas expressed in this thesis. Sometimes their help consisted of a chance remark, the preparation of an imaginative dinner, a wilderness trip, or something else, which provided me with inspiration, often unintentionally. I would like to thank my friends for their very important role in providing inspiration, or lateral thinking (or whatever it is called) without which this work would not have reached its goals and would have been a dull affair.

More specifically I am very grateful to:

my supervisor, Prof. J.R. Kramer, who kept me alert with his challenging remarks and his broad understanding of the subject;

Prof. W. Stumm, Y.K. Chau and W. Snodgrass, for very valuable discussions during the development of the research;

Prof. D.M. Shaw, Prof. B.J. Burley and Prof. D.R. Woods who formed my understanding and encouraging supervisory committee;

Dr. M. Schnitzer who generously sent an aliquot of fulvic acid, D.S. Jeffries who provided me with samples of dystrophic waters, R. Martella for analysis by X-ray diffraction of a MnO₂ sample;
P.T.S. Wong for his collaboration in the algae experiments, and Jill Gleed for her assistance (and mere presence) in the laboratory, and Helen Elliott for her very speedy and accurate typing.

I acknowledge furthermore the Social Sciences and Humanities Research Council of Canada for a Cultural Exchange Scholarship during the two and a half years of my stay in Canada.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER 1</th>
<th>INTRODUCTION</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Formation of complexes</td>
<td>5</td>
</tr>
<tr>
<td>1.3</td>
<td>Driving forces of complexation and adsorption of transition metals</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>Theory of adsorption and complexation</td>
<td>10</td>
</tr>
<tr>
<td>1.5</td>
<td>Summary</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 2</th>
<th>THEORY</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Stability constants</td>
<td>17</td>
</tr>
<tr>
<td>2.2</td>
<td>Competition by protons</td>
<td>19</td>
</tr>
<tr>
<td>2.3</td>
<td>Adsorption on MnO₂</td>
<td>25</td>
</tr>
<tr>
<td>2.4</td>
<td>Adsorption on MnO₂ in presence of a complexing ligand</td>
<td>28</td>
</tr>
<tr>
<td>2.5</td>
<td>Adsorption on MnO₂ in presence of two complexing ligands</td>
<td>31</td>
</tr>
<tr>
<td>2.6</td>
<td>The effect of a second ligand on the calculated Cu²⁺ concentration</td>
<td>39</td>
</tr>
<tr>
<td>2.7</td>
<td>Determination of a second ligand</td>
<td>43</td>
</tr>
<tr>
<td>2.8</td>
<td>Formation of other than 1:1 complexes</td>
<td>46</td>
</tr>
<tr>
<td>2.9</td>
<td>Determination of K' and L when 2:1 complexes are formed</td>
<td>53</td>
</tr>
<tr>
<td>2.10</td>
<td>Effects of temperature and ionic strength</td>
<td>55</td>
</tr>
<tr>
<td>2.11</td>
<td>Summary</td>
<td>62</td>
</tr>
</tbody>
</table>
### CHAPTER 3  
ORGANIC AND INORGANIC MATTER IN THE NATURAL ENVIRONMENT AND INTERACTIONS WITH METAL IONS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Surfaces in natural waters</td>
<td>64</td>
</tr>
<tr>
<td>3.2 Organic matter in natural waters</td>
<td>64</td>
</tr>
<tr>
<td>3.3 Land derived organic matter</td>
<td>67</td>
</tr>
<tr>
<td>3.4 Organic matter derived from planktonic organisms</td>
<td>71</td>
</tr>
<tr>
<td>3.5 Composition of humic substances</td>
<td>74</td>
</tr>
<tr>
<td>3.6 Nature of the groups responsible for complexing transition metals</td>
<td>81</td>
</tr>
<tr>
<td>3.7 Inorganic surfaces</td>
<td>96</td>
</tr>
<tr>
<td>3.8 Interactions between organic matter and inorganic particulates</td>
<td>102</td>
</tr>
<tr>
<td>3.9 Complexation and adsorption of metal ions by the combined presence of organics and inorganic particulates</td>
<td>111</td>
</tr>
<tr>
<td>3.10 Summary</td>
<td>116</td>
</tr>
</tbody>
</table>

### CHAPTER 4  
METHODS TO DETERMINE THE STATE OF HEAVY METALS IN NATURAL WATERS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>118</td>
</tr>
<tr>
<td>4.2 Methods to measure complexing capacities of natural waters</td>
<td>119</td>
</tr>
<tr>
<td>4.3 The measurement of, conditional, stability constants</td>
<td>125</td>
</tr>
<tr>
<td>4.4 Summary</td>
<td>140</td>
</tr>
</tbody>
</table>

### CHAPTER 5  
MnO₂ AS MEDIUM FOR THE DETERMINATION OF COMPLEXING CAPACITIES AND CONDITIONAL STABILITY CONSTANTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>141</td>
</tr>
</tbody>
</table>
5.2 Preparation of materials
5.3 Calibration of MnO₂
5.4 Titration of natural water samples
5.5 Statistical treatment of the data
5.6 Sample description
5.7 Summary

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>145</td>
</tr>
<tr>
<td>5.3</td>
<td>146</td>
</tr>
<tr>
<td>5.4</td>
<td>149</td>
</tr>
<tr>
<td>5.5</td>
<td>152</td>
</tr>
<tr>
<td>5.6</td>
<td>154</td>
</tr>
<tr>
<td>5.7</td>
<td>155</td>
</tr>
</tbody>
</table>

CHAPTER 6
RESULTS AND DISCUSSION

6.1 Calibration of MnO₂
6.2 Comparison of adsorption models
6.3 Test of the MnO₂ method on some known ligands
6.4 Application of the MnO₂ method to samples of lakes and rivers
6.5 Complexing capacities and conditional stability constants of natural waters
6.6 Effect of pH on conditional stability constants
6.7 Determination of conditional stability constants for mixed ligands and for complexes other than 1:1
6.8 Light absorption at 260 nm and complexing capacity
6.9 The use of MnO₂ with other metal ions
6.10 Molecular weight of FA
6.11 Adsorption of the metal complex on MnO₂
6.12 Summary

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>157</td>
</tr>
<tr>
<td>6.2</td>
<td>161</td>
</tr>
<tr>
<td>6.3</td>
<td>164</td>
</tr>
<tr>
<td>6.4</td>
<td>170</td>
</tr>
<tr>
<td>6.5</td>
<td>170</td>
</tr>
<tr>
<td>6.6</td>
<td>180</td>
</tr>
<tr>
<td>6.7</td>
<td>182</td>
</tr>
<tr>
<td>6.8</td>
<td>183</td>
</tr>
<tr>
<td>6.9</td>
<td>185</td>
</tr>
<tr>
<td>6.10</td>
<td>188</td>
</tr>
<tr>
<td>6.11</td>
<td>188</td>
</tr>
<tr>
<td>6.12</td>
<td>190</td>
</tr>
<tr>
<td>CHAPTER 7</td>
<td>MODELS TO ESTIMATE THE IMPACT OF COMPLEXATION ON THE NATURAL ENVIRONMENT</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>7.1 Introduction</td>
<td></td>
</tr>
<tr>
<td>7.2 Complexation of Cu by some known ligands and naturally occurring ligands at pH 8.3</td>
<td></td>
</tr>
<tr>
<td>7.3 Complexation of Cu at varying pH</td>
<td></td>
</tr>
<tr>
<td>7.4 Summary</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 8</th>
<th>MEASUREMENT OF COMPLEXING MATERIALS EXCRETED FROM ALGAE AND THEIR ABILITY TO AMELIORATE COPPER TOXICITY</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1 Introduction</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>8.2 Effects of dissolved organic matter on planktonic organisms</td>
<td></td>
<td>201</td>
</tr>
<tr>
<td>8.3 Excretion of organic matter by planktonic organisms</td>
<td></td>
<td>205</td>
</tr>
<tr>
<td>8.4 Algae and metal toxicity: a chemical model</td>
<td></td>
<td>208</td>
</tr>
<tr>
<td>8.5 Excretion of complexing ligands by different algal species and their ability to ameliorate copper toxicity: experimental</td>
<td></td>
<td>214</td>
</tr>
<tr>
<td>8.6 Results and discussion</td>
<td></td>
<td>216</td>
</tr>
<tr>
<td>8.7 Summary</td>
<td></td>
<td>226</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHAPTER 9</th>
<th>SUMMARY AND IMPLICATIONS FROM THIS STUDY</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1 Introduction</td>
<td></td>
<td>228</td>
</tr>
<tr>
<td>9.2 Implications from analyses with the MnO₂ method of natural waters</td>
<td></td>
<td>230</td>
</tr>
</tbody>
</table>

REFERENCES | | 233  |
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>48</td>
</tr>
<tr>
<td>Stability constants and conditional stability constants adjusted for pH 7.6 for copper and humic and fulvic acids.</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>57</td>
</tr>
<tr>
<td>Effect of temperature on stability constants and conditional stability constants of complexes of Cu$^{2+}$.</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>60</td>
</tr>
<tr>
<td>Effect of ionic strength on stability constants of copper and strontium complexes with some organic ligands.</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>65</td>
</tr>
<tr>
<td>World quantities of organic carbon</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>68</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>68</td>
</tr>
<tr>
<td>Decomposition</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>72</td>
</tr>
<tr>
<td>Excretion products of phytoplankton</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>75</td>
</tr>
<tr>
<td>Classification and characterization of humic material in soils, sediments and waters</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>78</td>
</tr>
<tr>
<td>Relative molar percentages of amino acids in humic matter</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>87</td>
</tr>
<tr>
<td>Stability constants for Cu$^{2+}$-humic matter complexes</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>99</td>
</tr>
<tr>
<td>ZPCs for a number of oxides, hydroxides and minerals</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>122</td>
</tr>
<tr>
<td>Methods to determine complexing capacity</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>126</td>
</tr>
<tr>
<td>Conditional stability constants and acidity constants for HAs, FAs and ligands in natural waters</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>6.1</td>
<td>Log conditional stability constants as determined with the MnO₂ method, compared with literature values, corrected for the pH</td>
</tr>
<tr>
<td>6.2</td>
<td>Complexing capacities and conditional stability constants for complexes of copper in natural waters, determined by the MnO₂ method</td>
</tr>
<tr>
<td>8.1</td>
<td>Production of complexing ligands by three phytoplankton species and the conditional stability constants of the copper complexes of the exudates and of natural waters</td>
</tr>
<tr>
<td>8.2</td>
<td>Conditional stability constants of copper complexes of algal exudates (after 10 days' growth) and of lake waters, and calculated free copper ion concentrations</td>
</tr>
<tr>
<td>8.3</td>
<td>Equations and stability constants used to calculate the free copper ion concentration in equilibrium with complexing ligands</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Energy-level diagram of the splitting of d-orbitals of Cu$^{2+}$ by an octahedral ligand field.</td>
<td>7</td>
</tr>
<tr>
<td>2.1</td>
<td>Effect of the ligand concentration on the species distribution of complexes of CuL and CuL$_2$.</td>
<td>51</td>
</tr>
<tr>
<td>3.1</td>
<td>Conditional stability constants for complexes of copper ions and HA, FA and ligands in natural waters.</td>
<td>91</td>
</tr>
<tr>
<td>3.2</td>
<td>Relative forces diagram.</td>
<td>110</td>
</tr>
<tr>
<td>6.1</td>
<td>Calibration of MnO$_2$ for the adsorption of copper.</td>
<td>158</td>
</tr>
<tr>
<td>6.2</td>
<td>Conditional stability constant, log K$_R$, for the adsorption of Cu on MnO$_2$ as function of pH.</td>
<td>159</td>
</tr>
<tr>
<td>6.3</td>
<td>Comparison of adsorption models for copper on MnO$_2$.</td>
<td>163</td>
</tr>
<tr>
<td>6.4</td>
<td>The titration of 8-OH-quinoline with Cu$^{2+}$ ions.</td>
<td>167</td>
</tr>
<tr>
<td>6.5</td>
<td>Titration with copper of FA and dystrophic river sample, in presence of 40 μM MnO$_2$.</td>
<td>174</td>
</tr>
<tr>
<td>6.6</td>
<td>Overestimation of K' and [L] by the presence of CO$_3^-$ in equilibrium with air-CO$_2$ in the sample.</td>
<td>177</td>
</tr>
<tr>
<td>6.7</td>
<td>Conditional stability constants for complexes of Cu and organic ligands in natural waters as function of pH of determination.</td>
<td>181</td>
</tr>
</tbody>
</table>
6.8 Absorption at 260 nm as function of complexing capacity of natural water samples. 184

7.1 The effect of complexation, at pH of 8.3 by naturally occurring ligands and some artificial ligands, on [Cu^{2+}]. 195

7.2 Model for complexation of copper by lake-organics. 198

8.1 ¹⁴C primary production of Chlorella vulgaris in exudates of other algae in various levels of total copper. 223

8.2 ¹⁴C primary production of Chlorella vulgaris in exudates of other algae as function of the calculated free copper ion concentration. 225
In the case of scholars, to be sure, in the case of really scientific men, things may be different - "better", if you will - there may really exist something like a drive to knowledge there, some little independent clockwork which, when wound up, works bravely on without any of the scholar's other drives playing any essential part. The scholar's real "interests" therefore generally lie in quite another direction, perhaps in his family or in making money or in politics.

Friedrich Nietzsche: On the prejudices of philosophers

In: Beyond Good and Evil
CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Heavy metals occur in natural waters in very low concentrations, which generally do not vary over more than one order of magnitude. Typical concentrations are $10^{-9}$M for Cd, $10^{-8}$M for Cu, and $10^{-7}$M for Zn. The residence times of copper and zinc in the oceans are $2 \times 10^4$ years, which puts them apart from a particulate-forming metal such as Fe, with a residence time of 200 years (Brewer 1975).

Several heavy metals are essential nutrients for organisms. The metals are used in enzymes for electron transport or to alter fat or water solubility. Cuproproteins form part of dehydrogenases and cytochrome oxidase. Copper enzymes have a relatively high oxidation potential and function in dehydrogenation, using oxygen as the acceptor in plants and animals (Mahler and Cordes 1969, p.567).

An algal cell of 2 μm thick and 10 μm long containing
the same concentration of heavy metals as the surrounding water \((10^{-7} \text{M})\), would have about 10 atoms of metal per cell. Since this is not sufficient to satisfy the demand of the enzymes, the organism has to increase its metal concentrations. Thus it has been found that phytoplankton concentrates copper \(3 \times 10^4\) times (Lowman et al. 1971).

The difference between a situation of not enough of an element in the water to sustain growth and a situation of excess which inhibits growth, may be only one order of magnitude (van den Berg 1975). Also, the Cu concentration necessary for optimum growth varies in different environments. A great scatter is observed for LD_{50} values (doses of heavy metal which produce a toxic effect for 50% of the test organisms) in different environments (Jackson and Morgan 1978).

The organisms obviously are not the only systems which are trying to get at the metals. Algae may have to compete with dissolved organic matter or organic molecules adsorbed on inorganic particles, which may have annoyingly (for the algae) high stability constants, but occur in extremely low concentrations in seawater (\(\pm 1\) mg/liter total organic carbon, Stumm and Brauner 1975) or higher in freshwater depending on the local situation. Another form
of competition may come in the form of inorganic complexation, such as by \(\text{OH}^-\), \(\text{Cl}^-\), \(\text{HCO}_3^-\), \(\text{H}_2\text{PO}_4^-\), or of adsorption on inorganic particulates, such as hydroxides and oxides. It is not yet certain, however, whether these associations make metals more or less acceptable to the organism. One might wish to compare the metal-organism interaction with an organic or inorganic ligand or particle and metal interaction. Then planktonic organisms could be modelled as complexing ligands or as surface active particles. In this case we could predict strong competition by (a) complexation by other ligands in the same environment, and (b) other cations such as \(\text{Ca}^{2+}\), \(\text{Na}^+\), \(\text{H}^+\), and other heavy metal ions. In fact, for copper, it has been shown that the \(\text{Cu}^{2+}\) ion, or the free copper ion, is the active form of the metal in relation to availability or toxicity (Anderson and Morel 1978; Jackson and Morgan 1978; Sunda and Guillard 1976) and that organic ligands may well detoxify copper (Gnassia-Barelli et al. 1978). Similarly for zinc it has been shown that \(\text{Zn}^{2+}\) is the active form (Anderson et al. 1978).

Apparently measurement of total metal concentrations is not sufficient to assess its availability or toxicity to organisms, or even its geochemical behaviour. Adsorption and complexation processes affect the stage of metals in natural waters and may be most important in determining
their associations. Copper is particularly known for its toxicity to organisms in natural waters at very low concentration and for its ability to form complexes, more so than other divalent heavy metals. This will be discussed in the following chapters.

This work therefore concerns itself with the state of heavy metals in natural waters, with special reference to copper. A method is described to determine complexation of copper ions in natural waters at very low concentrations of the complexing agent. The method is applied to a number of lakes and rivers, and the results are used in a model to quantify its implications.

Data of various natural waters are compared with measurements performed on laboratory algal cultures, in an attempt to clarify the source of the complexing matter.

In order to explain the copper distribution in natural waters, firstly associations with organic ligands are treated theoretically (Chapter 2). Then the nature and distribution of inorganic and organic reactants are stated (Chapter 3) before methods of analysis (Chapters 4 and 5), and results, general models, and biomass effects (Chapters 6-8) can be developed. The theory of the MnO₂ method is combined with the complexation theory in one chapter (Chapter 2) for reasons of unity of the theory and legibility of the thesis.
1.2 FORMATION OF COMPLEXES

In aqueous solution all transition metal ions are complexed to some degree. This is explained by the Ligand Field Stabilization Theory (Cotton and Wilkinson 1972, p. 555-619). In the non-complexed ion, the outer or d-electrons spread themselves with equal probability over the five d-orbitals, since all are equivalent. Depending on the environment, however, these orbitals are not equivalent at all. They split in a group of three (t-orbitals) and one of two orbitals (e-orbitals). In an octahedral environment the two e-orbitals are of higher energy than the three t-orbitals; in a tetrahedral environment the opposite is true. If only five electrons are present and distributed as one electron per d-orbital, or if ten electrons are present (two electrons per d-orbital), no energy is gained from ligand field effects, since the energy gained (from electrons in raised orbitals) is cancelled by the energy lost (from electrons in lowered orbitals). In all other cases, i.e. if not all orbitals are filled, the electrons will tend to fill the lower energy orbitals first, thus producing overall a more advantageous energetic level. Depending on the number of electrons that a metal ion has, it will prefer an octahedral or a tetrahedral environment. Cu$^{2+}$ has 9
electrons in its d-orbitals and prefers the octahedral environment.

The stability gained from the ligand field effects represents only about 10% of the total energy involved in complexation (Cotton and Wilkinson 1972, p. 596) but it still plays an important role in the thermodynamic properties of transition-metal compounds. For example it explains the order in the strength of complexes which is frequently observed, the Irving-Williams order: Mn$^{2+}$<Fe$^{2+}$<Co$^{2+}$<Ni$^{2+}$<Cu$^{2+}$<Zn$^{2+}$.

Cu$^{2+}$ in water is surrounded octahedrally by six water molecules. Yet for simplicity it is referred to as the free metal ion. Generally data for constants are determined for complexes of copper in water, in which this water-complexed copper ion is the reference state. But in reality the formation of a complex in aqueous solution involves the displacement of water molecules by ligands. If the ligand produces stronger orbital splitting, it will produce a more stable complex.

Some metals, notably those with 9 (Cu$^{2+}$) or 7 electrons in the d-orbitals, will gain additional stabilization in octahedral complexes due to the Jahn-Teller effect. Since electrons are not equally distributed over all the orbitals, the complex becomes distorted, which can
Figure 1.1  Energy level diagram of the splitting of d-orbitals of Cu\(^{2+}\) by an octahedral ligand field. The spin direction of the electrons is indicated by arrows on the d-orbitals.
$\text{Cu}^{2+}$

$3d^9$

Ligand Field Effect

Jahn Teller Effect
be explained by a further splitting of the e and t orbitals. This again is energetically advantageous. Figure 1.1 shows this schematically.

1.3 DRIVING FORCES OF COMPLEXATION AND ADSORPTION OF TRANSITION METALS

The two subjects, complexation and adsorption, are treated here as one. Especially for small charged ions the two processes can be thought largely as being the same. The van der Waals forces which become important for the adsorption of large, organic molecules, are still fairly insignificant. As a matter of fact, several adsorption models (Chapter 1.4) treat adsorption as surface complexation. Also the same sequence of binding strength, explained by the Irving Williams series, is frequently observed when metals are compared.

The entropy of small charged spheres in aqueous solution is very low. If the molal entropies of ions are referred to the aquated proton as zero, then, for monatomic ions, the following empirical relation is approximately correct:
\[ S^o = \frac{3}{2} R \ln M - \frac{270.\bar{z}}{(r+x)^2} + 37 \] (Powell and Latimer 1951)

in which \( z \) = charge on the ion

\( r \) = Pauling's crystal radius (in Å)

\( x \) = constant = 1.00 Å for anions
\( = 2.00 \) Å for cations

\( M \) = atomic weight

\( R \) = gas constant.

So, a small cation with a high charge will have a low entropy.

This may be correlated to the structure disturbing effect which these ions have on water. The extent to which charged surfaces influence the water structure has been a subject of several discussions. Apparently the thickness of the affected layer is somewhere between a couple of molecular water layers (about 5 Å) (Lyklema 1977), 10-24 Å (van Diemen and Stein 1978), to over several hundred molecular diameters (about 0.1 μm) (Drost-Hansen 1977).

An aquated metal ion such as copper or zinc possesses six water molecules immediately around it. Immediately surrounding this complex is a second layer of water molecules, still quite coherently bound to the first layer. From work in highly concentrated solutions it is apparent that the total hydration number of zinc, Zn\(^{2+}\), is 16 (Skou et al. 1977), and the complexation of one Cl\(^-\) ion releases
4 water molecules. One can appreciate that this must be a great gain of entropy for the solvent. So entropy can be expected to play an important role in complexation and adsorption processes.

In the case of water-metal complexes the water molecules are bound to the ions by ion-dipole forces. Most other complexes, organic or inorganic, are actually composed of ions of opposite charges. Coulombic forces are the most important factor in determining stability of complexes. They provide a favorable enthalpy term. But the fact that complexes can be formed in which the ligands more than compensate for the charge of the metal ion, such as in \( \text{CuCl}_3^- \), shows the importance of entropy. The same is valid for adsorption of positive metal ions on an originally negatively charged surface, which may reverse the charge on the solid.

1.4 THEORY OF ADSORPTION AND COMPLEXATION

Short introduction into models related to the theory of the double layer

A surface between two phases, whether they are liquid-liquid, solid-liquid, solid-air or liquid-air, will only under very special circumstances not bear a charge.
This charge results in a distribution of an equivalent amount of ionic charge of opposite sign in the solution phase near the interface, called the electrical double layer. Theoretical treatment of the double layer was first discussed by Gouy (1910) and Chapman (1913).

The charge on the solid ("wall") is treated as a surface charge smeared out uniformly over the surface. The space charge in the solution is considered to be built up by unequal distribution of point-like ions. This distribution is governed by a Boltzmann relation. The model does not consider the size of the ions and leads to calculated capacities of the double layer, depending on the surface potential, which are far too high.

This has been remedied by O'Stern (1924). His correction comes down to the application of the Gouy-Chapman theory with the first layer not immediately at the wall, but at a distance \( d \) away from it. Stern further considered the possibility of specific adsorption of the ions and assumed, that these ions were located in the plane \( d \), referred to as the Stern layer.

Stern's theory was improved by Grahame (1947). He supplies evidence, that in the Stern layer only anions really are adsorbed involving some chemical bonding, with loss of a part of their hydration shells, whereas the
cations remain hydrated and are only attracted to the surface by electrostatic forces. This results in an inner Helmholtz plane (distance to centre of anion), which previously was the Stern layer, and an outer Helmholtz plane (distance to centre of hydrated cation). The double layer description of Gouy-Chapman may be used from the outer Helmholtz plane on.

Grahame's approach seems to give the best accord with experiment. The high experimental capacities for large positive and negative potentials, as found for hydrophobic sols of metal oxides in water, are not explained by any of these theories.

Tadros and Lyklema (1968) determined for silica gel a surface charge and capacity higher than can be explained by hydrolysis of the silanol groups on the surface. Also Perram (1973), Schindler and Kamber (1968), and Stumm, Huang and Jenkins (1970) treat the same problem, each giving a different explanation. Their basic ideas will now be summarized.
The porous double layer (Lyklema 1971)

Lyklema mentions three trends:

1. The more porous the surface, the higher the surface charge at given difference between the pH and the pH of zero point of charge (Zpc).

2. The experimental surface charge can exceed the surface charge produced by full dissociation of all hydroxyl groups on the surface.

3. This high surface charge does not show up in colloid chemical properties, such as zeta-potential.

These observations led to the concept of a porous double layer: the surface charge as well as part of the countercharge is not restricted to the surface proper, but can be accommodated also behind this surface, inside the solid.

This model gives a qualitative explanation for the trends shown above.

The model of Perram (1973) proposes a comparable concept, in the form of a gel layer of infinite thickness, but this model seems to be less realistic, and may only serve as mathematical explanation of his observations, rather than as a chemical model.
Model of surface complexation (Stumm, Hohl, Dalang 1976 and Schindler, Gamsjäger 1972)

Schindler explains the high surface charge by a combination of acidity constants for the surface layer, and a potential, which depends on ionic strength and charge on the surface.

Stumm further develops this concept and uses the same hydrolyzed surface groups as ligands for complexation with heavy metals. The model explains at least quantitatively the effects of specific adsorption, but it is not certain, what the potential stands for. It is not the same as the zeta-potential or the wall-potential.

As an intermediate solution Anderson (Malotky and Anderson 1976) goes back to the Stern equation, calculates $\phi$ (thermodynamic meaning unknown) and the maximal adsorption capacity ($I_m$) at the isoelectric point (IEP). Then he models mathematically adsorption at varying pHs and potentials. The problem is that he assumes that adsorption depends on the pH at the IEP, which may well be the cause of non-linearity in his model. In cases of specific adsorption, however, adsorption depends more on the pH of ZPC. As a matter of fact adsorption co-determines the pH of the IEP.
The James-Healy Model (1971)

The model by James and Healy partially returns to the double layer theory by Grahame (1947), redefines $\phi$, assumes that adsorbed ions keep their first hydration layer, and applies then a summation of the energies of coulomb and solvation and a specific chemical adsorption energy. They find hydrolysis of the metal ion a prerequisite for adsorption. Grahame (1947) originally mentioned that $\phi$ might represent covalent bond energy.

The double layer theory does not work sufficiently for the hydrophobic metal oxides. The theory is not able to explain specific adsorption effects.

Transition metal ions in water are completely hydrated (complexed by water). There may well be hydrogen bonding between the primary hydration sphere and the hydroxyl groups on the surface of the metal oxide. Possibly $\phi$ can be explained in terms of hydrogen bonding.
1.5 SUMMARY

An introduction into trace metal interactions with particles, natural ligands, and aquatic organisms is given. The importance of knowledge about the form in which metals occur in natural waters is stated in relation to metal availability/toxicity to organisms and in relation to metal transport. It is possible to depict phytoplanktonic organisms as complexing ligands.

In a general discussion the forces driving complexation and adsorption of transition metals are compared. Ligand field effects provide only about 10% of the energy involved in complexation, the remaining energy is provided by the gain in entropy upon release of water molecules by complexation, and by Coulombic forces which provide a favourable enthalpy term.

Different chemical models to explain complexation and adsorption are compared qualitatively. There is not yet a model available which explains metal adsorption satisfactorily on all kinds of surfaces.
Es ist ein großer Irrtum zu glauben, dass Menschheits-probleme "gelöst" werden. Sie werden von einer
gelangweilten Menschheit liegen gelassen.

K. Tucholsky: Zwischen gestern und morgen
(1952), p.175. Rowohlt (Publ.)
CHAPTER 2

THEORY

2.1 STABILITY CONSTANTS

The conditional stability constant for the formation of the complex CuL is defined as

\[ K' = \frac{[\text{CuL}]}{[\text{L}'][\text{Cu}^{2+}]} \] (1)

where [L'] is the total concentration of ligand not bound to Cu\(^{2+}\). This notation is similar to the one used in Rossotti and Rossotti (1961, p.9), except for the use of Cu\(^{2+}\) instead of Cu'. In this study, to the extent possible, correction for the formation of other complexes with Cu\(^{2+}\), such as CuOH\(^+\) and CuCO\(_3\)\(^-\) will be made. Use of equation (1) assumes that copper is complexed by L only, i.e. that complex formation by other ligands in solution is negligible or that they are corrected for. This may not always be true. In these cases deviations will be apparent which will be
described in later sections.

If more than one type of site is available, on the surface of a large organic molecule for example, the binding of a second copper ion is described by the next stepwise conditional stability constant

\[ K'_2 = \frac{[\text{Cu}_2\text{L}]}{[\text{CuL}][\text{Cu}^{2+}]} \]

If 2:1 complexes are formed, the formation of the \( \text{CuL}_2 \) complex is described by the overall constant

\[ \beta'_2 = \frac{[\text{CuL}_2]}{[\text{L}'][2][\text{Cu}^{2+}]} \]  \hspace{1cm} (2)

If the ligand is a macromolecule such as HA and two sites are involved in binding one copper ion, the same notation may be used. L stands then for the concentration of sites rather than the ligand concentration.
2.2 COMPETITION BY PROTONS

In equation (1) \( L' \) is used to represent all ligands which are not bound to copper. Therefore \( K' \) is called a conditional stability constant, since it depends on environmental factors such as pH. Protons are particularly important since naturally occurring complexing ligands may well have very high acidity constants, such as \( pK_{a2} = 8.8 - 9.5 \) (Takamatsu and Yoshida 1978). So these organics are partially protonized at the pH of natural waters.

The acidity constant(s) for the dissociation of the ligand-proton complex are:

\[
K_{a1} = \frac{[H^+][HL^-]}{[H_2L]} \quad \text{and} \quad K_{a2} = \frac{[H^+][L^{2-}]}{[HL^-]}
\]

or generally

\[
K_{an} = \frac{[H^+][H_{N-n}L^{-n}]}{[H_{N-n+1}L^{-n}]}.
\]

where \( N \) is the total number of exchangeable protons, and \( n \) is the number of protons which stepwisely is exchanged.

To describe complexation of Cu by a ligand, equation (1) is used here. \([L']\) stands for all ligand not bound to
copper, and is really

\[ [L'] = [L^-] + [HL] \quad (3), \]

when the ligand has only one exchangeable proton, and where \( L^- \) is the unprotonated ligand. Substitution into (1) and combination with the acidity constant, \( K_a \), gives

\[ K' = \frac{[CuL]}{[Cu^{2+}][L^-](1 + \frac{H^+}{K_a})} \quad (4). \]

When \( H^+ \ll K_a \) (high pH),

\[ K' = \frac{[CuL]}{[Cu^{2+}][L^-]} = K \quad (5). \]

\( K' \) is then constant, independent of the pH, and equal to the stability constant \( K \). When \( [H^+] \gg K_a \) (low pH),

\[ K' = \frac{[CuL]}{[Cu^{2+}][L^-]\frac{H^+}{K_a}} \quad \text{or} \quad K' = \frac{K}{[H^+]} \quad (6). \]

So, the conditional stability constant, \( K' \), which will be determined, varies with the pH when the pH is smaller than the logarithm of the acidity constant of the ligand.
When the ligand is a large molecule with more than one functional group one can imagine that two protons may be exchanged for one two-valent metal ion. In such a case the mass balance becomes $[L'] = [L^2_] + [HL^-] + [H_2L]$, and then substitution gives

$$K' = \frac{[CuL]}{[Cu^{2+}][L^2-] \left( \frac{[H^+]}{K_{a_2}} + \frac{[H^+]^2}{K_{a_1} K_{a_2}} + 1 \right)}$$

(7)

Three limits will now be considered in which the pH varies from larger than both acidity constants, in between both, and smaller than both acidity constants.

1. $pH > pK_{a_1} > pK_{a_2}$ : now approximately

$$K' = \frac{[CuL]}{[Cu^{2+}][L^2-]} = K$$

This case is comparable to the association of two charged ions without the release of protons. So, no competition by protons can be present in this region.

2. $pK_{a_1} > pH > pK_{a_2}$ : then approximately
\[ K' = \frac{[\text{CuL}]}{[\text{Cu}^2+][\text{L}^2-][\text{H}^+]} K_{a_2}^2 \quad \text{or} \quad K' = K_{a_2} \frac{K_a}{[\text{H}^+]} \], equivalent to (6).

In this pH region the ligand behaves like a monoprotonated ligand. In a plot of log \( K' \) vs. pH, log \( K' \) would follow a line with a slope equal to one.

3. \( \text{pK}_{a_1} > \text{pK}_{a_2} > \text{pH} \) : then approximately,
\[ K' = \frac{[\text{CuL}]}{[\text{Cu}^2+][\text{L}^2-]\text{H}^+} K_{a_1} K_{a_2} \quad \text{or} \quad K' = K_{a_1} \frac{K_{a_2}}{[\text{H}^+]^2} \] (8)

Now log \( K' \) varies with 1/2pH: a plot of log \( K' \) vs. pH would have a slope of two in this pH region.

In the case of formation of a 2:1 complex the conditional stability constant, \( \beta_2' \) (equation 2), becomes
\[ \beta_2' = \frac{[\text{CuL}_2]}{[\text{Cu}^2+][\text{L}^-]^2(1 + \frac{[\text{H}^+]}{K_a}^2)} \] (9).

In this case \( \text{CuL}_2 \) may describe either bidentate binding by two identical sites on a single molecule, or the binding of one \( \text{Cu}^2+ \) ion by two ligand molecules.
Similarly
\[ \beta_2' = \beta_2 = \frac{[\text{CuL}_2]}{[\text{Cu}^{2+}][\text{L}^-]^2} \]  \hspace{1cm} (10)

when pH > \log K_a, and
\[ \beta_2' = \beta_2 \left( \frac{K_a}{[\text{H}^+]} \right)^2 \]  \hspace{1cm} (11) when pH < \log K_a.

So \( \beta_2' \) depends then on \([\text{H}^+]^2\).

In the literature one also finds a different way of describing these equilibria (Bjerrum 1923). It is possible to use a constant, \( B \), independent of the pH, by incorporating the protonized form, \( \text{HL} \), of the ligand in the equation:

\[ B = \frac{[\text{CuL}][\text{H}^+]}{[\text{Cu}^{2+}][\text{HL}]} \]  \hspace{1cm} (12).

The relationships of \( B \) with \( K \) and \( K' \) are found by incorporating the acidity constant, and are

\[ B = K \cdot K_a \]  \hspace{1cm} (13) and \[ B = K' (K_a + [\text{H}^+]) \]  \hspace{1cm} (14).

So \( B \) is simply the product of the acidity constant and the stability constant. In the case of a 2:1 complex:

\[ B_2' = \frac{[\text{CuL}_2][\text{H}^+]^2}{[\text{HL}]^2[\text{Cu}^{2+}]} \]  \hspace{1cm} (15).
\[
B_2 = \beta_2 \cdot K_a^2 \quad \text{(16)} \quad \text{and} \quad B_2 = \beta_2^1 (K_a + [H^+])^2 \quad \text{(17)}.
\]

For determination of any of \( K, \beta, B, \) or \( B_2 \), it is necessary to have knowledge of the acidity constant(s). \( K' \), and sometimes \( \beta_2 \), can be determined without this knowledge, but they are only valid at the pH at which they have been determined.

When the ligand is a macromolecule such as HA, often a similar notation is used, but 2:1 complexation then has the meaning of two complexing sites per metal ion. Complexation is then described by two stepwise stability constants, \( b_1 \) and \( b_2 \), representing

\[
b_1 = \frac{[\text{CuL}^+][H^+]}{[\text{LH}][\text{Cu}^{2+}]} \quad \text{and} \quad b_2 = \frac{[\text{CuL}_2][H^+]}{[\text{LH}][\text{CuL}^+]}
\]

Now \( B_2 = b_1 \cdot b_2 = \frac{[\text{CuL}_2][H^+]^2}{[\text{HL}]^2[\text{Cu}^{2+}]} \). Since only one ligand is involved this equilibrium can be more meaningfully represented by an average stability constant

\[
B_{AV} = (b_1 \cdot b_2)^{1/2}
\]

which is equivalent to \( B \) in equation (12).
2.3 ADSORPTION ON MnO₂

In this section the theory is developed for the adsorption of copper on a weak ion exchanger, as exemplified by MnO₂. In the following sections the theory will be discussed for adsorption in presence of complexing ligands. Later in this thesis (Chapter 5) a method will be described which uses a dispersion of MnO₂ as ion exchange medium to substitute existing methods to determine copper complexation. I developed this method especially to function at the very low ligand concentration in natural waters. The theory of the MnO₂ method will be discussed in this chapter (sections 2.3-2.9), however, because of its very close connection to the equations developed in the previous sections. Thus unity is obtained of the theory concerning this thesis, rendering the other chapters in a, hopefully, more legible form.

When no metal complexing ligands are present, the adsorption of Cu²⁺ on MnO₂ can be described by the Langmuir equation:

\[ \Gamma_{\text{ads}} = \Gamma_{\text{max}} \cdot \frac{[\text{Cu}^{2+}]}{B + [\text{Cu}^{2+}]} \]  (18)
in which $\Gamma_{\text{ads}} = \text{amount of copper adsorbed per mole MnO}_2$ in
\[ \frac{\text{mole Cu}_{\text{ads}}}{\text{mole MnO}_2} \]

$[\text{Cu}^{2+}] = \text{concentration of free Cu}^{2+} \text{ions in equilibrium with the MnO}_2$

$\Gamma_{\text{max}} = \text{the maximum amount of Cu}^{2+} \text{which can be adsorbed, or the maximum number of sites available}$

$B = \text{constant, related to the energy of adsorption.}$

The amount of copper adsorbed is calculated from

$[\text{Cu}_{\text{ads}}] = \text{Cu} - [\text{Cu}_{\text{dissolved}}]$, where Cu is the total concentration of copper present. At very low $[\text{Cu}^{2+}]$ and therefore low coverage of the MnO$_2$, (18) reduces to

\[ \Gamma_{\text{ads}} = K_R \cdot [\text{Cu}^{2+}] \]

in which $K_R = \Gamma_{\text{max}} \cdot B$, in mole$^{-1}$liter. $K_R$ is then the conditional stability constant for adsorption of copper on MnO$_2$ and can be calibrated as a function of pH, when temperature and ionic strength are constant. A similar constant is frequently used in ion exchange experiments.

Very strong (high B) ion exchange resins, and relatively large amounts, are generally used however, so the percentage coverage is low (Ardakani and Stevenson 1972; Schubert 1952).

The distribution of metal ions over resin and solvent is then assumed to be constant. In the experiments described here, the amount of ion exchanger in equilibrium with the
metal ions is very small, so up to about 50% of all the available sites may be occupied. For this reason the Langmuir equation, which can be made to represent a good fit to the data, is used.

Equation (18) is readily transformed into

$$\frac{1}{I_{ads}} = \frac{1}{I_{max}} + \frac{1}{I_{max}} \frac{1}{B} \frac{1}{[Cu^{2+}]} \quad (19).$$

A plot of $\frac{1}{I_{max}}$ vs. $\frac{1}{[Cu^{2+}]}$ results in a straight line, where the Y-intercept represents $\frac{1}{I_{max}}$ and the slope $\frac{1}{I_{max}} \cdot \frac{1}{B}$.

Since the reciprocal of the Cu$^{2+}$ concentration is the independent variable, a few data points, representing very low copper concentrations, will be at high $\frac{1}{[Cu^{2+}]}$, and these data will have a strong influence on the slope, even though they may contain relatively larger errors in the measurement (from contamination). Also, the data at higher copper concentrations tend to group together. Therefore it is better to use a different linearized form of the Langmuir equation:
\[
\frac{[\text{Cu}^{2+}]}{\Gamma_{\text{ads}}} = \frac{1}{\Gamma_{\text{max}}} + \frac{1}{B} + \frac{[\text{Cu}^{2+}]}{\Gamma_{\text{max}}} \quad (20).
\]

From a plot of \( \frac{[\text{Cu}^{2+}]}{\Gamma_{\text{ads}}} \) vs. \( [\text{Cu}^{2+}] \), \( \Gamma_{\text{max}} \) is now obtained from the slope, and constant \( B \) from the slope divided by the Y-intercept.

2.4 ADSORPTION ON MnO₂ IN PRESENCE OF A COMPLEXING LIGAND

A complexing ligand, \( L \), in equilibrium with copper ions and MnO₂ will effectively compete for \( \text{Cu}^{2+} \) with the MnO₂. The distributions of \( L \) and \( \text{Cu}^{2+} \) over complexes and free ions can be described by the conditional stability constant, \( K' \), in (1) and by a mass balance

\[
L = [L'] + [\text{Cu}L] \quad (21),
\]

where \( L \) is the total ligand concentration present, and \( L' \) and CuL are as before.

It will now be assumed that only \( \text{Cu}^{2+} \) adsorbs and that the CuL complex does not adsorb. The validity of this assumption depends on the likelihood of any adsorption of CuL on the MnO₂ surface and is discussed in sections 3.6-3.9. Adsorption of CuOH⁺, if at all, would not influence
the values of the conditional stability constants determined by this method, although it would change the formal representation of the chemistry. But, since the adsorption of copper on MnO₂ is calibrated at the same pH as the experimental pH, any errors resulting from assumptions concerning the adsorption of CuOH⁺ (or of Cu(OH)₂ for that matter) will cancel out in the mass balance when the CuL concentration is calculated from the total amount of dissolved copper (equations 24 and 25). The probability of adsorption of CuOH⁺ is further discussed using model calculations of experimental data in section 6.2.

So, assuming that CuL does not adsorb substitution of (21) into (1) gives

\[
\frac{1}{[\text{CuL}]} = \frac{1}{K'} \cdot \frac{1}{L} \cdot \frac{1}{[\text{Cu}^{2+}]} + \frac{1}{L} \quad (22) \quad \text{or}
\]

\[
\frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K' \cdot L} + \frac{[\text{Cu}^{2+}]}{L} \quad (23).
\]

Both equations are very similar to the Langmuir equation. In fact, if one substitutes adsorption sites for ligands, one obtains the Langmuir equation. Equations (22) and (23) give straight lines when \( \frac{1}{[\text{CuL}]} \) is plotted vs. \( \frac{1}{[\text{Cu}^{2+}]} \), (22), or \( [\text{Cu}^{2+}]/[\text{CuL}] \) vs. \( [\text{Cu}^{2+}] \), (23). But, for the same
reason as mentioned for the Langmuir equation, equation (23) is the preferable one, in that it gives a better spread of the data. The total ligand concentration, $L$, is then found from the $(slope^{-1})$ and the conditional stability constant, $K'$, from the slope divided by the $Y$-intercept.

The Langmuir equation describes $[Cu^{2+}]$ as a function of $B$, $\Gamma_{\text{max}}$, and $\Gamma_{\text{ads}}$. So by titrating, in a stepwise manner, MnO$_2$ with Cu, in the presence of a complexing ligand, we may calculate $[Cu^{2+}]$, in equilibrium with both MnO$_2$ and the ligand, by determining $\Gamma_{\text{ads}}$, from the mass balance

$$Cu = [Cu_{\text{dis}}] + [Cu_{\text{ads}}] \quad (24),$$

where Cu is the total concentration of copper, $[Cu_{\text{ads}}]$ is the concentration of copper adsorbed on the MnO$_2$. $[Cu_{\text{dis}}]$ is the concentration of copper in solution, which is composed of

$$[Cu_{\text{dis}}] = [Cu^{2+}] + [CuL] + [CuOH^+] \quad (25).$$

From (25) we may then calculate $[CuL]$, after which we proceed to calculate $K'$ and $L$ by plotting $[Cu^{2+}]$ vs. $[Cu^{2+}]/[CuL]$ as described by (23).

If, for some reason, we know the ligand concentration, $K'$ may be calculated from a single data point of a titration.
with copper, using

\[ K' = \frac{L[Cu^{2+}]}{[CuL]} - [Cu^{2+}]^{-1} \quad (26). \]

**Vice versa** we may calculate \( L \) from one point if we know \( K' \), using

\[ L = \frac{[CuL]}{[Cu^{2+}]} \cdot \frac{1}{K'} + \frac{[CuL]}{K'} \quad (27). \]

Such a calculation will have a much larger uncertainty, however, compared to results using all titration data.

Until now it has been assumed that copper is complexed by \( L \) only, in 1:1 complexes, i.e. that complexation by other anions in the solution is negligible, except \( OH^- \) and \( CO_3^{2-} \), for which it is possible to correct by measuring the \( pH \) and removing \( CO_2 \). This may not always be correct. In such cases deviations from linearity, of (23), will occur. These are described in the next sections.

### 2.5 ADSORPTION OF \( MnO_2 \) IN PRESENCE OF TWO COMPLEXING LIGANDS

Calculation of \( K' \) and \( L \) involves plotting of

\[ \frac{[Cu^{2+}]}{[CuL]} \quad \text{vs.} \quad [Cu^{2+}], \text{following} \]
\[
\frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K \cdot L} + \frac{[\text{Cu}^{2+}]}{L}
\]  \hspace{1cm} (23)

The concentration of CuL is calculated by deduction, using (25). In the presence of a second ligand, L2, (25) becomes:

\[
[Cu_{\text{dis}}] = [\text{Cu}^{2+}] + [\text{CuL}] + [\text{CuL2}].
\]

So the calculated [CuL] really is [CuL] + [CuL2]. We have to transform (23) in such a way that it will describe a plot of \[
\frac{[\text{Cu}^{2+}]}{([\text{CuL}] + [\text{CuL2}])}
\] vs. \[ [\text{Cu}^{2+}] \].

This goes as follows:

\[
\frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K \cdot L} + \frac{[\text{Cu}^{2+}]}{L} \quad (23) \rightarrow \quad [\text{Cu}^{2+}] = \frac{[\text{CuL}]}{K' \cdot L} + \frac{[\text{Cu}^{2+}][\text{CuL}]}{L}
\]

Then:

\[
[Cu_{\text{dis}}] = \frac{[\text{CuL}] + [\text{CuL2}]}{K' \cdot L} - \frac{[\text{CuL2}]}{K' \cdot L} + \frac{[\text{Cu}^{2+}][([\text{CuL}]+[\text{CuL2}])]}{L}
\]

\[
- \frac{[\text{Cu}^{2+}][\text{CuL2}]}{L} \quad (28).
\]

[CuL] is replaced by [CuL] + [CuL2].and to retain the balance, the difference is deducted. So (28) is completely
equivalent to (18) in form.

Now rewriting of (28):

\[
\frac{[\text{Cu}^{2+}]}{[\text{CuL}]^* + [\text{CuL2}]} = \frac{1}{K' \cdot L} \left( 1 - \frac{[\text{CuL2}]}{[\text{CuL}] + [\text{CuL2}]} \right) \]

\[
+ \frac{[\text{Cu}^{2+}]}{L} \left( 1 - \frac{[\text{CuL2}]}{[\text{CuL}] + [\text{CuL2}]} \right)
\]

(29).

Within certain limits a plot of

\[
\frac{[\text{Cu}^{2+}]}{[\text{CuL}] + [\text{CuL2}]} \text{ vs. } [\text{Cu}^{2+}]
\]

will provide the conditional stability constant for the formation of the stronger complex and approximately its real ligand concentration. The error in the detected ligand concentration depends on the difference in strength of the complexes which are formed and on the difference in ligand concentrations.

In (29) \( a = 1 - \frac{[\text{CuL2}]}{[\text{CuL}] + [\text{CuL2}]} \).

Since \([\text{CuL2}]\) and \([\text{CuL}]\) vary with \([\text{Cu}^{2+}]\), the plot will be curved for parts where \([\text{CuL}]\) and \([\text{CuL2}]\) are of similar magnitude. The influence of \(a\) will be minimal when \([\text{CuL2}]\)
is minimal: \( a \) is then close to unity.

We will now try to put this on more of a quantitative basis. Combination with mass balance equations
\[ L = [L'] + [CuL] \text{ and } L_2 = [L_2'] + [CuL_2], \]
and substitution gives
\[
\frac{K'L}{1 + K'[Cu^{2+}]} = \frac{K'L}{1 + K'[Cu^{2+}]} + \frac{K_2'L_2}{1 + K_2'[Cu^{2+}]} \quad (30).
\]

The only variable in (30) is the \( Cu^{2+} \) concentration. But its effect depends on the relative magnitudes of the stability constants, \( K' \) and \( K_2' \), and the ligand concentrations, \( L \) and \( L_2 \).

Limits will now be considered. \emph{A priori} assumptions are that:

(a) the ligand concentrations are high enough to give copper complexation, or \( L > K' \) and \( L_2 > K_2' \),

(b) \( K_2' < K' \), or \( L_2 \) is the weaker ligand.

Further discussion deals with low and with high copper concentrations.
1. Low \( \text{Cu}^{2+} \) concentrations

\[ [\text{Cu}^{2+}] \ll \frac{1}{K}, \frac{1}{K_2'} \text{, or } p[\text{Cu}^{2+}] > \log K' > \log K_2'. \]

In this situation most copper is complexed, but the ligands are only partially filled. Considering the high complexing capacities (micro mole range) which are observed in natural waters, and the generally very low copper concentrations (10^{-7} molar range or less) this is the situation we can expect in the natural water environment.

At low \( [\text{Cu}^{2+}] \ll [\text{Cu}^{2+}] \cdot K' \ll 1 \) and \( [\text{Cu}^{2+}] \cdot K_2' \ll 1 \), so approximately \( (1 + [\text{Cu}^{2+}] \cdot K') \approx 1 \) and \( (1 + [\text{Cu}^{2+}] \cdot K_2') \approx 1 \). Then

\[ a = \frac{K' \cdot L}{K' \cdot L + K_2' \cdot L_2} \]

So \( a \) becomes constant at low \( [\text{Cu}^{2+}] \), and the plot of

\[ \frac{[\text{Cu}^{2+}]}{[\text{CuL}] + [\text{CuL}_2]} \text{ vs. } [\text{Cu}^{2+}] \]

will be straight. The ligand concentration is then calculated from: slope^{-1} = \( \frac{L}{a} \), the stability constant is \( \frac{\text{slope}}{Y_{\text{intercept}}} = K' \).

Interestingly \( K' \), as measured, is not affected by the presence of a second, weaker, ligand. The measured ligand concentration will be larger than \( L \).
2. **High Cu\(^{2+}\) concentrations**

If we continue the titration of the ligands with copper until they are largely saturated, then

\[ [\text{Cu}^{2+}] > \frac{1}{K_{2'}} > \frac{1}{K'} \text{, and } [\text{Cu}^{2+}] \cdot K' > [\text{Cu}^{2+}] \cdot K_{2'}' \]

so approximately

\[
a = \frac{K' \cdot L}{[\text{Cu}^{2+}] \cdot K'} = \frac{L}{L + L_{2}}
\]

This is constant, and only dependent on the ligand concentration. The ligand concentration which is determined from the slope, is

\[
\frac{L (L + L_{2})}{L} = L + L_{2}, \text{ or the sum of both ligand concentrations present. The stability constant remains } K', \text{ the value of the stronger ligand.}
\]

From this, we may conclude that continued titration with Cu in presence of more than one ligand results in curvature and a new slope representing both ligands.

Now for some special cases it will be shown what happens to the measured ligand concentrations and stability
constants.
A. \( L = L_2, K' = K_2' \). A trivial case. Two ligands of identical concentrations and strength. As a result \( a = 1/2 \), so the determined ligand concentration is \( 2L \). It is measured as one ligand present in double its concentration.

B. \( L = L_2, K' > K_2' \). Two ligands in the same concentration, but with different stability constants. At low \([Cu^{2+}]\), \[ a = \frac{K'}{K' + K_2'} \]. The determined ligand concentration becomes

\[
\frac{K' + K_2'}{K'} \cdot L.
\]

If \( K' >> K_2' \), then it will approach \( L \). If \( K' = 10 \times K_2' \), the determined ligand concentration is \( \frac{11}{10} L \), or 9% too large.

At high \([Cu^{2+}]\), \( a = 1/2 \), with the result that the double ligand concentration is measured, with \( K' \) as stability constant.

C. \( L \neq L_2, K_2' < K' \).

\( L_2 > L \). If the concentration of the second, weaker, ligand is high enough, it can actually overcome the weaker strength of its complex and become the most important ligand. In other words \( K_2'.L_2 \) becomes larger than \( K'.L \).

We start off with a case where this is not yet so: \( K_2'.L_2 < K'.L \). At low \([Cu^{2+}]\), approximately \( a = 1 \) and the determined ligand concentration will approach \( L \), with \( K' \) as
constant.

When \( K'\cdot L_2 \) and \( K'\cdot L \) become similar, then

\[
\alpha = \frac{K'\cdot L}{K'\cdot L + K'\cdot L_2}
\]

and we determine a ligand concentration of

\[
\text{slope}^{-1} = L \left( \frac{K'\cdot L + K'\cdot L_2}{K'\cdot L} \right).
\]

The error in \( L \) depends on the relative sizes of \( K'\cdot L \) and \( K'\cdot L_2 \). When \( K'\cdot L_2 \) actually becomes larger than \( K'\cdot L \), then the error will be large. At high \([\text{Cu}^{2+}]\), \( \alpha = \frac{L}{L + L_2} \), and the determined ligand concentration will be \( L + L_2 \), the sum of both ligands.

Overall we may conclude that the strongest ligand is quite well defined when the stability constants are sufficiently different and this difference is not balanced by an equal difference in ligand concentration, in other words, when \( K'\cdot L >> K'\cdot L_2 \).

Through continued titration to higher \([\text{Cu}^{2+}]\) the plot will become linear again after a period of curvature. Then the difference of the new slope and the old one depends on the relative ligand concentrations. The concentration which we determine now equals \( L + L_2 \). A large change in
the slope means that \( L2 \gg L \).

If \( K' L \) and \( K2' L2 \) are of approximately the same magnitude or especially when \( K2' L2 > K' L \), the approximations we made, will be valid only for extremely low and high concentrations of \([Cu^{2+}]\) only. So, large parts of the plot will be curved.

Since we use the determined \( K' \) and \( L \) to calculate the free \( Cu^{2+} \) concentration in the original water, it is interesting to calculate what the \( Cu^{2+} \) concentration is in presence of two ligands, using both concentrations and stability constants, and to compare this with the \( Cu^{2+} \) concentration calculated from the single ligand and stability constant as we would determine it from a plot at low \([Cu^{2+}]\). The determined \( L \) is higher than the real \( L \) and it may well make up, in complexation terms, for the second ligand, if that one is not determined.

2.6 THE EFFECT OF A SECOND LIGAND ON THE CALCULATED \( Cu^{2+} \) CONCENTRATION

A situation is now assumed in which two ligands are present in the sample. Similarly to the experimental procedure \( K \) and \( L \) will be calculated from linear parts of the
resulting plot of \( \frac{[Cu^{2+}]}{[CuL]} \) vs. \( [Cu^{2+}] \). A test case is chosen where the deviations of the approximations are thought to be maximal but still realistic. Then the concentration of the weaker ligand, \( L_2 \), is larger than that of the stronger ligand, \( L \), but the conditional stability constants, \( K_{2'} \) and \( K' \), are sufficiently different to overcome the concentration effect: \( K'.L > K_{2'}.L_2 \).

The following values have been taken:

\[
\begin{align*}
L & = 2 \times 10^{-7} \text{M} \\
L_2 & = 5 \times 10^{-7} \text{M} \\
K' & = 10^8 \\
K_{2'} & = 10^7 \\
\text{Cu} & = 3 \times 10^{-8} \text{M (total copper concentration)}
\end{align*}
\]

At low \( [Cu^{2+}] \) the ligand concentration, \( \text{Lig}_T \), and its stability constant, \( K' \), are determined as

\[
\text{Lig}_T = \left( \frac{K'.L + K_{2'}.L_2}{K'.L} \right) \cdot L = 2.5 \times 10^{-7} \text{M}
\]

and \( K' = 10^8 \).

We see that the ligand concentration is 25% higher than the concentration of \( L \). From this we can now calculate the free copper ion concentration, assuming that only one ligand is present and using the determined data.
\[ K' = \frac{[CuL]}{[Cu^{2+}].L'} \quad (1) \]

\[ L = L' + [CuL] = 2.5 \times 10^{-7} \quad (21) \]

\[ Cu^+ = [Cu^{2+}] + [CuL] \rightarrow [CuL] = Cu - [Cu^{2+}] \quad (31). \]

Combination of (28) and (29) gives CuL:

\[ [CuL] = K'.[Cu^{2+}] . (L - [CuL]) \quad (32) \]

This combines with (31) to give

\[ Cu - [Cu^{2+}] = K'.[Cu^{2+}].L - K'.[Cu^{2+}].Cu + K'.[Cu^{2+}]^2, \text{ or} \]

\[ K'.[Cu^{2+}]^2 + (1 + K'.L - K'.Cu).[Cu^{2+}] - Cu = 0 \quad (33). \]

From this \([Cu^{2+}]\) is calculated by

\[ [Cu^{2+}] = \frac{-b + (b^2 - 4.a.c)^{1/2}}{2.a} \]

in which \(a = K'\)

\[ b = 1 + K'.L - K'.Cu \]

and \(c = -Cu\)

When \(L_{eq} = 2.5 \times 10^{-7} M: \quad p[Cu^{2+}] = 8.887 M, \text{ but when}\)

we use the real concentration \(L = 2 \times 10^{-7} M: \quad p[Cu^{2+}] = 8.782 M.\)
Now this will be compared with the $[\text{Cu}^{2+}]$ as calculated in presence of the second ligand, $L_2$, as well:

$$L_2 = 5 \times 10^{-7} \text{M}$$

$$K_2' = 10^7$$

$$L = [L'] + [\text{CuL}] \rightarrow [L'] = L - [\text{CuL}] \quad (34)$$

$$L_2 = [L_2'] + [\text{CuL}_2] \rightarrow [L_2'] = L_2 - [\text{CuL}_2] \quad (35)$$

$$\text{Cu} = [\text{Cu}^{2+}] + [\text{CuL}] + [\text{CuL}_2] \quad (36)$$

$$\frac{[\text{CuL}]}{[\text{Cu}^{2+}][L']} = K_1' \rightarrow [\text{CuL}] = K_1'.[L'].[\text{Cu}^{2+}] \quad (37)$$

$$\frac{[\text{CuL}_2]}{[\text{Cu}^{2+}][L_2']} = K_2' \rightarrow [\text{CuL}_2] = K_2'.[L_2'].[\text{Cu}^{2+}] \quad (38)$$

Combination of (34) through (38) gives

$$\text{Cu} = [\text{Cu}^{2+}] + \frac{K_1'.[\text{Cu}^{2+}].L}{1 + K_1'.[\text{Cu}^{2+}]} + \frac{[\text{Cu}^{2+}].K_2'.L_2}{1 + K_2'.[\text{Cu}^{2+}]}$$

which converts into

$$a.[\text{Cu}^{2+}]^3 + b.[\text{Cu}^{2+}]^2 + c.[\text{Cu}^{2+}] + d = 0$$

in which $a = K_1'.K_2'$

$$b = -Cu.K_1'.K_2' + K_1' + K_2' + K_1'.K_2'.L + K_1'.K_2'.L_2$$

$$c = 1 + K_1'.L + K_2'.L_2 - K_1'.Cu - K_2'.Cu$$

and $d = -Cu$. 
This has been solved graphically and iteratively by choosing values for Cu$^{2+}$, resulting into $p[\text{Cu}^{2+}] = 8.897$ M, compared with $p[\text{Cu}^{2+}] = 8.887$ M from the determined data: a theoretical error of 2% when a second ligand is present in higher concentration than the other, stronger, ligand. So, the error in the determination of the first ligand partially makes up for the error which would result from not having considered the second ligand.

It is, however, possible to determine the second ligand as well. After that the concentration of the first ligand can be further corrected.

2.7 DETERMINATION OF A SECOND LIGAND

When curvature is observed, followed by a second straight line, this indicates that a second site or ligand is available. Using the data and results obtained for the first ligand (2.6), we may calculate the concentration of the CuL2 complex from

$$[\text{CuL2}] = \text{Cu} - [\text{Cu}^{2+}] - [\text{CuL}].$$
We know Cu and [Cu$^{2+}$], and we calculate [CuL] from

$$[\text{CuL}] = K'\cdot[\text{Cu}^{2+}] \cdot (L - [\text{CuL}]) \text{ or } [\text{CuL}] = \frac{K'\cdot[\text{Cu}^{2+}] \cdot L}{1 + K'\cdot[\text{Cu}^{2+}]}$$

Then we plot $\frac{[\text{Cu}^{2+}]}{[\text{CuL}]}$ vs. $[\text{Cu}^{2+}]$, and we obtain $K2'$ and $L2$ in the usual way. Knowing $L2$ and $K2'$ we can then correct $L$ and $K'$ by calculating [CuL] from

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

in which [CuL2] is calculated from

$$[\text{CuL}2] = \frac{K2'\cdot[\text{Cu}^{2+}] \cdot L2}{1 + K2'\cdot[\text{Cu}^{2+}]}$$

using the calculated values for $K2'$ and $L2$. And then of course we can calculate a corrected $L2$ and $K2'$.

The example used previously (2.6) showed an error of 25% in $L$. If this error is reduced to another 25%, or 6% of the original, by one correction, two or three iterations should give values for $L$, $L2$, $K'$, and $K2'$, with an error much smaller than the error which probably is introduced by the measurements itself.

This method of calculating $K'$ and $L$ for a second
ligand is rather inaccurate in practice. It involves deduction of a calculated \([\text{CuL}]\) from the combined free copper and complexed copper concentrations \( ([\text{CuL}] + [\text{CuL}_2] + [\text{Cu}^{2+}] ) \) to get \([\text{CuL}_2]\). \([\text{CuL}] \) forms a large fraction of the total and is much larger than \([\text{CuL}_2]\), so large errors may result.

It would have been ideal if the slope of the plot of \( \frac{[\text{Cu}^{2+}]}{[\text{CuL}] + [\text{CuL}_2]} \) vs. \([\text{Cu}^{2+}]\) would have given information concerning the second ligand. The only information it gives is about the ligand concentration \(\text{L}_2\), nothing about its \(K_2'\).

It should be noted that the presence of a second site on the same ligand cannot be discerned from a case in which two ligands are present in the same concentration but with different stability constants: \(L = \text{L}_2\) and \(K \neq K_2\).

A further complication would arise when the formation of the first complex affects the stability of the second complex, or when the sites are differently affected by the pH. Then the one site could be more stable at advantageous pH, and vice versa in an electrostatically different environment.
2.8 FORMATION OF OTHER THAN 1:1 COMPLEXES

Various ligand to metal ratios in complex formation have been observed. The number of metal ions per organic molecule has been shown to be larger than one (Bresnahan et al. 1978; Coleman et al. 1956; Matsuda and Ito 1970), less than one (Bresnahan et al. 1978; Buffle et al. 1977; Courpron 1967) and also exactly equal to one (Gamble et al. 1970; Geering and Hodgson 1969; Schnitzer and Hansen 1970). The variations are at least partially due to the fact that molecular weights are not exactly known and can be determined by several methods, each with its inherent errors.

These ratios depend on pH, ionic strength, ligand concentration and metal concentration. Schnitzer and Hansen (1970) showed that 1:1 complexes are formed if the ratio of complexed ligand to total ligand is kept sufficiently low to prevent the formation of 2:1 (CuL2) complexes. Stevenson (1976) observed that the bond strength decreased with increasing metal (Cu$^{2+}$) concentration, and concludes that mixed 1:1 and 2:1 (CuL2) complexes are formed: 2:1 at low $M^{2+}$/HA ratios, 1:1 at high $M^{2+}$/HA ratios. In one case the stability constant for the 2:1 complex, $K_2$, became larger than the constant for the 1:1 complex, $K_1$ (Takamatsu and Yoshida 1978), above a certain pH. It is hard to understand that a 2:1
with its problems of steric hindrance and less Coulombic attraction would have an energetic advantage over the 1:1 complex. Takamatsu and Yoshida (1978) explain the phenomena by the formation of a more stable chelate ring at higher pH.

By the following calculation of a realistic example the importance of the ligand concentration on the determination of stability constants is shown.

In the methods of continuous variations, ion exchange, and gel permeation, rather high ligand concentrations are used, usually varying from $10^{-3} - 10^{-4}$ M. A small amount of data suggests that concentrations of HA (humic acids) and FA (fulvic acids) are indeed in this range in the soil environment (Schnitzer and Hansen 1970), but in natural waters, the concentration of complexing molecules generally is in the micro molar to sub micro molar range (van den Berg and Kramer 1979b) which is two or three orders of magnitude less. Stability constants for natural organics are mostly given as overall constants for 2:1 complexes, $\beta^\prime_2$, sometimes including competition by $H^+$, $B_2$. Also they tend to be measured at very low pH, pH 3-4, and not at constant pH. Extrapolation of the data to higher pH will most certainly bring in some errors. In Table 2.1 some data have been collected and have been adjusted to pH 7.6 assuming that from each site that binds a Cu$^{2+}$ ion one proton is released.
<table>
<thead>
<tr>
<th>ligand</th>
<th>pH of measurement</th>
<th>type of constant</th>
<th>log constant</th>
<th>type of constant</th>
<th>log constant at pH 7.6</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>4.0</td>
<td>$\beta_2'$</td>
<td>8.4</td>
<td>$\beta_2'$</td>
<td>15.6</td>
<td>Stevenson 1976</td>
</tr>
<tr>
<td>HA</td>
<td>3.5</td>
<td>$\beta_2'$</td>
<td>-2.1</td>
<td>$\beta_2'$</td>
<td>13.1</td>
<td>Stevenson et al. 1973</td>
</tr>
<tr>
<td>PA</td>
<td>3.5</td>
<td>$\beta_2'$</td>
<td>-3.6</td>
<td>$\beta_2'$</td>
<td>11.6</td>
<td>Ibid.</td>
</tr>
<tr>
<td>FA</td>
<td>3.0</td>
<td>K'</td>
<td>3.3</td>
<td>K'</td>
<td>7.9</td>
<td>Schnitzer and Hansen 1970</td>
</tr>
<tr>
<td>PA</td>
<td>5.0</td>
<td>$\beta_2'$</td>
<td>8.65</td>
<td>$\beta_2'$</td>
<td>7.1</td>
<td>Takamatsu and Yoshida 1978</td>
</tr>
<tr>
<td>FA, lake</td>
<td>8.0</td>
<td>K'</td>
<td>8.4</td>
<td>K'</td>
<td>8.0</td>
<td>Mantoura and Riley 1975</td>
</tr>
</tbody>
</table>

Takamatsu and Yoshida (1978) used as acidity constants $pK_{la}^1 = 4.9$ and $pK_{la}^2 = 9.1$. 
A very good (coincidental?) relation is apparent for the conditional stability constants observed by Schnitzer and Hansen (1970) and by Mantoura and Riley (1975), when adjusted to pH 7.6, even though they were determined at widely differing pHs. Such a correlation is indeed coincidental since at low pH possibly different sites are involved in binding than at high pH. This will be treated in Chapter 4.

The following example calculation is performed at the typical pH of natural waters, pH 7-8. Constants have been chosen as log $K' = 8$, and log $\beta'_2 = 13$. The ligand concentration will vary from $10^{-4}$ M to $2 \times 10^{-7}$ M. The fraction distribution of the total amount of ligands over CuL and CuL$_2$ is calculated. The result is plotted in Figure 2.1. It involves the following considerations:

Mass equations:

\[
\frac{[\text{CuL}]}{[\text{Cu}^{2+}].[L']} = K' \quad \frac{[\text{CuL}_2]}{[\text{Cu}^{2+}].[L']^2} = \beta'_2
\]

\[
\frac{[\text{CuL}_2]}{[\text{CuL}].[L']} = \frac{\beta'_2}{K'} = K'_2 \quad L = [L'] + [\text{CuL}] + 2 \times [\text{CuL}_2]
\]
Combination gives

\[ L = \frac{[\text{CuL}]}{[\text{Cu}^{2+}], K'} + [\text{CuL}] + \frac{2.K'_2.[\text{CuL}]^2}{[\text{Cu}^{2+}], K'} \]

Then

\[ \frac{2.K'_2}{K'.[\text{Cu}^{2+}]} [\text{CuL}]^2 + [\text{CuL}] \left( \frac{1}{[\text{Cu}^{2+}], K'} + 1 \right) - L = 0 \]

[CuL] is then calculated as a function of [Cu\(^{2+}\)] from

\[ [\text{CuL}] = \frac{-b + (b^2 - 4.\lambda.\alpha.\sigma)^{1/2}}{2.\lambda} \]

(only the positive solution has a realistic meaning)

in which

\[ \lambda = \frac{2.K'_2}{K'.[\text{Cu}^{2+}]} \]

\[ b = \frac{1}{[\text{Cu}^{2+}], K'} + 1 \]

\[ c = -L \]

The result has been plotted in Figure 2.1.
Figure 2.1 Effect of the ligand concentration on the species distribution of complexes of CuL and CuL₂.

The solid lines denote the low ligand concentration found in natural waters, the broken lines represent the concentration which frequently is used to determine stability constants.
[Cu\textsuperscript{2+}] varies from 10\textsuperscript{-9} to 10\textsuperscript{-5} M. In natural waters the Cu\textsuperscript{2+} concentration probably is less than 10\textsuperscript{-9} M, but during the analysis this is brought to the values as presented here. We see that at a total ligand concentration of 2 \times 10\textsuperscript{-7} M, the concentrations of CuL and L\textsuperscript{1} never become high enough to induce the formation of CuL\textsubscript{2}, the 2:1 complex. When L = 10\textsuperscript{-4}, however, CuL\textsubscript{2} is the most important species until a Cu\textsuperscript{2+} concentration of 10\textsuperscript{-7.5} M; at higher [Cu\textsuperscript{2+}] it still is an important species. At higher ligand concentration, [CuL\textsubscript{2}] would be even higher and it would extend to a higher [Cu\textsuperscript{2+}]. Now it becomes very understandable, that various ligand to metal ratios are observed during a titration with Cu, and, that the apparent overall stability constant varies with increasing [Cu\textsuperscript{2+}] (Takamatsu and Yoshida 1978), since at higher [Cu\textsuperscript{2+}] the stronger 1:1 complex is formed.

If β\textsubscript{2} (and K\textsubscript{2}) is higher than the value used here, similar effects will occur at lower ligand and metal concentrations.

Conclusions: both the total ligand concentration and the metal concentration are important in determining whether 1:1 or 2:1 complexes are formed;
at low ligand concentrations 2:1 complexes are less likely to be formed.

2.9 DETERMINATION OF $K'$ AND $L$ WHEN 2:1 COMPLEXES ARE FORMED

If one suspects the formation of 2:1 complexes (CuL₂) then it is still possible to come to a reasonable estimate of both stability constants and the ligand concentration, but only if one is able to continue the titration to high concentrations of Cu$^{2+}$ ions.

The following equations are used:

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

$$L = [L'] + [\text{CuL}] + [2 \text{CuL}_2]$$

$$\frac{[\text{CuL}_2]}{[\text{CuL}].[L']} = K_2'$$

$$\frac{[\text{CuL}]}{[\text{Cu}^{2+}].[L']} = K'$$

Approximations:

low [Cu$^{2+}$]: $L = [L'] + 2[\text{CuL}_2]$

high [Cu$^{2+}$]: $L = [L'] + [\text{CuL}]$
Firstly the data for high $[\text{Cu}^{2+}]$ are used:

Approximately $[\text{CuL}] \gg [\text{CuL}_2]$, so \[ \frac{[\text{CuL}]}{[\text{Cu}^{2+}]} \gg \frac{[\text{CuL}_2]}{[\text{Cu}^{2+}]} \]

Then \[ \frac{[\text{CuL}]}{[\text{Cu}^{2+}]} = K' (L - [\text{CuL}]) \]

Conversion gives \[ \frac{[\text{Cu}^{2+}]}{[\text{CuL}]} = \frac{1}{K' \cdot L} + \frac{[\text{Cu}^{2+}]}{L} \]

and a plot of \[ \frac{[\text{Cu}^{2+}]}{[\text{CuL}]} \text{ vs. } [\text{Cu}^{2+}] \] has a slope equal to \[ \frac{1}{L} \]

and a $Y$-intercept equal to \[ \frac{1}{K' \cdot L} \].

Secondly the low $[\text{Cu}^{2+}]$ data are used:

\[ \frac{[\text{CuL}_2]}{[\text{CuL}]} = K'_2 (L - 2[\text{CuL}_2]) \]

conversion:

\[ \frac{[\text{CuL}]}{[\text{CuL}_2]} = \frac{1}{L} \cdot \frac{1}{K'_2} + \frac{\text{CuL}}{L/2} \]

Now $L = 2 \times \frac{1}{Y$-intercept$}$ and $K'_2 = \frac{slope \times 2}{Y$-intercept$}$
Only for a ligand with high stability constants is one able to reached sufficiently high \([\text{Cu}^{2+}]\) so that previous approximations will be valid.

2.10 EFFECTS OF TEMPERATURE AND IONIC STRENGTH

For comparative purposes, measurements are performed at constant temperature and ionic strength. In the natural environment, temperature and ionic strength vary and it is important to assess the effect of these variations for the real world.

The discussion is complicated by the competition for complexation sites by protons. Any temperature, or ionic strength, related effect on the stability constants for copper complexes, will also be valid, to some degree, for the proton complexes. When the affinity of the ligand increases for the one, so will it for the other, though maybe not in the same amount. So the result will be that the changes in the two stability constants, for the proton and the cupric ion, may at least partially cancel each other.

TEMPERATURE EFFECTS

For isothermal reactions
\[ \Delta G = \Delta H - T \Delta S \]  

(39)

\( \Delta G \) is the change in free energy, \( \Delta H \) is the change in enthalpy, \( T \) is the absolute temperature, and \( \Delta S \) is the change in entropy of the reaction. The relation between the free energy of reaction and a stability constant, \( K \), is given by

\[ -\Delta G = R \cdot T \ln K \]  

(40).

The free energy of reaction is affected by a change in temperature depending on the entropy change of reaction

\[ \left( \frac{\delta \Delta G}{\delta T} \right) = -\Delta S \]  

(41)

For entropy driven reactions \( \Delta S \) is positive, and an increase in temperature produces a decrease in free energy. The relation (40) between the stability constant, \( K \), and the free energy includes the temperature as well, so that the final effect on \( K \) depends on the magnitude of \( \Delta S \). Since we do not know \( \Delta S \) we cannot predict the effect of temperature on \( K \).

In Table 2.2 acidity constants, stability constants for Cu complexation, and conditional stability constants, calculated for pH 8.0 at different temperatures, have been
Table 2.2  Effect of temperature on stability constants and conditional stability constants of complexes of Cu\(^{2+}\) with glycine and NTA

<table>
<thead>
<tr>
<th></th>
<th>Ka(_1) H L</th>
<th>K CuL</th>
<th>K(_2) CuL(_2)</th>
<th>K(_1)'</th>
<th>K(_2)'</th>
<th>temp. (^\circ)C</th>
<th>(\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>10.20</td>
<td>8.85</td>
<td>7.36</td>
<td>6.65</td>
<td>5.16</td>
<td>10</td>
<td>+0</td>
</tr>
<tr>
<td></td>
<td>9.78</td>
<td>8.58</td>
<td>7.09</td>
<td>6.80</td>
<td>3.31</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.41</td>
<td>8.42</td>
<td>6.58</td>
<td>7.01</td>
<td>5.17</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>10.39 +0</td>
<td>13.21</td>
<td>0.1 M KNO(_3)</td>
<td>10.82</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.33</td>
<td>13.16</td>
<td></td>
<td>10.83</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.28</td>
<td>13.10</td>
<td></td>
<td>10.82</td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.23</td>
<td>13.15</td>
<td></td>
<td>10.92</td>
<td></td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Intermediate temperatures between given values were found by graphical extrapolation.

Acidity constants and stability constants from Sillen and Martell (1964).
collected for glycine and NTA. It appears that for NTA the variations of the acidity constant and the stability constant with the temperature cancel each other completely, when the conditional stability constants, \( K'_1 \), are calculated. For glycine a resulting variation remains, in \( K'_1 \) more than in \( K'_2 \). Additionally, measurements of conditional stability constants, \( K'_1 \), for the strontium-citrate complex showed that \( K'_1 \) does not change, within experimental error (Schubert 1952).

**Conclusion:** Experimental data confirm that temperature dependent variations in stability and acidity constants cancel each other partially, and sometimes completely, when conditional stability constants are calculated.

IONIC STRENGTH EFFECTS

The ionic strength affects the activity of the ions. In freshwater environment and in the experimental set-up (described in Chapter 5) the ionic strength is relatively quite low, 0.01, so that the activities change following a predictable, and negligible, manner. There is a second effect, however, which can have stronger repercussions. The ligands in natural waters may be of a very complicated nature, and have molecular weights of moderate, \( 10^3 \), to
large, $10^5$, size. Such molecules can behave as charged particles in that the double layer becomes suppressed in increasing ionic strength. Also, the configuration may change, or the form of the complex may be affected. Indeed, very strong effects have been noted on stability constants when the ionic strength is varied. In Table 2.3 some stability constants, each determined by the same method at different ionic strengths have been calculated.

Since $K'$ is a constant for the reaction between a metal ion and charges on a colloidal particle, $K'$ should vary with the degree of neutralization and the ionic strength to the same extent as the acidity constants, but in the opposite direction. On the other hand $B$, which is essentially the product of $K'$ and $K_a$, should be independent of ionic strength (Gregor et al. 1955). This is indeed apparent in the data of Coleman et al. (1956).

The conditional stability constant, $K'$, for the complex of strontium with citrate, molecular weight only 192, is quite considerably affected by the ionic strength (Schubert 1952). The extrapolated value of $K'$ at infinite dilution agrees satisfactorily with the value calculated from the individual ion activity coefficients (Schubert 1952). This indicates that the change in $K'$ with the ionic strength
Table 2.3  Effect of ionic strength on stability constants of copper and strontium complexes with some organic ligands

<table>
<thead>
<tr>
<th>ligand</th>
<th>$pK_a$</th>
<th>n</th>
<th>stability constant</th>
<th>ionic strength</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\log \beta^2$</td>
<td>$B_2$</td>
<td></td>
</tr>
<tr>
<td>peat/Cu$^{2+}$</td>
<td>5.5</td>
<td>2.2</td>
<td>7.9</td>
<td>2.5</td>
<td>0.01 M KNO$_3$</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>2.2</td>
<td>6.9</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>2.2</td>
<td>6.2</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>log $K'$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA/Cu$^{2+}$</td>
<td>1</td>
<td>4.7</td>
<td>0.00 M KCl</td>
<td></td>
<td>Schnitzer and Hansen 1970</td>
</tr>
<tr>
<td>pH 3.0</td>
<td>1</td>
<td>3.3</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.6</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate/Sr$^{2+}$</td>
<td>2.82</td>
<td></td>
<td>0.16</td>
<td></td>
<td>Schubert 1952</td>
</tr>
<tr>
<td>pH 7.2-7.3</td>
<td>2.99</td>
<td></td>
<td>0.076</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>3.36</td>
<td></td>
<td>0.039</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.41</td>
<td></td>
<td>0.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.57</td>
<td></td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.67</td>
<td></td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $\beta^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil HA/Cu$^{2+}$</td>
<td>7.22</td>
<td></td>
<td>0.1 M KCl</td>
<td></td>
<td>Stevenson 1977</td>
</tr>
<tr>
<td>pH 4-5</td>
<td>7.87</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA/Cu$^{2+}$</td>
<td></td>
<td></td>
<td>log $\beta^2=8.9-4.9.(\mu)^{0.5}$</td>
<td>KCl</td>
<td>Stevenson 1976</td>
</tr>
<tr>
<td>pH 4.9, 25°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
is entirely due to the effect that the ionic strength has on the individual activity coefficients of the citrate and strontium ions. It should be noted that when the ionic strength is 0.01, extrapolation to infinity increases $K'$ by a factor of 2.8 in the case of strontium and citrate. The measurements reported in this dissertation are performed at an ionic strength of 0.01 M KNO$_3$.

Stevenson (1976) also showed that the $pK_a$ of HAs increases slightly with decreasing concentration of HA. In his determinations $B_2$ appears to vary slightly with the ionic strength: $\log B_2 = -1.5$ when $\mu = 0.01$, and $\log B_2 = -1.7$ when $\mu = 0.1$, at pH 4. But he emphasizes that results obtained at lower ionic strength are subject to error, because small changes in salt concentration can have a significant effect on the ionization of COOH groups.

It is important to consider that the concentration of the ions used to increase the ionic strength is higher than the metal and ligand concentration. At very high ionic strength even weak interactions between these ions and the ligands or the metal ions may effect a decrease in $K'$, difficult to discern from an ionic strength effect.
2.11 SUMMARY

After a recapitulation of complexation theory, a theory is developed for the determination of conditional stability constants for mono- and bidentate complexes and for 2:1 complexes (CuL₂) by the MnO₂ method (experimental description in Chapter 5). This theory can be applied in principle to other ion exchange methods that make use of weak ion exchange media with an adsorption behavior of metal ions similar to Langmuir adsorption. From this theory and from thermodynamic considerations it has been deduced that:

- titration with Cu²⁺ of a single ligand in presence of MnO₂ can be graphically represented by a straight line; slope and intercept with the y-axis provide information on the conditional stability constant and the ligand concentration;

- the presence of a second ligand (or site) will produce a knick in the straight line; the change in the slope depends on the concentration of the second ligand; if the two ligands differ sufficiently in complexing ability, the conditional stability constant for Cu²⁺ can be obtained for each ligand by one titration with Cu²⁺ at fixed pH;

- the concentration of a ligand will be overestimated by the MnO₂ method, if a second ligand is present but not
noticed; this overestimation partially makes up for the loss of the second ligand in calculations and a calculated $[\text{Cu}^{2+}]$ differs very little from the real $[\text{Cu}^{2+}]$;

- any change in the ligand concentration may alter the ligand-to-metal ratio when there is a tendency to form complexes other than 1:1; therefore it is advisable to determine complexation at the low ligand concentration present in natural waters;

- increases or decreases of conditional stability constants as result of variations in temperature or ionic strength are partially cancelled by similar effects on acidity and stability constants.
True security of the individual does not lie in isolated personal efforts but in general human solidarity.

F.M. Dostoyevski: The Brothers Karamazov
CHAPTER 3

ORGANIC AND INORGANIC MATTER IN THE NATURAL
ENVIRONMENT AND INTERACTIONS WITH METAL IONS

3.1 SURFACES IN NATURAL WATERS

Surfaces of oxides and of organical molecules get
electrical charge due to ionization in water, and can become
sites for binding for dissolved ions. The following
discussion is divided into organic matter and inorganic
matter.

3.2 ORGANIC MATTER IN NATURAL WATERS

The amount of dissolved organic carbon (DOC) in
water is huge, being about ten times greater than the esti-
mated world oil reserves (see Table 3.1). The amount of
particulate organic carbon (POC) is two orders of magnitude
less, and the amount of carbon in planktonic organisms again
Table 3.1 World quantities of organic carbon

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of dissolved organic matter in world ocean, assuming</td>
<td>$1-2 \times 10^{18}$ g C</td>
<td>(le Williams 1975, p.303)</td>
</tr>
<tr>
<td>700 $\mu$g C.L$^{-1}$ (le Williams) and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 mg L$^{-1}$ (Duursma and Marchand 1974) and volume $1.369 \times 10^9$ km$^3$</td>
<td></td>
<td>(Bowden 1965)</td>
</tr>
<tr>
<td>Particulate organic carbon,</td>
<td>$1.4-3 \times 10^{16}$ g C</td>
<td></td>
</tr>
<tr>
<td>10 $\mu$g C.L$^{-1}$ (Gordon 1971, Parsons 1975) to 20 $\mu$g L$^{-1}$</td>
<td></td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td>Plankton</td>
<td>$5 \times 10^{14}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td>Total amount of hydrocarbons, assuming ultimate estimated oil reserves of $1.7 \times 10^{12}$ barrels (Tiratso 1976) and assuming 40% C in oil</td>
<td>$2 \times 10^{17}$ g C</td>
<td></td>
</tr>
<tr>
<td>Annual inputs into oceans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net primary productivity</td>
<td>$3.6 \times 10^{16}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td>assuming: 100 g C fixed m$^{-2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 g C fixed m$^{-2}$.yr$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rain 1 mg C.L$^{-1}$</td>
<td>$2.2 \times 10^{14}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td>rivers 5 mg C.L$^{-1}$</td>
<td>$1.8 \times 10^{14}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
</tbody>
</table>
Table 3.1 continued

Into dissolved fraction:

phytoplankton excretions
assuming 10% of assimilation excreted
Resistant material from phytoplankton
Hydrocarbons from tankers, etc.

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides and PCB's</td>
<td>$3.6 \times 10^{15}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td></td>
<td>$1.8 \times 10^{15}$ g C</td>
<td>(le Williams 1975)</td>
</tr>
<tr>
<td></td>
<td>$10^{12}$ g C</td>
<td>(Reed 1972)</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{11}$ g</td>
<td>(Duursma and Marchand 1974)</td>
</tr>
</tbody>
</table>
is one to two orders of magnitude less.

Organic matter in water is partially land derived and partially produced in situ by primary production.

3.3 LAND DERIVED ORGANIC MATTER

Humic acids are formed from a combination of decomposed plant material tissues and metabolic products of microorganisms (Kononova 1973). Lignin is formed from plants on land, ultimately from CO₂, via carbohydrates, aromatization, dimerization and polymerization (Nord 1964); see Table 3.2. Lignin serves to give the plants strength. It is difficult to break it down. During the decomposition with help of microorganisms, the lignin is transformed into humic acids (HA); see Table 3.3. The nitrogen content increases, and the methoxyl content decreases. It is thought that polymerization is formed simultaneously with degradation of the side chain of lignin. Cross-linkage of the polymers through the side chains decreases with continued decomposition, and linkage of the rings takes place to an increasing degree. Increasing demethylation enables increasing condensation with nitrogen-containing compounds (Flaig 1964).
Table 3.2 Photosynthesis

- CO₂
  carbohydrates
  aromatization
  dimerization
  polymerization
- Lignin abstracted from Nord (1964)

Table 3.3 Decomposition

- Lignin
  polymerization
  degradation of side chain
  cross linkage
  linkage of rings
  decrease in nitrogen
  condensation
- Humin abstracted from Flaig (1964)
  microbial degradation
  decrease of molecular weight
- Humic Acids
  increase in carboxyl groups
  decrease of molecular weight
- Fulvic Acids abstracted from Schnitzer and Khan (1972)
It is not sure what is formed first. One theory is that the higher molecular weight HAs and humic fractions represent the first stages of humification, before further degradation into FA and ultimately to CO₂ and H₂O. This can take a very long time, however. Radiocarbon dates (Campbell et al. 1967) varied from 1400 years, for very stable Ca-humates, to 25 years, for labile HA hydrolysates which consisted of amino acids, peptides, carbohydrates, etc.; mobile HA was intermediate (780 years) and FA was younger (550 years). Thus material can accumulate during hundreds and even thousands of years (Bada and Lee 1977).

A rather different pathway is mentioned by Stevenson and Goh (1971), in that FAs represent the initial products of humification and that further condensation results in the formation of humic acids and ultimately coal. Lignin is still considered to be the source of building blocks. The pathways may be partially reversible, depending on the presence of an oxidative or reductive environment. In reducing environments high concentrations of stable free radicals are formed from FA, in powder form and in aqueous solution (Senesi and Schnitzer 1977); their presence increases going from lignin to HA (Steelink 1964). Irradiation of FA with white light increases reversibly the concentration of
free radicals (Senesi and Schnitzer 1977). These free radicals are excellent sites for linking of molecules. Conceivably this could be a pathway along which FAs are converted into HAs. Processes going in the reverse direction can be deduced from the use of FA by bacteria (e.g. de Haan 1975).

The different forms of humic matter are subdivided according to their solubility, and extractability, in acids and bases. HAs are soluble in weakly alkaline environment, but precipitate in acid. FAs are soluble both in acid and base. The substance which can not be extracted by acid or base, is called Humin (Schnitzer and Khan 1972).

The FAs are the most labile and soluble and will be flushed out most easily by groundwater (Schnitzer 1971), so land-derived humic material in natural waters is most likely FA. This has been confirmed by de Haan (1975). He extracted humic matter from a lake in the Netherlands, and this was completely soluble after acidification to pH 1.0. The classification into HAs and FAs using acidification is very arbitrary. It appears that the fractions are still molecularly heterogeneous, and are structurally similar to each other, but they differ in molecular weight and functional group content (Schnitzer and Khan 1972, p. 3).

It is very difficult to discriminate between humic
substances from different sources. Combination of age measurements with spectrophotometric (visible and UV) measurements may be used to distinguish between HAs derived from different soils (Campbell et al. 1967). Also comparisons of elementary composition, using IR spectra, and fractionation by gel permeation have been made (Kononova 1973). Even to distinguish between land-derived and water-produced humic matter appears to be very difficult. Chapter 3.5 discusses how minimal the differences are between humic substances from different sources and even between HAs and FAs.

3.4 ORGANIC MATTER DERIVED FROM PLANKTONIC ORGANISMS

In a number of cases it has been shown that algae, and sometimes zooplankton excrete organic matter. The measurements are difficult, however, since it is easy to damage the cell walls during the separation of the dissolved and particulate phases.

There has been a difference of opinion on this subject. Sharp (1977) claimed that practically all measured
<table>
<thead>
<tr>
<th>Tested algal group</th>
<th>Excretion products</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ochromonas danica</td>
<td>ascorbate, biotin, vit. B₆, thiamine, nicotinate</td>
<td>Aaronson et al. 1971</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>folate, biotin, pantothenate</td>
<td>Pogg et al. 1965</td>
</tr>
<tr>
<td>Scenedesmus obliquus</td>
<td>pyridoxal carboxylic acid, amino acids: ala, asp, citr, ser, fum, glt, glycerol, gly+</td>
<td>Jütten and Friz 1974</td>
</tr>
<tr>
<td>Anabaena cylindrica</td>
<td>mainly glycolic acid, hydroxamate chelators</td>
<td>Murphy et al. 1976</td>
</tr>
<tr>
<td>Ochromonas malhamensis</td>
<td>glycolic acid, 14C</td>
<td>Nalewajko 1966</td>
</tr>
<tr>
<td>Mixed culture of lake</td>
<td>pyridoxal carboxylic acid, amino acids: ala, asp, citr, ser, fum, glt, glycerol, gly+</td>
<td>Nalewajko et al. 1963</td>
</tr>
<tr>
<td>Ochromonas danica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella</td>
<td>phosphatase, proteins, carbohydrates, no lipids or nucleic acids</td>
<td>Patni et al. 1977</td>
</tr>
<tr>
<td>Various (16 Chlorophyceae, 7 Bacillariophyceae, 1 Cyanophyceae)</td>
<td></td>
<td>Penhale and Smith 1977</td>
</tr>
<tr>
<td>Zostera marina (eelgrass)</td>
<td>ectocrine of tanoid nature</td>
<td>Pratt 1966</td>
</tr>
<tr>
<td>Oligoschides luteus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested algae</td>
<td>Excretion products</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Skeletonema</td>
<td>Cu$^{2+}$ complexing ligands</td>
<td>Stolzberg and Rösin 1977</td>
</tr>
<tr>
<td>Mixed culture of lake</td>
<td>$^{14}$C</td>
<td>Storch and Saunders 1978</td>
</tr>
<tr>
<td>Gloeocystis gigas and other species</td>
<td>only Gloeocystis: complexing</td>
<td>Swallow et al. 1978</td>
</tr>
<tr>
<td>Mixed marine</td>
<td>ligands</td>
<td>Thomas 1971</td>
</tr>
<tr>
<td>Mixed estuarine</td>
<td>$^{14}$C</td>
<td>Wiebe et al. 1977,</td>
</tr>
</tbody>
</table>
excretions were due to cell rupture, while others claimed that certain molecules were excreted and others not (Aaronson 1978; Fogg 1977). In Table 3.4 a listing is given of excretion products and the species that excreted them. The amounts which are excreted vary and seem to depend on the density of the organisms present. At lower density, relatively more is excreted (Anderson and Zeutschel 1970; Nalewajko et al. 1963). In estuaries <7%, in coastal surface waters <13%, coastal below surface waters <21%, and in the Sargasso sea <44% of the production is excreted (Thomas 1971). Generally 7-50% of total carbon fixed is being excreted (Fogg et al. 1965; Fogg 1977), which is an amazing quantity indeed. It seems a rather wasteful habit! This is further discussed, in more detail, in Chapter 8, which also describes some experiments with algal culture filtrates.

3.5 COMPOSITION OF HUMIC SUBSTANCES

Many different methods have been attempted to get an idea of the very complex nature of humic substances. A review is given in Table 3.5, where the sometimes conflicting
Table 3.5 Classification and characterization of humic material in soils, sediments and waters

<table>
<thead>
<tr>
<th>Class</th>
<th>Recognized groups</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA, soil</td>
<td>acidic polysaccharides, peptide components, carboxylic</td>
<td>IR, chemical</td>
<td>Anderson and</td>
</tr>
<tr>
<td></td>
<td>groups</td>
<td>degradation</td>
<td>Hepburn 1977</td>
</tr>
<tr>
<td>FA, HA</td>
<td>E₄/E₆ ratios not related to concentration of condensed</td>
<td>spectrophotometry</td>
<td>Chen et al. 1977</td>
</tr>
<tr>
<td></td>
<td>rings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA, FA</td>
<td>E₄/E₆ ratio is correlated to degree of condensation of</td>
<td></td>
<td>Kononova 1966</td>
</tr>
<tr>
<td></td>
<td>aromatic C network</td>
<td></td>
<td></td>
</tr>
<tr>
<td>humic matter</td>
<td>saturated and unsaturated sterols in plankton and</td>
<td>GLC and MS</td>
<td>Nishimura and Koyama</td>
</tr>
<tr>
<td></td>
<td>sediments</td>
<td></td>
<td>1977</td>
</tr>
<tr>
<td>HA, lake</td>
<td>Chlorophyll remnants, lipids</td>
<td>extraction, gas</td>
<td>Povolo et al. 1972</td>
</tr>
<tr>
<td>sediments</td>
<td></td>
<td>chromatography</td>
<td></td>
</tr>
<tr>
<td>HA, FA</td>
<td>decrease in COOH content with increasing molecular</td>
<td>IR</td>
<td>Stevenson and Goh 1971</td>
</tr>
<tr>
<td></td>
<td>weight and humification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>humic matter</td>
<td>algae products: more aliphatic, ESR, stable N-rich</td>
<td></td>
<td>Stuermer et al. 1978</td>
</tr>
<tr>
<td></td>
<td>terrestrial: more aromatic humic matter</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5 continued

<table>
<thead>
<tr>
<th>Class</th>
<th>Recognized groups</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA, FA</td>
<td>FA/HA ratios are changed by the environment</td>
<td>IR</td>
<td>Tan 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tan 1977</td>
</tr>
<tr>
<td>HA, FA</td>
<td>molecular weight: FA: 30,000, 15,000 and &lt;15,000; HA:</td>
<td>gel permeation</td>
<td>Tan and Giddens 1972</td>
</tr>
<tr>
<td></td>
<td>&gt;30,000 and &lt;15,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>molecular weight FA: 951</td>
<td>vapor pressure osmometry</td>
<td>Hansen and Schnitzer 1969</td>
</tr>
</tbody>
</table>
results of these investigations are given. Use of mass spectrometry breaks the complex molecules up and renders little pieces. Combination of destructive (mass spectrometry) with non-destructive (e.g. I.R. spectroscopy) methods is necessary to try to elucidate the problem. Simulation of processes which may occur in the natural environment has been performed by creating rather weakly oxidizing or reducing circumstances. Changes in the molecules are extrapolated to explain the formation and breakdown of humic substances in nature (Schnitzer and Ortiz de Serra 1973; Neyroud and Schnitzer 1977).

The very nature of extraction used to concentrate organic matter from water and soil, necessarily introduces artifacts. Organical solvents, sodium hydroxide or phosphate solutions, extract mixtures of molecules, which may well form their linkages in the concentrated environment of the extract.

Generally, HA and FA have been shown to be composites of lipids, saccharides, amino acids, phenol groups and carboxyl groups, with (e. g. Kononova 1966) or without (Wilson and Goh 1977) an aromatic core. The molar ratio of amino acids to FA and HA is very high. Analyses of HA and FA isolated from soils, lake sediments or lake waters.
Table 3.6  Relative molar percentages of amino acids in humic matter

<table>
<thead>
<tr>
<th></th>
<th>HA soil¹</th>
<th>lake sed.²</th>
<th>HA lake sed.²</th>
<th>FA lake sed.²</th>
<th>FA soil³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARG</td>
<td>2.6</td>
<td>3.5</td>
<td>3.3</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>HIS</td>
<td>2.0</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>LYS</td>
<td>3.8</td>
<td>5.6</td>
<td>4.7</td>
<td>3.8</td>
<td>2.1</td>
</tr>
<tr>
<td>ORN</td>
<td>-</td>
<td>0.9</td>
<td>1.0</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>total</td>
<td>8.4</td>
<td>11.3</td>
<td>10.2</td>
<td>6.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Acidic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td>10.5</td>
<td>9.9</td>
<td>12.4</td>
<td>14.4</td>
<td>12.9</td>
</tr>
<tr>
<td>GLU</td>
<td>8.4</td>
<td>9.1</td>
<td>8.4</td>
<td>10.0</td>
<td>9.1</td>
</tr>
<tr>
<td>total</td>
<td>18.9</td>
<td>19.0</td>
<td>20.8</td>
<td>24.4</td>
<td>22.0</td>
</tr>
<tr>
<td>Neutral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THR</td>
<td>5.4</td>
<td>6.9</td>
<td>7.5</td>
<td>7.9</td>
<td>6.8</td>
</tr>
<tr>
<td>SER</td>
<td>3.9</td>
<td>9.7</td>
<td>8.6</td>
<td>8.5</td>
<td>6.0</td>
</tr>
<tr>
<td>PRO</td>
<td>5.8</td>
<td>2.2</td>
<td>7.0</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>GLY</td>
<td>9.8</td>
<td>17.5</td>
<td>11.8</td>
<td>12.8</td>
<td>13.8</td>
</tr>
<tr>
<td>ALA</td>
<td>9.8</td>
<td>11.0</td>
<td>10.0</td>
<td>10.7</td>
<td>10.8</td>
</tr>
<tr>
<td>VAL</td>
<td>6.3</td>
<td>5.6</td>
<td>6.0</td>
<td>5.7</td>
<td>6.7</td>
</tr>
<tr>
<td>ILEU</td>
<td>5.0</td>
<td>3.3</td>
<td>3.9</td>
<td>3.7</td>
<td>3.8</td>
</tr>
<tr>
<td>LEU</td>
<td>6.9</td>
<td>5.6</td>
<td>5.4</td>
<td>5.3</td>
<td>2.7</td>
</tr>
<tr>
<td>TYR</td>
<td>1.1</td>
<td>1.5</td>
<td>3.2</td>
<td>3.1</td>
<td>0.8</td>
</tr>
<tr>
<td>PHE</td>
<td>3.2</td>
<td>3.1</td>
<td>3.4</td>
<td>2.9</td>
<td>1.9</td>
</tr>
<tr>
<td>total</td>
<td>57.2</td>
<td>66.4</td>
<td>66.7</td>
<td>64.6</td>
<td>58.5</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total mg/g</td>
<td>201</td>
<td>21.5</td>
<td>214.5</td>
<td>126.1</td>
<td></td>
</tr>
<tr>
<td>Glucosamine mg/g</td>
<td>0.3</td>
<td>1.94</td>
<td>7.9</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Galactosamine mg/g</td>
<td>0.6</td>
<td>1.15</td>
<td>11.4</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>
Notes to Table 3.6

1 Anderson and Hepburn 1977. These data have been converted to relative molar percentages. The total amount of amino acids in mg/g is calculated by using an overall estimated molecular weight of 150. The data were converted from N-molar basis to amino acid molar basis.


3 Schnitzer and Khan 1972, p.35.
show molar ratios of around 1/2 (mole amino acids/mole FA). This has been calculated from the data of Table 3.6, using an average molecular weight of 150 for the amino acids, and of 2000 for the FA. For a HA with a molecular weight of 20,000 this ratio would be 1/10 (mole amino acids/mole HA).

The molar percentages of the individual amino acids are quite similar for HAs and FAs from different sources (see Table 3.6), which might indicate a structural relationship between the FA and HA molecules. Products of marine algae have been shown to be slightly different, especially by their distribution of less-acidic amino acids, but neutral and acidic amino acids remain dominant (Pelet and Debyser 1977).

Due to their large molecular weight and partially aliphatic structure, humic matter is quite apolar and hydrophobic. The presence of electrically charged groups, especially the carboxyl groups in FAs (Stevenson and Goh 1971; Anderson et al. 1977), increase the water solubility and can form bonding via Coulombic interaction. Therefore, surfaces of less polar nature than water, and especially with electrically charged groups of charge opposite to that on the humic matter, may well interact with dissolved HAs and FAs by adsorbing them using van der Waal's and Coulombic
attractions. These interactions are treated in section 3.8.

3.6 NATURE OF THE GROUPS RESPONSIBLE FOR COMPLEXING TRANSITION METALS

The sites that are responsible for binding transition metal cations will have to be negatively charged or they must have the ability to ionize readily into negatively charged sites in the presence of positive metal ions. These sites have to compete with water molecules and other ligands, so it will certainly be to their advantage when sites are further along the spectrochemical series than other competing complexing sites, or to be positioned in such a way in the structure of a molecule that two or more groups can cooperate in forming a bidentate or polydentate complex. Amine, ethylene diamine (in EDTA), and NO₂⁻ are further along the spectrochemical series than OH⁻ and H₂O and of possible importance to natural ligands. Complexation by natural organics, however, has never been correlated to such groups.

By subsequently blocking different groups which were thought to be responsible for binding, and by measuring the metal retention the following complexing groups have been
discerned (Schnitzer and Skinner 1965): acidic carboxyl
groups and phenolic hydroxyl groups, and to a smaller
degree, less acidic carboxyls; alcoholic hydroxyls do not
participate in the organo-metallic reactions. Blocking of
either phenolic hydroxyls or of carboxyls resulted in a
similar effect, suggesting that the complexes are formed
following a salicylic type of complexes. It should be noted,
however, that due to the very complex nature of humic matter,
changes in the molecular structure may well have occurred as
a result of the treatment to block reactive groups which
were not accounted for. Metal retention capacity was deter-
mined and compared with that of the untreated sample, but
stability constants were not determined. Vinkler et al
(1976) confirmed more recently by similar work that COOH
groups are mainly responsible for binding of metal ions.
Apparently, depending on ionic strength and pH, metal ions
are bound, either by the combined action of carboxyl groups
and phenolic hydroxyl groups in ortho position to the carboxyl
groups (Gamble et al. 1970), or by COOH groups alone or in
pairs (Stevenson et al. 1973), originating from one molecule
or two. Indeed, two different binding sites have been
observed on FA from soil, the one having a much smaller condi-
tional stability constant than the other for complexes
with copper ions (Bresnahan et al. 1978). Also iron ions are bound by two binding sites, one producing strongly bound tetra and octahedral Fe$^{3+}$, the other adsorbing Fe$^{3+}$ superficially (Senesi et al. 1977).

Cooperation of 2 FA or HA molecules in forming 2:1 complexes has sometimes been shown to occur (Buffle et al. 1977; Stevenson 1977). For FA from natural waters both the presence of two binding sites with different stability constants (Mantoura and Riley 1975) and the formation of 2:1 complexes (Buffle et al. 1977) have been claimed. It is difficult to discern between these two possibilities, however, and Scatchard plots alone as used for example by Mantoura and Riley (1975) are not sufficient to make the decision, unless the molecular weight of the ligand is known (Chapter 2.7).

Carboxyl groups are much more acidic than the phenolic hydroxyl groups. But acidity constants are determined more readily at low pH than at high pH; at high pH, one has to bother about the exclusion of carbonate from the solution. So acidity constants for the carboxyl groups have more readily been determined: in 0.1 M KCl and pH 3, $pK_a = 3.56$ and 4.90 for two different types of carboxyl groups in FA (Gamble 1970).
These acidity constants vary with the pH. More recently much larger acidity constants have been determined. These acidity constants, $pK_{a_2}$, vary from 8.80 to 9.40 for HAs from eleven different sources, determined at pH varying from about 7.3-10.0 (Takamatsu and Yoshida 1978). This range of acidity constants constitutes the larger $pK$ values of two sets for each HA, while the smaller $pK_{a_1}$ values range from 4.6-5.1. One could imagine that at pHs greater than the more acidic acidity constants of carboxyl groups ($pH > pK_{a_1} = 4.6-5.1$), the conditional stability constants, $K'$, for complexes with metal ions, would be independent of the pH, if only carboxyl groups are involved in the formation of complexes. When both phenolic hydroxyl groups and carboxyl groups are involved, $K'$ will vary with the pH until the pH is larger than $pK_{a_2}$, pH $> 9.4$. Such a simplification may not be sufficient to explain the behavior of stability constants. The logarithm of the conditional stability constants has been observed to vary with the pH, but on less than a 1:1 basis (Takamatsu and Yoshida 1978; Schnitzer and Hansen 1970). In addition to the competing effect of protons for complexing sites on the molecules, the overall electrical surface charge is affected by the presence of potential determining ions, such as protons and other metal ions.
resulting in a change in the surface potential. This surface potential in turn affects both the acidity and the stability constants for complexes with metals.

The number of carboxyl groups per FA is larger than per HA (Schnitzer and Desjardins 1962) and the carboxyl group content decreases progressively with increasing molecular weight (Stevenson and Goh 1971). There are about 3.1 meq carboxyl groups which are thought to be in the ortho position to hydroxyl groups per gram FA. In addition, there are about 7.7 meq of other carboxyl groups (Gamble 1970). It is possible, however, that the number of carboxyl groups has been overestimated in certain cases, since Perdue (1979) found that FA and HA have similar concentrations of carboxyl groups. He found a considerable amount of phenolic hydroxyl groups in river water humics, slightly more than in soil HAs, but generally less than the concentration of carboxyl groups. Anderson et al. (1977) found an abundance of carboxyl groups in FAs but not many phenolic compounds.

From possible binding sites I would at present certainly not exclude NH₂ groups, which have rather been neglected in the literature. Acidity constants, pKₐ, for such groups on amino acids are in the neighborhood of 9.7 (glycine, glutamic acid), 9.8 (alanine) and higher, such
as 10.4 (cysteine). The stability constants for the NH$_3$ complexes with copper start from 10$^{4.1}$, a bit below OH$^-$, though N-groups are actually further down the spectrochemical series and produce a stronger ligand field effect. The negative charge on a hydroxyl group will favor it over the neutral NH$_2$ group, but once Cu$^{2+}$ has been neutralized by a carboxyl group, this advantage is minimized and amine groups might then be good sites for binding. In FAs and HAs large quantities of amino groups are always present (Chapter 2), even in molar ratios around 1. I do not think that (a) anybody has been able to remove amino acids from FAs and HAs without removing their essential groups, and (b) anybody has shown convincingly why amino acids should not be involved in binding heavy metals.

**Comparison of conditional stability constants for the formation of complexes of natural ligands and copper ions**

For comparative purposes the conditional stability constants for copper-humic matter complexation have been collected in Table 3.7. In a few cases sufficient data were available to calculate conditional stability constants from stability and acidity constants provided for the same ligand
| 1. seawater | 7.46 | 8.2 |  |  |  |  |  |  | Branica 1978 |
| 2. FA water | 6.1 | 6.0 |  |  |  |  |  |  | Bresnahan et al. 1978 |
| K₂ | 3.8 |  |  |  |  |  |  |  |  |
| FA soil | 6.3 |  |  |  |  |  |  |  |  |
| K₂ | 3.8 |  |  |  |  |  |  |  |  |
| 3. FA lake | 5.0 | 9.5 | 6.0 |  |  |  |  |  | Buffle et al. 1977 |
|  | 4.8 | 10.1 |  |  |  |  |  |  |  |
| 4. Peat | 5.4* | 5.5 | 0.20 | 0.40 | 7.9 |  |  |  | Coleman et al. 1956 |
| 5. FA soil |  |  | 3.5 |  |  |  |  |  | Courpron 1967 |
| HA soil | 7.0 | 5.0 |  |  |  |  |  |  | Geering and Hodgson 1969 |
| 6. HA soil | 5.6 | 7.0 |  |  |  |  |  |  | Guy and Chakrabarti 1976 |
| 7. HA soil | 6.20 | 6.8 |  |  |  |  |  |  | Mantoura and Riley 1975 |
| (2) | 5.08 |  |  |  |  |  |  |  |  |
| 8. FA lake | 8.4 | 8.0 |  |  |  |  |  |  | Schnitzer and Hansen 1970 |
| FA peat | 7.85 |  |  |  |  |  |  |  |  |
| 9. FA soil | 3.3 | 3.0 |  |  |  |  |  |  | Stevenson et al. 1973 |
|  | 4.0 | 5.0 |  |  |  |  |  |  |  |
| 10. HA soil | 3.9* | 5.25 | -1.05 |  |  |  |  |  |  |
| HA peat | 3.7* | 5.32 | -1.28 |  |  |  |  |  |  |
| FA soil | 2.7* | 4.85 | -1.80 |  |  |  |  |  |  |
Table 3.7 continued

<table>
<thead>
<tr>
<th>ligand</th>
<th>log $K'$</th>
<th>log $\beta'_2$</th>
<th>pH</th>
<th>log $B$ &amp; log $B_{ave}$</th>
<th>log $B_2$</th>
<th>log $K$</th>
<th>log $\beta_2$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. HA soil</td>
<td>3.9*</td>
<td></td>
<td>5.05</td>
<td>-0.85</td>
<td></td>
<td>8.4</td>
<td></td>
<td>Stevenson 1976</td>
</tr>
<tr>
<td>12. HA soil</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td>3.69*</td>
<td>7.38</td>
<td></td>
<td>Stevenson 1977</td>
</tr>
<tr>
<td>13. HA soil</td>
<td></td>
<td>6.5</td>
<td></td>
<td></td>
<td>4.8*</td>
<td>9.63</td>
<td></td>
<td>Takamatsu and Yoshida 1971</td>
</tr>
<tr>
<td>14. HA soil</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>van Dijk 1971</td>
</tr>
</tbody>
</table>

*these stability constants are not provided by the reference, but have been calculated from its data.

In some cases this calculation involved use of the relationship $B = K \cdot K'_a$, or $B = K' (K'_a + H^+)$, in other it meant using $B_{ave} = (B_2)^{0.5}$ or $K_{ave} = (\beta'_2)^{0.5}$, to put the constant in a comparable form.

(2) in this case a second, weaker, site was observed on the same ligand.
by the same reference. In Chapter 4.3 the methods used to determine these constants are discussed.

I have shown (Chapter 2.2) that \( \log K' \) varies with the pH when protons are in a position to compete with \( \text{Cu}^{2+} \) ions. When one proton is exchanged per \( \text{Cu}^{2+} \) ion the slope of such a plot will be equal to one, and when two protons are exchanged the slope will be equal to two.

Usually a slope smaller than unity has been observed, however, and this effect is ascribed to electrostatically charging of the molecule as function of ionization (Gamble et al. 1970; Takamatsu and Yoshida 1978), rather than using the simpler acidity constant approach which I will use here. Also, the increase in \( \log K' \) has been ascribed to selective binding to a different functional group which became available upon an increase in pH, and to steric stabilization derived from the formation of chelate rings (Takamatsu and Yoshida 1978).

It is possible that in the lower pH region, pH < 4-5, protonation of the carboxyl groups causes additional proton release upon complexation of \( \text{Cu}^{2+} \) ions. So the slope of a plot of \( \log K' \) as function of the pH could be curved towards a steeper slope equal to 2. Some measurements at pH 3.5 do indeed suggest that two protons are released upon the binding
of a Cu\(^{2+}\) ion (Stevenson et al. 1973) but it was claimed that
two acidic groups were binding Cu\(^{2+}\). In that case \(K'\) should
not vary with the pH when pH > \(K'_{\text{COOH}}\) or when pH > 9.5.

Even when it was thought that a basic phenolic
hydroxyl group is involved in binding, titrations have not
been performed at sufficiently high pH to determine its
acidity constant, until very recently. Thus it was noticed
on several occasions that metal complexation resulted in the
release of more protons than could be accounted for by acid
base titrations (Stevenson 1976; Stevenson 1977; Geering
and Hodgson 1969). The data of these references are included
in Figure 3.1 and have contributed to the conclusions. A
possible explanation for the divergence of their data with
the general trend is due to the high experimental concentra-
tions possibly resulting in 2:1 binding for part of the
complexes.

In Figure 3.1 the conditional stability constants,
log \(K'\), have been plotted against the pH for which \(K'\) was
calculated or determined. A very interesting relationship
emerges: the individual points can be represented by a
straight line having a slope of 1.00, ± 0.37 on the 2 \(\sigma\)
level, ± 0.18 on the \(\sigma\) level. In other words: log \(K' = \) pH
-0.5 when 5<pH<8.5. (This was calculated by linear regression,
Figure 3.1 Conditional stability constants, $K'$, for complexes of copper ions and HA, FA and ligands in natural waters. The figures refer to references in Table 3.7.

Linear regression of log $K'$ as function of pH produces log $K' = (1.00 \pm 0.37) \cdot \text{pH} - 0.45$. The data fit a chemical model which describes complexation by the combined action of acidic carboxyl and phenolic hydroxyl groups. The effect of various acidity constants is shown by the broken curves.
using a least square fit, of the values for pH and K' in Table 3.7).

It is important to realize that variations from this line, or from any curve describing these data for that matter, may be due to:

(a) the different methods that have been used in determining these constants, and

(b) the different composition of the ligands, since each reference used its own source and isolation methods.

It is actually surprising that a linear relationship within such small confidence limits evolved at all from these data, unless one accepts that basically the same groups are responsible for binding Cu^{2+} ions in all these instances. Apparently a limited variation in rest-group composition and in molecular weight has only little influence on the behavior of the complexing sites. Or, the effect on the slope evens out when determinations are performed over a range of pHs.

Clearly, the conclusion of the data as plotted in Figure 3.1 is that complexation of copper ions by ligands in the natural environment, for the major part of the pH in natural waters is a process whereby somehow one proton is exchanged for each complexed Cu^{2+} ion. This result should
not be very surprising for those authors who supported a mechanism which allows just that: the combination of an acidic carboxyl group, \( pK_a \sim 3-5 \), and a phenolic hydroxy group, \( pK \sim 9-10 \), in binding a \( Cu^{2+} \) ion (e.g. Schnitzer and Skinner 1965; Gamble et al. 1970).

The hydroxyl/carboxyl hypothesis has further been tested by calculating the variation of \( \log K' \) with the pH and by plotting the result in Figure 3.1. Calculation has been performed using equations (4) and (8) in Chapter 2.2. Note that this model is only partially quantitative: in order to reach \( \log K' \) values similar to the data in the plot, a stability constant, \( \log K \), was chosen as 9.5. Choice of a different \( \log K \) shifts the plot vertically without changing the slope. Acidity constants, \( pK_{a1} \) and \( pK_{a2} \), were chosen as 5 and 10 respectively. The acidity constants determine where the plot will curve from a slope of 2 to 1 at \( pH = pK_{a1} \), and from a slope of 1 to 0, at \( pH = pK_{a2} \). A second curve has been drawn, using \( pK_{a1} = 4 \) to show this effect. The curvature in the lower pH region could explain some of the discrepancy of the data points from the linear regression. But, there are not enough data available to explain any effects on \( \log K' \) for the pH region smaller than 5.

Similarly, there are not enough data available for
the high pH region. A $pK_a \sim 10$ is a rather well proven value (Takamatsu and Yoshida 1978; Perdue 1979) and is too high to show an effect on the data on $\log K'$ that are available now.

It is conceivable that, due to competition by protons, at low pH a complex is favored between a metal ion and 1 or 2 carboxyl groups, while at neutral and higher pH a complex involving a carboxyl group and a phenolic hydroxyl group is favored. This site-swapping is not very likely in the case of FAs and HAs, however. The complexation of metal ions at low pH, pH $< 5$, has such a low stability constant, $\log K' \sim 5$, that it could well represent the bonding between a single ligand site, COOH group, and the metal ion, although bonding by two COOH groups has been claimed in one case (Stevenson et al. 1973). One would expect bidentate binding to be of higher strength however, unless the low strength is a result of electrostatic effects. At higher pH a much stronger bidentate complex is formed by a hydroxyl group and a carboxyl group. Such a model is compatible with the data available on acidity constants for such groups.

Between pH 5-8.5, complexation can be described using the Bjerrum constant, B, for a monoprotonated ligand. By fitting B to the data used in Figure 3.1 one finds that
\[ B = \frac{[\text{CuL}] [H^+]}{[\text{Cu}^{2+}] [\text{HL}]} = K_a K_a = 10^{-0.5} = 0.3. \]

In other words

\[ \frac{[\text{CuL}]}{[\text{HL}]} = 0.3 \frac{[\text{Cu}^{2+}]}{[H^+]} \]

or \([\text{CuL}] = [\text{HL}] \) when \([H^+] = 0.3 [\text{Cu}^{2+}] \). So the affinity of the ligands is slightly larger for protons than for \(\text{Cu}^{2+}\) ions.

**Summary and conclusions**

(1) The complexation of \(\text{Cu}^{2+}\) ions by ligands in natural waters, in the pH range 5-8.5, is a process whereby a single proton becomes exchanged for each \(\text{Cu}^{2+}\) ion which is complexed.

(2) A model which describes \(\text{Cu}^{2+}\) complexation by ligands in the natural environment by the combined action of acidic carboxyl and phenolic hydroxyl groups, is compatible with the data available on conditional stability constants and acidity constants.
(3) Within pH limits, \( 5 < \text{pH} < 8.5 \), conditional stability constants, \( \log K' \), of natural ligands for complexes with \( \text{Cu}^{2+} \) ions, bear the following relationship with the pH:
\[
\log K' = \text{pH} - 0.5.
\]

(4) When the pH is between 5 and 8.5 the affinity of humic and fulvic acids for protons is about 3x, larger than for \( \text{Cu}^{2+} \) ions.

3.7 INORGANIC SURFACES

Some metals in aqueous environment form oxides or solid hydroxides with an extremely low solubility due to their high oxidation state. \( \text{Fe}^{3+} \) for example, in equilibrium with goethite as solid phase, has a solubility of \( 10^{-20} \) M at pH 8 (\( \log K_{\text{So}} = -44 \), Sillen and Martell 1964), although its total solubility increases again through complex formation with hydroxyl ions. Such hydroxides will occur more or less aggregated as particles ranging from colloidal size (Stumm and Giovanoli 1976) to actual nodules, found on the bottom of the oceans (Broecker 1974) and also in lakes and soils. Besides such chemically precipitated particles, a variety of erosion and weathering products are present as clay
particles.

The surface of these particulates acquires a charge by one or more of several effects; adsorption of potential determining ions is among these effects of primary importance.

For oxide water interfaces the adsorption of $H^+$ and $OH^-$, as potential determining ions, provides a charge corresponding to the $pH$ of the environment. This adsorption is at least formally related to the protolysis of surface $OH$ groups (Schindler and Gamsjäger 1972). The adsorption of hydrogen ions can thus be described by the equation

$$-M-OH + H^+ \rightarrow -M-Oh^+$$

whereas the uptake of hydroxyl ions can be presented as a deprotonation

$$\cancel{-M-OH} \rightarrow -M-O^- + H^+.$$

Schindler and Gamsjäger (1972) introduce two acidity constants to formulate the adsorption equilibria:

$$K_{a1} = \frac{[H^+][-M-OH]}{[-M-Oh^+]},$$

$$K_{a2} = \frac{[H^+][-M-O^-]}{[-M-OH]}.$$
in which \([-M-OH^+], [-M-O^-], [-M-OH]\) are concentrations of protonated, deprotonated and unchanged surface OH groups (mole/kg).

Other metal ions, such as Cu\(^{2+}\), can substitute for protons, form a chemical bond with the surface and bring a change about in the charge on the surface.

A review of the solids which can be found in sediments or suspended in waters is given in Table 3.8. The charge is given as function of the pH. The zero point of charge (ZPC) is the pH at which the wall or surface potential is zero; the isoelectric point (IEP) is the pH at which the \(\zeta\)-potential is zero.

Crystalline minerals obtain part of the surface charge by lattice defects: tetravalent Si atoms may be replaced by trivalent Fe or Al, resulting in a surplus negative charge (Follett 1965). The anions on broken crystal edges can function as potential determining ions when they are capable of ionization, such as \(-\text{OH}\) or \(-\text{CO}_3\). This will produce a pH dependent charge, which may be different from the charge on a crystal face (e.g. Thiessen 1942).

Some hydrophobic colloids (solids of apolar nature, without hydrophilic groups such as \(\text{OH}^-\) or \(\text{CO}_3^{2-}\)) have a surface charge entirely due to adsorption of potential
Table 3.8 ZPC's for a number of oxides, hydroxides and minerals which may occur as particulates in natural waters

<table>
<thead>
<tr>
<th>Substance</th>
<th>ZPC pH</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>9.1</td>
<td>Tewari and Lee 1975</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>Wiese and Healy 1975</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>Huang and Stumm 1973</td>
</tr>
<tr>
<td>CaCO₃ (calcite)</td>
<td>8-9</td>
<td>Parks 1967</td>
</tr>
<tr>
<td>α-Cr₂O₃</td>
<td>6.35±0.1</td>
<td>Yates and Healy 1975</td>
</tr>
<tr>
<td>Fe₂O₄</td>
<td>7.0</td>
<td>Tewari and Lee 1975</td>
</tr>
<tr>
<td>α-FeOOH (Goethite)</td>
<td>7.5±0.1</td>
<td>Yates and Healy 1975</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>Forbes 1974</td>
</tr>
<tr>
<td></td>
<td>8.0±0.3</td>
<td>Kavenagh et al. 1977</td>
</tr>
<tr>
<td>Fe₂O₃ (α-Hematite)</td>
<td>8.5</td>
<td>Parks and de Bruyn 1962</td>
</tr>
<tr>
<td>Fe(OH₃)</td>
<td>7.9</td>
<td>Davis and Leckie 1978</td>
</tr>
<tr>
<td></td>
<td>7.4-8.4</td>
<td>Parks 1965</td>
</tr>
<tr>
<td>HgS (Cinnabar)</td>
<td>3-4</td>
<td>James and Parks 1974</td>
</tr>
<tr>
<td>Kaolinite, edges plate</td>
<td>7.2</td>
<td>Williams and Williams 1978</td>
</tr>
<tr>
<td></td>
<td>&lt;3.5</td>
<td>Parks 1967</td>
</tr>
<tr>
<td>δ-MnO₂</td>
<td>3.3±0.5</td>
<td>Gray et al. 1978</td>
</tr>
<tr>
<td></td>
<td>2.8±0.3</td>
<td>Morgan and Stumm 1964</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>Murray 1975</td>
</tr>
<tr>
<td>γ-MnO₂</td>
<td>5.9±0.3</td>
<td>Gray et al. 1978</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>&lt;2.5</td>
<td>Parks 1967</td>
</tr>
<tr>
<td>SiO₂</td>
<td>3</td>
<td>Yates and Healy 1976</td>
</tr>
<tr>
<td>TiO₂</td>
<td>5.5</td>
<td>Tewari and Lee 1975</td>
</tr>
<tr>
<td></td>
<td>5.9</td>
<td>Wiese and Healy 1975</td>
</tr>
<tr>
<td>ZrO₂</td>
<td>6.8</td>
<td>Tewari and Lee 1975</td>
</tr>
</tbody>
</table>

The data of Parks (1967) are quoted by him. For the original author one should look there.

Some ZPC's were determined as IEP's, but when the measurement is performed in absence of specifically adsorbing ions, the IEP can be taken as equal to the ZPC.
determining ions other than OH\(^-\) or H\(^+\). The potential on AgI-sols (Lyklema 1971) for example is dependent on the relative concentrations of Ag\(^+\) and I\(^-\) in solution.

Of course there are many surfaces in natural waters with a charge resulting from a mixture of these causes. An amorphous hydroxide, aging into more crystalline material, may lose part of its charge by polymerization. Thus ferric hydroxide loses 3/4 of its charge when aged for 120 days, at pH > 8.5, into goethite (Lahann 1976). Apparently the IEP, although not measured by Lahann, changes accordingly. Similarly an increasing amount of surface hydroxyl groups is formed on kaolinite with increasing imperfection of the crystals (Vasilev et al. 1976). Increasing crystallinity, as observed in aging precipitates, shifts the IEPs for oxides in the acid direction (Parks 1965). A wide range of IEPs has been observed for the various polymorphs of MnO\(_2\) (Healy and Fuerstenau 1965). They showed that a linear relationship exists between the ZPC and heat of immersion in water (or in any other liquid), and measured it by calorimetry. It has been shown, that there exists an almost continuous solution range of manganese oxides. Jenne (1977), for example, gives a review of these. Wadsley (1950) was able to synthesize many of these substituted oxides by cation exchange and heating to increase the rate.
Giovanoli et al. (1975) made a series of crystalline, 10 Å, manganese oxides in which different transition metals were built into the structure. Equilibration with a smaller sized metal (H⁺) by lowering the pH, makes the structure collapse into a 7 Å layered system.

From Table 3.8 one realizes that there will be surfaces in natural waters of a series of hydroxides, oxides and carbonates, with a charge, which depends on the pH and the presence of other potential determining ions. At neutral pH this charge ranges from negative (in most of the cases) to positive. Manganese oxides may be expected to have a negative charge. The charge on iron hydroxides may be slightly positive in freshwater systems, and slightly negative in seawater (higher pH).

Not usually, however, will these surfaces occur as "clean" surfaces. Iron hydroxides tend to coat clay surfaces (Jenne 1977) and also organic matter will adsorb for reasons of electrostatical and van der Waals attraction. Thus a rather complex system is produced, which can exert its influence on the trace elements in the water column by many means. Such effects will be treated in the next chapters.
3.8 INTERACTIONS BETWEEN ORGANIC MATTER AND INORGANIC PARTICULATES

There is a massive amount of literature concerning the interaction of organics with solids. Most are studies on the whereabouts and effects of organic matter in soils, trying to explain the impact on agriculture. The soil is a natural leaching system: the groundwater seeps from decaying organic matter along inorganic particulates, equilibrating the phases with each other. One can imagine that the agriculturist is interested in knowing how the nutrient availability is affected, where his organic or inorganic fertilizers are going, whether his insecticides and herbicides will be reactive in contact with this mixture. Environmentalists wonder whether their fertilizers, herbicides and insecticides will reach the surface waters or will be adsorbed by the soil.

The decaying organic matter and respiration of microorganisms (CO₂ development) results in a low pH (4-6) and high concentrations, relative to surface waters, of dissolved organics in the groundwater. This mixture is highly aggressive in that it can solubilize oxides, hydroxides and carbonates, and it prevents the ions to precipitate and
adsorb by complexation with the organics and competition with hydrogen ions for sites on solids.

For better understanding it is necessary to simplify the system. In the following discussion the organic matter will be divided into three groups, based on their adsorption properties. Firstly small, polar molecules will be discussed, molecular weight not more than 200-300, with relatively many ionized groups; then the large, apolar molecules, molecular weight in the thousands; more ionized groups may be present but they do not dominate the adsorption chemistry; thirdly the group of intermediate molecules, where both size and polarity play a role in adsorption.

1. Adsorption of small, polar molecules

In this group Coulombic forces generally play the most important role in determining capacity and strength of adsorption. Depending on the pH and on the presence of complexing cations, reactive sites, such as carbonate, hydroxide and amine groups, will be ionized and provide a charge favourable for adsorption. Organic anions are adsorbed at the edges of the clay particles. Organic cations, on the other hand, are adsorbed on the negative, face surfaces of the clay (van Olphen 1977, p.171). As a result there is
a much larger adsorption capacity of the clay for these cations. When a molecule is in the cation state (pH smaller than its $K_a$) and the clay has a positive charge as well (pH < ZPC) very little adsorption will occur (Lailach and Brindley 1969; Kumanomido 1978) and there will be strong competition with other, inorganic, cations for sites (Kodama and Schnitzer 1968; Lailach et al. 1968b; Mortland 1970). A similar, but opposite effect occurs at high pH when both the inorganic solid and the organic molecule are negatively charged.Adsorption will be inhibited by competing anions (Kosiur 1977). Maximum adsorption occurs when $K_a < \text{pH} < \text{ZPC}_{\text{solid}}$ (Kosiur 1977; Davis and Leckie 1978; Huang et al. 1977; Kumanomido et al. 1978). The organic is then negatively charged, the solid positively. Adsorption of the organic cation moves the ZPC of the particles to a lower pH (Kumanomido et al. 1978). The maximum adsorbing capacity ($\Gamma_{\text{max}}$) is determined by the cation exchange capacity (CEC) of the clay (Hayes et al. 1978a; Eirich 1977).

Specific adsorption can play a role. Carter (1978) showed that out of a mixture of amino acids asparagine becomes specifically adsorbed onto CaCO$_3$ surfaces in seawater environment, which explains the relatively high concentrations of asp. found in non-biogenic oolites. If there are
metal ions in the surface, one can imagine that surface complexation occurs. It is suggested that amino acids adsorb on hydroxyapatite by complexation with Ca\(^{2+}\) (Kresak et al. 1977). Montmorillonite saturated with different cations showed strongest adsorption of parathion and its derivatives when it was covered with a cation with a high tendency to form complexes: Fe\(^{3+}\) > Ca\(^{2+}\) > Na\(^{+}\) (Bowman and Sans 1977). Also the size of the adsorbing molecule is of importance, when it has to fit in between layers of a clay as montmorillonite. The ethylene diamine complex of copper, Cu(en)\(^{2+}\), is too large to fit, but the Cu(en)\(^{2+}\) adsorbs and coordinates with O-atoms on the clay (Burba and McAtee 1977).

2. Adsorption of large, basically apolar, molecules

The adsorption of molecules of high molecular weight is mainly governed by non-ionic forces, such as van der Waals attraction and entropy of displaced water molecules. Maximum adsorption is independent of the number of cation exchange sites available on the solid; it now is dependent on the size of the molecule and its orientation. In case of a poly-electrolyte the size may be pH dependent: at the pH of the iso electric point (IEP) the size will be minimal due to
minimal repulsion between similarly charged groups. In such a case adsorption will be maximal at the IEP.

With increasing chain length of long-chain fatty acids, Allen and Patel (1971) have found that total adsorption decreases, but that the heat developed per adsorbed molecule increases. Going from 6 to 7 C-atoms the chain tries to form a loop: the COOH-group apparently goes to the surface, even when both have opposite, negative, charge. The same is noticed by Norde and Lyklema (1978a). They calculate that the surplus negative charge generated by this seemingly illogical behavior is counteracted by a high concentration of cations in the same region (Norde and Lyklema 1978b). Two high molecular weight proteins adsorb mainly endothermically. The surface potential apparently has very little effect on maximal adsorption (Norde and Lyklema 1978a); maximal adsorption is around the IEP of the protein, when the size is minimal. Increase in ionic strength (more charge-shielding action) does not affect the affinity at a pH<IEP when there is Coulombic attraction, but \( \Gamma_{\text{max}} \), the maximal adsorption, increases. It is concluded that conformational effects of the organic molecules are most important, which points to entropy as being one of the main driving forces for adsorption (Norde and Lyklema 1978a). In non-ionic polymers this is even more so the case. The thickness of an adsorbed layer parallels the intrinsic viscosity
of the polymer in a given solvent (Eirich 1977). In other words: a stiff polymer will give a thicker layer, the coils will be sticking further from the surface. It should be noted that adsorption of molecules of large size, as discussed above, may be extremely irreversible.

3. Adsorption of molecules of intermediate size. In this group fall the fulvic acids (FA) and the smaller humic acids (HA).

In molecules of intermediate size the properties, which I mentioned before, are combined. Both Coulombic forces, van der Waals attraction and entropy will be effective. Depending on the environment, on the properties of the solution and of the solid, one or the other force will dominate. Mortland (1970) mentions that at Mw. > 400 entropy dominates (entropy of release of H₂O molecules); in his proposal FA competes with H₂O for ligand positions on the clay. It follows the ideas of Kodama and Schnitzer (1968) who note a marked effect of exchangeable cations in FA adsorption on montmorillonite at low pH. They measure the increase of the interlamellar spacing between the layers of montmorillonite and conclude that spacing depends on the ease with which FA displaces water molecules which are associated with the counterions. At higher pH (pH 6) less
FA adsorbs and there is no increase in the d-spacing (Schnitzer and Kodama 1966). More recently it has been noted that adsorption of FA on goethite and gibbsite strongly decreases going from pH 3 to pH 6, and that large quantities of OH-groups are released upon adsorption (Parfitt et al. 1977). They call it a ligand exchange mechanism. For organic molecules the van der Waals attraction and the entropy both favour adsorption, the Coulombic force will either attract or repulse.

In several cases it has been observed that the maximal adsorption is higher than expected, especially noticeable at pH > ZPC when very little adsorption is expected (Hayes 1978a; Kavanagh 1977). This adsorption may be explained by surface complexation and depends on the cation exchange capacity. Porphyrins, which are very strong metal chelators and occur in heme and chlorophyll, still adsorb as anions at pH 9 on kaolinite and montmorillonite (Kosiur 1977). But there is strong competition by other anions such as phosphates, and the major difference between the two clay minerals is due to the cation exchange capacity.

The presence of cations in the clay is important to favour surface adsorption by anionic organics. The adsorption of parathion and derivates is both dependent on the presence
of certain complexing cations (higher valency gives stronger adsorption) and on the inverse of the water-solubility of the organic molecule (less soluble adsorbs better; Bowman and Sans 1977). The last effect points to hydrophobicity as a driving force, which basically can be thought the same as entropy.

The adsorption of similar herbicides on goethite appears to be mainly controlled by Coulombic forces since there is no adsorption when the pH > ZPC and when both the solid and the organic are negatively charged. But at pH < ZPC the adsorption is higher than expected due to van der Waals attraction (Kavanagh 1977).

The double layer is depressed by increased ionic strength. This effect promotes the adsorption of HAs by clay minerals in seawater (Rashid 1972). Both the clay and the HA have a negative charge in this environment. By depressing the double layer the HA can reach the surface and will adsorb by van der Waals attraction.

**Summary of 3.8**

The previous discussion on the adsorption of organics can best be summarized in the following diagram (Figure 3.2).
increasingly: irreversible adsorption
: dependent on surface quantity

increasingly: dependent on surface quality, such as ZPC
: dependent on solution properties, such as ionic strength, pH, inorganic ions

Figure 3.2 Relative forces diagram for adsorption of organics, of different molecular weight, on a charged surface. In vertical direction the relative strengths of surface attractive forces and entropy are indicated in relation to their effectiveness in promoting surface adsorption.
This diagram is meant to be qualitative rather than quantitative. Especially the relative strength of the van der Waals attractions and the entropy, gained by the release of $\text{H}_2\text{O}$ molecules, is still very dubious. There is however some quantitative impression to be obtained at the intersections of the curves, relating this to the molecular weight of the molecules on the $X$-axis. The polarity or hydrophobicity of the molecule is important in determining the relative effects of the forces at higher molecular weight. To relativate the molecular magnitude and composition better, a second-scale has been set along the $X$-axis representing the reverse of the number of ionizable groups per number of $C$-atoms in the molecule.

3.9 COMPLEXATION AND ADSORPTION BY THE COMBINED PRESENCE OF ORGANICS AND INORGANIC PARTICULATES

In the previous discussion we have seen that organics in natural waters are mainly composed of FA (de Haan 1975; Schnitzer 1971). FA is some sort of collection or aggregate of amino acids, lipids and sugars (de Haan 1975; Kemp and Mudrochova 1973) and with a more or less aromatic core (Schnitzer 1971; Wilson and Goh 1977), with a total molecular
weight of around 1000 (Hansen and Schnitzer 1969). As such it falls in the group of intermediate size molecules, and adsorption will be determined both by Coulombic forces and by van der Waals forces and entropy.

Adsorption will be relatively most strong when FA and solid have opposite electrical charge. Since FA is negatively charged over most of the pH-range of importance in natural systems, the solid preferably ought to be positively charged, or \( \log K_a,FA < pH_{adsorption} < pH_{ZPC} \). The COOH groups in FA ionize at pH > 3-4 (Gamble 1970) thereby giving FA a negative charge. ZPCs for a number of solids can be found in Table 3.8. A mineral of very ubiquitous nature and favourable charge is iron hydroxide, Fe(OH)₃, with a \( pH_{ZPC} \) of 7.5-8.5 (Parks 1965; Davis and Leckie 1978). In many freshwaters it will be positively charged and will be a suitable substrate for FA to adsorb upon. In seawater the charge may be slightly negative due to the higher pH of 8.3. But the double layer which prevents adsorption is compressed by the high salt concentration, so that the van der Waals forces get their chance and FA adsorbs (Rashid 1972).

What is now the effect on metal complexation due to adsorption of FA onto a solid? FA is itself a jumble of organics, presumably held together by the same sort of forces which bind it to the solid. Apparently FA is perfectly able
to complex metals and it may well continue to do so when adsorbed. There is, however, more evidence in this direction. It is thought that COOH groups of organic molecules, though close to the surface of the solid when adsorbed, are still surrounded by a high concentration of cations (Norde and Lyklema 1978b). Experimentally it has been shown that cations at certain pH intervals will promote adsorption of organics (Bowman and Sans 1977) and, vice versa, that complexing matter such as glutamic acid promotes adsorption of metal ions onto a solid surface (Davis and Leckie 1978), again for a limited pH-range. In both cases the pH at the ZPC of the solid represents the upper limit, after which all adsorption strongly diminishes as a result of electrical repulsion. The studies showing enhanced adsorption of organics by metal ions generally were performed at less than neutral pH on solids which were saturated with metal ions in very high concentration. They may well represent coprecipitates, a solid covered by the precipitated metal hydroxide or metal carbonate: a situation which is rather unusual for natural water systems. Considering the potentiality for an organic molecule to adsorb at higher than pH\textsubscript{ZPC}, the situation seems bleak. One could imagine an originally negative surface which becomes positive due to specific adsorption of metal ions and is then a suitable place for
negative organic molecules to adsorb as well. The problem is, however, that organic complexing material may well have the same or similar preference for metal ions, and thus will provide itself of the same positive charge, and obstruct its chance for adsorption onto the solid. The ultimate result depends on the relative affinities of both solid inorganics and dissolved organics for the metal ions, and on the concentrations of metals, surfaces and complexing matter present. A very high concentration of metals would saturate both the solid and the organics, and make adsorption unlikely to happen.

More conceivably the metal concentration will be low, not sufficient to saturate either of them ("undersaturation is the rule in nature", Szalay 1964). The affinity of the organics for metal ions in the natural water environment is not very well known, but may well be larger than that of inorganic solids. Depending on whether the free metal ion concentration in equilibrium with the organic-metal complex is still high enough to give the solid a positive, repulsing charge, mixed adsorption will occur. But very little is known about this as yet. Adsorption of a negatively charged organic on a positively charged surface will bring the overall pH_{ZPC} down to a point between the two potentials
Kumanomido et al. 1978). The organic molecule probably loses some of its capacity to complex metals due to occupied groups and diminished electrical potential relative to the positive metal ions.

For the natural water situation one might be able to conclude that those metals with a strong affinity for FA will occur as a complex with FA. The complex as a whole has a fair chance to adsorb on the surface of a hydroxide such as Fe(OH)$_3$, but this adsorption probably has not a great impact on the free metal concentration as such. It could, however, have some impact on the ion exchange method which is described in the next chapters. Also it may affect the situation in soils, where generally the pH is lower and the concentrations of all constituents, organics and solids, is higher. One has to realize, however, that in most studies mentioned in this discussion, the experiments were performed at high concentrations of both organics and solid surfaces, resulting in high concentrations of dissolved organics in the equilibrium situation. Often only a fraction of the total organics is adsorbed in those cases. This is somehow comparable to the real world situation, where the total amount of dissolved matter is thought to be two orders of magnitude larger than the total amount of particulate organic matter (Table 3.1): another argument that dissolved organics in natural waters are largely free and not adsorbed.
3.10 SUMMARY

Various types of organic and inorganic surfaces and sites are discussed which offer opportunities for metal ions to adsorb or form complexes. Analysis of the composition of familiar complexing ligands in nature, such as FAs and HAs (literature data), leads to the conclusion that amino acids form a very important fraction. This conclusion is especially apparent when the data are considered on a mole per mole basis, rather than on a weight per weight basis as normally in the literature.

In dystrophic waters FAs form the main constituent of the dissolved organic matter. Phytoplanktonic algae, however, excrete a range of organic compounds. It is impossible to discern between FAs from different sources (soil or sediments) on the basis of chemical composition.

A review of literature data concerning copper complexation by FAs and HAs provides information concerning the types of groups involved in binding. The data can be described satisfactorily by a model which allows for the exchange of a proton for each complexed \( \text{Cu}^{2+} \) ion. This complexing site must have an acidity constant, \( pK_{a2} \), of approximately 9 or larger. The model confirms the established idea that possibly complexation by FAs is a bidentate process in which a
carboxyl group and a phenolic hydroxyl group (high $pK_{a_2}$) are involved.

The main conclusions of this chapter are:

- the molar ratio of amino acids to FAs and HAs in humic matter varies from 0.1 to 1.0;

- by complexation of Cu$^{2+}$ by FAs and HAs one proton is exchanged from a group with a high $K_a$ ($pK_a > 8$); a model of bidentate binding by a carboxyl group and phenolic hydroxyl group fits the literature data, but amine groups would fit the data as well;

- conditional stability constants of HAs and FAs for complexes with Cu$^{2+}$ vary with the pH: $\log K' = pH - 0.5$, when $5 < pH < 8$; for the same pH limits, the affinity of HAs and FAs for protons is about 3x higher than for Cu$^{2+}$ ions;

- adsorption of FA-like molecules on solid surfaces is for a large part governed by Coulombic forces; very little adsorption is expected on MnO$_2$, but Fe(OH)$_3$(s) may adsorb natural organics when pH > pH$_{ZPC}$ of Fe(OH)$_3$(s).
The appropriate technology is needed for things that are small and beautiful.

Variant on E.F. Schumacher
CHAPTER 4

EXISTING METHODS TO DETERMINE COMPLEXATION
OF HEAVY METALS IN NATURAL WATERS

4.1 INTRODUCTION

Methods are discussed which were in existence before the development of the method which is proposed in this thesis.

For comprehension of the state in which metals occur in natural waters, it is necessary to know both the ligand concentration and the, conditional, stability constants for the complexes that may be formed. Knowledge merely of the ligand concentration, or complexing capacity, of a natural water is of limited use in understanding the processes which govern the behavior of these metals. Several methods have been developed which measure only the complexing capacity, but one could imagine that combination of such a method with some other means to measure stability constants for the complexes that may be formed, might give satisfactory
information on the subject. Therefore the measurements of complexing capacities and of stability constants will be treated separately.

4.2 METHODS TO MEASURE COMPLEXING CAPACITIES OF NATURAL WATERS

The complexing capacity of a natural water represents the maximum amount of metals that can be bound or complexed by ligands, or sites, present in the water. Due to the usually reversible behavior of the complexes that are formed (one can create circumstances that make the complexes split into ligands and metal ions) the ligands are never completely saturated with metal ions. In the presence of a surplus of metal ions most or only part of the sites may be occupied, depending on the stability constants. So, the methods that measure complexing capacities of natural waters cannot actually determine this capacity as the concentration of metals bound at any given situation. At best one might be able to measure a concentration approaching the complexing capacity by working at a high total metal concentration, and at high pH in order to minimize competition by protons. But generally methods that measure ligand concentrations
try to make some sort of extrapolation to the ligand concentration, by inferring proportionally to the amount of metal ions which is bound when the ligand is titrated with these ions.

It is a sensible idea to use a metal for the titration of the ligands, which will actually form a complex appreciably, at a reasonable, low metal concentration. In theory it is possible to titrate a ligand with, for example, sodium ions, but one may have to add more than 0.1 mol/L Na⁺ before an appreciable proportion of the ligand is complexed. And then it will be rather difficult to determine how much has been complexed, when the ligand concentration is 6 orders of magnitude less in concentration. Contrarily, one tries to use a metal for which the ligand is expected to have a strong affinity.

For electrostational reasons a metal ion of high valency will be complexed stronger than a metal ion of low valency. A problem is formed by competition of hydroxyl ions. High valent metal ions also tend to form hydroxides of very low solubility. So one may have to compromise a bit in this search for a suitable metal to titrate the complexing ligands.

The methods that have been developed and the metals
employed are summarized in Table 4.1. Further specifications of the methods have been added to notes in the table. Two of the methods make use of the toxic effect that copper ions have on microorganisms; these are quite sensitive methods if one works in pure, laboratory systems, but they may be subject to unknown interferences when applied to natural waters. One method uses Co$^{2+}$ ions which are oxidized, after complexation, into Co$^{3+}$ to give a robust complex. Two methods, 5 and 6, make use of the solubility of copper complexes; one, 5, separates the dissolved complexes from solid copper hydroxide by filtration at high pH, the other one, 6, by filtration through an ion exchange column. The most simple method probably is the one, 1, which uses a.s.v. to measure free copper ions in a buffered solution, titrated with copper. This method is subject to interference from organics adsorbing on the mercury electrode, resulting in peak shifts and peak widenings, which easily can be mistaken for complexation effects. Unless the complex is quite robust, it will tend to split during the preconcentration step of Cu$^{2+}$ in the electrode, whereby an artificially high free metal concentration is measured. So this method really works in a state of disequilibrium. If the problems concerning surface active effects of the organics on the mercury electrode and splitting of the complex can be solved,
Table 4.1 Methods to determine complexing capacity

<table>
<thead>
<tr>
<th>Method</th>
<th>Limit of Detection (μM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. d.p.a.s.v. in presence of NaAc buffer of pH 6.0, log K'&gt;10</td>
<td>0.1</td>
<td>Chau et al. 1974</td>
</tr>
<tr>
<td>2. growth suppression of diatom Thalassiosira pseudonana</td>
<td>0.1</td>
<td>Davey et al. 1973</td>
</tr>
<tr>
<td>3. growth suppression of bacteria</td>
<td>0.05</td>
<td>Gillespie and Vaccaro 1978</td>
</tr>
<tr>
<td>4. complexation of Co^{2+}; log K'&gt;17</td>
<td>0.4</td>
<td>Hanck and Dillard 1977b</td>
</tr>
<tr>
<td>5. complexation of Cu^{2+} at pH 10; log K'&gt;13</td>
<td>?</td>
<td>Kunkel and Manahan 1973</td>
</tr>
<tr>
<td>6. filtration through Chelex-100 column</td>
<td>0.05</td>
<td>Stolzberg and Rosin 1977</td>
</tr>
</tbody>
</table>

Ref.
1. the method can be improved by working at lower temperature and using a dual electrode (Hanck and Dillard 1977a).
2. growth suppression is measured by $^{14}$C uptake.
3. complexed Co$^{2+}$ is oxidized to Co$^{3+}$ assuming that free Co$^{2+}$ is not oxidized as well.
4. at pH 10 the solubility of Cu$^{2+}$ is only $10^{-12}$ M; K' has to be greater than $10^{13}$ to get significant, 90%, complexation of the ligand at pH 10; this means that a ligand as strong as NTA can not yet be measured.
5. free Cu$^{2+}$ will adsorb on the column, while complexed Cu is assumed to go through; some loss of ligand (EDTA) is noted; since a strong resin is used, only strong complexing ligands will be measured.
polarography could be a very promising method, because of its sensitivity. It could be applied to unfiltered waters, and sites on inorganic particles would be co-determined. Solutions to the polargraphical problems, such as using very short pulses in d.p.p. (differential pulse polarography) unfortunately tend to diminish the sensitivity as well. There is a possibility that use of rotating disk and ring electrodes mixed with a.s.v., as used by Shuman and Michael (1975, 1978) gives more clarification about the rate of splitting of complexes. Such knowledge would make correction possible for the production of free metal ions during polargraphic measurement. It must be very difficult, however, to discern between diffusion and dissociation of the complex. The rate of one of these has to be known in order to determine the other one. Additionally, high ligand concentrations are required for an accurate measurement, or high rate constants in presence of lower concentrations.

Generally, in all these methods the complex needs to have a very high stability constant in order to be measured accurately. Artificial ligands such as EDTA and NTA form indeed such strong complexes, but ligands in the natural environment may tend to be much weaker. Their stability constants will be discussed in the next section.

In this thesis a method will be proposed which makes
use of the equilibrium between an ion exchange medium, metal ions and complexing ligands, which it is hoped will solve most of the problems of the previous methods.

But firstly the measurement of the ligand itself should be considered, employing methods not related to metal complexation. Theoretically it is possible to determine the concentration of organic molecules in a given solution, without even knowing the composition of these molecules. This can best be done by vapor pressure osmometry (Hansen and Schnitzer 1969). But, the organics will have to be preconcentrated many times before this is possible, whereby new linkages and molecules may be formed. Only an average molecular weight is determined for all the molecules, complexing ligands and other. It is not known how many sites are available on these molecules. The conclusion is that an analysis of organic matter alone, with respect to molecular weight and concentration of acid-titratable groups, gives very little information concerning the complexing capacity of natural waters.
4.3 THE MEASUREMENT OF, CONDITIONAL, STABILITY CONSTANTS

When considering the different methods that exist to determine stability constants for complexes between organical ligands and metal ions, it is important to discern between methods in which the ligand concentration is not limiting and is expected to be very high, and those methods which measure at the very low ligand concentration present in the natural environment. In Table 4.2 a survey is given of stability constants for complexes of metals and humic matter from different sources, and of the methods that have been used.

When measurement is preceded by concentration and separation steps to increase the ligand concentration, a few methods are open for application. A good description of such methods may be found in Rossotti and Rossotti (1961). Basically there are the following methods:

- method of continuous variations: the metal to ligand ratio is varied and the specific adsorption of the complex is measured by spectrophotometer;

- potentiometric titrations: acid/base titrations are performed on the ligand while varying the metal concentration and measuring the pH;
<table>
<thead>
<tr>
<th>ligand</th>
<th>metal</th>
<th>type of constant</th>
<th>log constant</th>
<th>pH</th>
<th>ionic strength</th>
<th>temp. °C</th>
<th>method</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>Zn$^{2+}$</td>
<td>$K'$</td>
<td>5.13</td>
<td>6.5</td>
<td>0.16 KCl</td>
<td>?</td>
<td>I.E.</td>
<td>Ardakani and Stevenson 1972</td>
</tr>
<tr>
<td>water</td>
<td>Cu$^{2+}$</td>
<td>$K'$</td>
<td>7.46</td>
<td>8.2</td>
<td>0.7 NaCl</td>
<td>25</td>
<td>dpp</td>
<td>Branica 1978</td>
</tr>
<tr>
<td>FA, water</td>
<td>Cu$^{2+}$</td>
<td>$K'_1$</td>
<td>6.1</td>
<td>6.0</td>
<td>0.1 KNO$_3$</td>
<td>25</td>
<td>I.S.E.</td>
<td>Bresnahan et al. 1978</td>
</tr>
<tr>
<td>FA, soil</td>
<td></td>
<td>$K'_2$</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA, lake</td>
<td>Cu$^{2+}$</td>
<td>$K'_1$</td>
<td>5.0</td>
<td>6.0</td>
<td>0.1 NaNO$_3$</td>
<td>25</td>
<td>I.S.E.</td>
<td>Buffle et al. 1977</td>
</tr>
<tr>
<td>FA, 2</td>
<td>Pb$^{2+}$</td>
<td>$K'_2$</td>
<td>9.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA, 2</td>
<td>Pb$^{2+}$</td>
<td>$K'_2$</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA, 3</td>
<td>Pb$^{2+}$</td>
<td>$K'_2$</td>
<td>10.1</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA, 3</td>
<td>Pb$^{2+}$</td>
<td>$K'_2$</td>
<td>10.4</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA, 3</td>
<td>Pb$^{2+}$</td>
<td>$K'_2$</td>
<td>6.3</td>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peat</td>
<td>H$^+$</td>
<td>$K_a$</td>
<td>-5.5</td>
<td></td>
<td>0.01 KNO$_3$</td>
<td>?</td>
<td></td>
<td>Coleman et al. 1956</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\beta_2$</td>
<td>-4.8</td>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-4.3</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu$^{2+}$</td>
<td>$K'_2$</td>
<td>7.9</td>
<td></td>
<td>0.01 KNO$_3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu$^{2+}$</td>
<td>$K'_2$</td>
<td>6.9</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu$^{2+}$</td>
<td>$K'_2$</td>
<td>6.2</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 continued

| 6. FA, soil | Cu^{2+} | Zn^{2+} | Bave | \( K' \) | 3.23 | 3.5 | 0.1 KCl | ? | C.V. Courpron 1967 |
| HA, soil | Cu^{2+} | Zn^{2+} | 2.83 | 3.5 | 7.0 | 5.0 | 2.87 | 5.0 |
| 7. FA | H^{+} | \( K_{a,1} \) | -2.33 | 2.66 | 0.1 KCl | 25 | pH Gamble 1970 |
| | | \( K_{a,1} \) | -2.6 | 3.8 |
| | | \( K_{a,2} \) | -5.04 | 4.9 |
| 8. FA | Cu^{2+} | Bave | 1.11 | 2.62 | 0.1 KCl | 25 | I.E. Gamble et al. 1970 |
| 9. HA, soil | Cu^{2+} | K' | 5.6 | 7.0 | 0.2 KNO\(_3\) | 25 | I.E. Geering and Hodgson 1969 |
| 10. PAA | H^{+} | \( K_a \) | -6.17 | 0 | 25 | P.T. Gregor et al. 1955 |
| | | | -4.91 | 0.2 NaNO\(_3\) | |
| | | | -4.48 | 1 |
| | | | -4.30 | 2 |
| | | | -1.19 | 0 |
| | | | -1.17 | 0.2 |
| | | | -0.99 | 2 |
| 11. HA, soil | Cu^{2+} | K' | 6.20 | 6.8 | 0.01 KNO\(_3\) | ? | dial. Guy and Chakrabarti 1976 |
Table 4.2 continued

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>K_0</th>
<th>K_1</th>
<th>K_2</th>
<th>K_3</th>
<th>K_4</th>
<th>pH</th>
<th>NaCl</th>
<th>TRIS</th>
<th>pH</th>
<th>Perm.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.</td>
<td>FA, lake</td>
<td>Cu^{2+}</td>
<td>8.42</td>
<td>8.0</td>
<td>0.01</td>
<td>NaCl</td>
<td>0.01</td>
<td>TRIS</td>
<td>20</td>
<td>Gel- perm.</td>
<td>Mantoura and Riley 1975</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FA, peat</td>
<td>Cu^{2+}</td>
<td>8.80</td>
<td>8.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>FA, lake</td>
<td>Ni^{2+}</td>
<td>5.14</td>
<td>8.51</td>
<td>7.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FA, peat</td>
<td>Ni^{2+}</td>
<td>4.98</td>
<td>5.64</td>
<td>4.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>FA, lake</td>
<td>Zn^{2+}</td>
<td>5.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FA, peat</td>
<td>Zn^{2+}</td>
<td>5.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>FA, soil</td>
<td>H^+</td>
<td>K_{a,2}</td>
<td>-10.5±0.3</td>
<td>?</td>
<td>calorimetry</td>
<td>Perdue 1978</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>HM, river</td>
<td>H^+</td>
<td>K_{a,2}</td>
<td>-10.1±0.2</td>
<td>?</td>
<td>calorimetry</td>
<td>Perdue 1979</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>FA, soil</td>
<td>Pb^{2+}</td>
<td>3.21</td>
<td>2.8</td>
<td>0.1</td>
<td>NaClO_4</td>
<td>25</td>
<td>P.T.</td>
<td>Ramamoorthy and Manning 1975</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table 4.2 continued

| 17. FA, soil Cu<sup>2+</sup> | K<sup>'</sup> | 3.3 | 3 | 0.1 KCl | ? | I.E. Schnitzer and C.V. Hansen 1970 |
| Ni<sup>2+</sup> | 4.0 | 5 |
| Co<sup>2+</sup> | 3.2 | 3 |
| Pb<sup>2+</sup> | 4.2 | 5 |
| Ca<sup>2+</sup> | 2.8 | 3 |
| Zn<sup>2+</sup> | 4.1 | 5 |
| Mn<sup>2+</sup> | 2.7 | 3 |
| Mg<sup>2+</sup> | 3.3 | 5 |
| Fe<sup>3+</sup> | 2.2 | 3 |
| Al<sup>3+</sup> | 3.6 | 5 |

18. lake 1 Cu<sup>2+</sup> K<sup>'</sup> 5.72 6.5 ? ? A.S.V. Shuman and Woodward 1977

<p>| 2 | 4.87 | 5.7 |
| 4.99 | 6.0 |
| 5.15 | 6.5 |
| 5.20 | 7.0 |</p>
<table>
<thead>
<tr>
<th>Table 4.2 continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>lake 3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>19. HA, soil</td>
</tr>
<tr>
<td>H⁺</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Ka</td>
</tr>
<tr>
<td>Bave</td>
</tr>
<tr>
<td>4.51</td>
</tr>
<tr>
<td>0.1 KCl</td>
</tr>
<tr>
<td>25 P.T.</td>
</tr>
<tr>
<td>Stevenson et al.</td>
</tr>
<tr>
<td>1973</td>
</tr>
<tr>
<td>HA, peat</td>
</tr>
<tr>
<td>H⁺</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Ka</td>
</tr>
<tr>
<td>Bave</td>
</tr>
<tr>
<td>4.81</td>
</tr>
<tr>
<td>6.5</td>
</tr>
<tr>
<td>HA, soil</td>
</tr>
<tr>
<td>H⁺</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Ka</td>
</tr>
<tr>
<td>Bave</td>
</tr>
<tr>
<td>5.25</td>
</tr>
<tr>
<td>0.1 KCl</td>
</tr>
<tr>
<td>25 P.T.</td>
</tr>
<tr>
<td>Stevenson et al.</td>
</tr>
<tr>
<td>1973</td>
</tr>
<tr>
<td>20. HA, soil</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Pb²⁺</td>
</tr>
<tr>
<td>Cd²⁺</td>
</tr>
<tr>
<td>β₂</td>
</tr>
<tr>
<td>8.9-4.9 (μ)</td>
</tr>
<tr>
<td>8.7-4.7 (μ)</td>
</tr>
<tr>
<td>6.9-5.4 (μ)</td>
</tr>
<tr>
<td>&lt;4</td>
</tr>
<tr>
<td>var.</td>
</tr>
<tr>
<td>25 P.T.</td>
</tr>
<tr>
<td>Stevenson 1976</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Bave</td>
</tr>
<tr>
<td>0.85</td>
</tr>
<tr>
<td>0.1 KCl</td>
</tr>
<tr>
<td>HA, soil</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Pb²⁺</td>
</tr>
<tr>
<td>Cd²⁺</td>
</tr>
<tr>
<td>H⁺</td>
</tr>
<tr>
<td>Ka</td>
</tr>
<tr>
<td>5.05</td>
</tr>
<tr>
<td>21. HA, soil</td>
</tr>
<tr>
<td>Cu²⁺</td>
</tr>
<tr>
<td>Pb²⁺</td>
</tr>
<tr>
<td>Cd²⁺</td>
</tr>
<tr>
<td>β₂</td>
</tr>
<tr>
<td>7.22-7.38</td>
</tr>
<tr>
<td>7.87-8.25</td>
</tr>
<tr>
<td>7.00-7.03</td>
</tr>
<tr>
<td>7.65-8.11</td>
</tr>
<tr>
<td>6.01-6.06</td>
</tr>
<tr>
<td>6.69-6.92</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 4.2 continued

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>22. HA, soil H⁺</td>
<td>Kₐ₁</td>
<td>4.68±9.10</td>
<td>0.1 NaClO₄</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Kₐ₂</td>
<td>8.8-9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>β₂</td>
<td>8.65±0.65 (pH-5) var.</td>
<td></td>
<td>P.T. I.S.E.</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td></td>
<td>8.35±0.30 (pH-5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd²⁺</td>
<td></td>
<td>6.25±0.63 (pH-5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. HA, soil Cu²⁺</td>
<td>K'</td>
<td>7</td>
<td>6</td>
<td>0.1 KNO₃</td>
</tr>
</tbody>
</table>

I.E. = ion exchange  
dpp = differential pulse polarography  
I.S.E. = ion specific electrode  
P.T. = potentiometric titration  
C.V. = continuous variations  
dial. = dialysis  
Gelperm. = gel permeation  
A.S.V. = anodic stripping voltammetry
Notes to Table 4.2

1. For different HA's log K varied from 3.14 to 5.13, at a HA concentration of $10^{-3}$ M. Only 1:1 binding was observed, and K did not increase with the degree of humification of the HA's.

2. Using a vibrating disk electrode an unknown ligand was titrated in seawater. The ligand concentration was 0.15 μM.

3. Two different binding sites were observed. The total number of binding sites increased with the pH from 0.6-2.6 per molecule water-FA, from 0.8-4.2 per molecule of soil-FA. Molecular weight was determined separately.

4. The stability constants have been calculated on a molar basis of the FA's involved, of which the molecular weights were determined by vapor pressure osmometry. FA, FA,2 and FA,3 stand for FA's from different sources (lakes). They observed 2:1 complexes, two ligand molecules per metal ion.

5. Only one, rather acidic, acidity constant was observed and two binding sites per copper ion.

6. Molar ratios in the complexes: ZnFA$_{1.17}$, ZnHA$_{1.0}$, CuFA$_{1.35}$. Possibly these ratios represent mixed 1:1 and 2:1 complexes.
7. Two acidity constants were observed at low pH. $K_{a,1}$ is ascribed to a carboxyl group in ortho position to a hydroxyl group. About 3.1 meq. per gram FA were present of this first COOH type, about 7.7 meq. per gram FA of a second type.

8. $\log B_{ave}$ varied from 0.70 to 1.18 between pH 3.35 and pH 2.49 respectively; KCl was used to vary the ionic strength. It might affect the measurements of copper complexation at higher than 0.1 M KCl.

9. The carboxyl group concentration is used as ligand concentration. Since not all these groups may be in a position to react, such an assumption may produce an underestimation of the stability constant.

10. PAA is a poly-carboxylic acid. Complexation of $Cu^{2+}$ is through bidentate binding with carboxyl groups. Because of the small difference between the two carboxyl groups involved in binding, the spreading between the two stepwise stability constants is very small, so $B_{ave}$ is considered to be more meaningful. PAA is used as a model for complexing by humic matter.

11. Two types of binding sites were found sufficiently differing in binding strength to use two stability constants.
12. It is not mentioned whether care has been taken to exclude CO₂. The molecular weight of FA as determined by gelfiltration was 5000. This method usually overestimates the molecular weight of complex materials, so the stability constant will be erroneous as well. K₀ is an average stability constant, obtained from a single run. K₁ and K₂ represent two different sites.

13. K'ave is an average value for the data of 40 soil samples. K increased with increasing humification, a large scattering of complexing capacities per gram FA and HA was observed. As ligand concentration, the concentration of complexing sites was used.

14. and 15. Kₐ₁,₂ is the acidity constant for a phenolic OH group. The particular which is determined, however, is not in ortho position to a carboxyl group, so it very probably is not involved in complexing metals. Acidity constants for OH groups in ortho position to COOH groups may well be affected by electrical charge distribution of these groups.

16. Acidity constants as given by Gamble (1970) were used to calculate K'.

17. By taking care that all assumptions were valid, they only found 1:1 complexes with FA. With increasing ionic strength the conditional stability constants decreased.
18. It was assumed that the complex does not kinetically dissociate during electrode deposition, that Cu$^{2+}$ is much smaller than the total Cu concentration, and that other complexes give continuously the same response. The ligand concentrations varied from 20 μM to 126 μM.

19. Two acidic (carboxylic) groups per metal ion were involved in binding while liberating two protons. Only one acidity constant was found for these groups. The given $B_{ave}$ values are an average of the two formation constants involved in the binding of copper ions.

20. $pK_a$ increases with decreasing ionic strength and with decreasing HA concentration. By the addition of metal ions protons are released from otherwise non-titratable functional groups, which is explained by the release of $H^+$ from water bound by the metal, upon pH increase, i.e. the formation of (HA-M-OH)$^-$, which is a rather unsatisfactory explanation. 1:1 complexes are formed at high M/L ratios, 2:1 (2 sites per metal ion) at low M/L ratios.

21. The ranges given for $\beta_2$ are values for HA s from three different sources, and the values are averages for determinations at pH 4 and 5. $\beta_2$ is used for two sites per metal ion, but they suggest the formation of 2:1 complexes by two molecules linked by a metal ion, because of structural considerations (not given).
22. Two sites per molecule involved in binding. Though they give values for pH dependent stability constants, their measurements were performed at pH 6.5 and lower due to the sensitivity limitations of specific ion electrodes.

23. The formation constant was estimated by competition with some known ligands. It is thought that an hydroxy complex is formed (by measurements of pH), similar to Stevenson (1977).
- a variant of the previous method: the metal concentration is measured as well by ion specific electrode;

- ion exchange: in presence of a surplus of resin the ratio of metal to ligand is varied. Total metal is determined and the pH is measured (sometimes). The ratio, metal ions in solution/metal ion adsorbed, is assumed to be constant.

Sometimes combinations of these methods have been performed.

Possibly the earliest report on the use of ion exchange resins goes to Günther-Schulze (1922a,b). A natural zeolite, permutite, was brought into equilibrium with copper ions and various inorganic anions. It was assumed that, beside Cu$^{2+}$ ions, monovalent complexes such as CuCl$^+$ adsorb on the resin, which is not a bad assumption considering that quite similar assumptions have been made more recently in that monovalent hydroxyl complexes adsorb preferentially (James et al. 1975) and even that uncharged Co(OH)$_2$ complexes adsorb (Healy et al. 1968). Then, total copper, chloride and exchanged potassium were determined, in equilibrium with the zeolite. The ligand concentration varied from 5 x 10$^{-2}$ M upward.

Due to the very low sensitivity of all these methods, even more recent experiments still had to be done at high ligand, 10$^{-3}$ to 10$^{-4}$ M, concentrations and equally high metal
concentrations. Generally the determinations were performed at low pH, pH 3 to 4, to keep the metals in solution.

Much information on a complex becomes available when acidity constants and stability constants are known, and conditional stability constants can be calculated, valid at higher pH. But, as has been stated in Chapter 2: electrostatical effects may influence the complexing behavior when the pH is changed considerably, so extrapolation from pH 3-4 to neutral pH and higher may not be valid, and: different types of complexes may be formed at higher ligand concentrations than at lower; generally 2:1 complexes are favored, unless one works at very low free metal concentrations.

Polarographical methods have been developed (Shuman and Woodward 1977) which enable us to measure the free metal ion concentration directly, and which in certain conditions allow us to work at low ligand concentrations. High molecular weight organics such as FAs and HAS tend to exert a surface active effect, however, which renders the measurement of a stability constant on the basis of the shift of the peak potential impossible (Brezonik et al. 1976). Even when adsorption effects can be avoided, success of the measurement still depends on the complete reversibility of the complex. (In d.p.p. an electrode potential is measured while the
complex is being split, and in a.s.v. a potential is measured during the formation of the complex.) It is tried to measure the free metal ion, copper, concentration, and its change when more metal is added. During the determination of the free metal ion concentration, its concentration is lowered by the amount taken up by the mercury electrode, which produces a shift in the equilibria: part of the complexes will split to release metal ions. As a result the constants that are determined will be too small. The use of rotated ring and disk electrodes to obtain information on the kinetics of these processes apparently only works well at rather high ligand concentrations (Shuman and Michael 1975, 1978) and it is questionable whether they are able to differentiate between diffusion of the whole complex and its subsequent delivery of a Cu$^{2+}$ ion to the electrode, and the splitting up of the complex.

It is desirable to develop a method for natural water situations, where the complexing ligands occur in low concentrations. In other words: the appropriate technology is needed for things that are small and beautiful.
4.4 SUMMARY

In this chapter existing methods are discussed that determine metal complexation in water. There are a number of sensitive methods to measure the complexing capacity of a ligand solution. Two of these methods are biological (they use toxic effects on organisms), a third method uses anodic stripping voltammetry (ASV) at pH 6. The ASV method functions accurately only when the ligand-copper complex has a stability constant, log K, greater than 10. Biological methods are subject to various interferences.

There are several methods to determine conditional stability constants for metal complexes with natural ligands. But all of these methods function only for strong complexes or at a high concentration of ligands, normally at $10^{-3}$ to $10^{-4}$ M ligands. A polarographical method might possibly be effective at a lower ligand concentration, but is subject to interferences related to kinetic effects of complex splitting and of adsorption onto the electrode.

No existing method determines satisfactorily both the ligand concentration and the conditional stability constant for complexes with copper ions (or with any other metal ions for that matter) at the very low ligand concentration in natural waters.
If the percentage of the gross national product that goes through the hands of the government shows the degree of socialism, then the U.S.A. are for 40% socialistic.

Data of Milton Friedman, Manchester Guardian, 116, 20 (1977) 17
CHAPTER 5

MnO₂ AS MEDIUM FOR THE DETERMINATION OF COMPLEXING CAPACITIES AND CONDITIONAL STABILITY CONSTANTS

5.1 INTRODUCTION

Ion exchange materials have been used already quite a while to quantify complexation of transition metal ions by either inorganic (Günther-Schulze 1922a,b) or organic ligands (Allen et al. 1976; Ardkani and Stevenson 1972; Courpron 1967; Gamble et al. 1976; Geering and Hodgson 1969; Li et al. 1957; Matsuda and Ikuta 1969; Schnitzer and Hansen 1970; Schubert 1948, 1952; Stevenson et al. 1973). The main difference between those methods and the one that I describe here, the MnO₂ method, is that MnO₂ is a weak ion exchanger: it does not hold metal ions, specifically copper ions, very tightly, while previous methods involved very strong, chelator type, ion exchange resins. Therefore solutions with low complexing capacities and weak stability constants can be
estimated by the MnO₂ method without solution modification.

The idea of the method is that an ion exchanger is brought into equilibrium with metal ions, copper ions in this case, and complexing ligands. The complexing ligands compete with the ion exchanger for metal ions. If one knows the concentration of ligands and the affinity of the ion exchanger for the metal ions, then one determination of the amount of metal ions that the ligands withhold from the ion exchanger is sufficient to determine the affinity of the ligands for the metal ions.

If the ligand concentration is not known, one has to make a number of such measurements while varying either the metal or the ligand concentration, or both. Linear relationships have been developed (Chapter 2) from which both the ligand concentration and its affinity for metal ions, the conditional stability constant, can be readily calculated.

Firstly, the most significant difference between this method and other methods is that a weak rather than a strong ion exchange medium is used. A ligand either needs to have a high affinity for metal ions, or it has to be present in a high concentration if it wants to compete significantly with a strong ion exchanger. A low affinity ion exchanger, such as MnO₂, however, will give a relatively weak, complexing ligand the opportunity to compete successfully for metal ions,
even when the ligand is present in such a low concentration that it only just forms a complex with metal ions.

Since

$$\frac{[CuL]}{[Cu^{2+}] [L']} = K',$$

the complexed copper concentration, $[CuL]$, is equal to the free copper concentration, $[Cu^{2+}]$, when the free ligand concentration, $[L']$, is equal to the conditional stability constant, $K'$: $[CuL] = [Cu^{2+}]$ when $[L'] = K'^{-1}$. In other words, a ligand which is present in a concentration equal to, or higher than, its conditional stability constant will give a sufficient increase in the dissolved metal concentration to determine its affinity for the metal.

In this case the limit of detection depends on the analytical sensitivity of copper in solution and on sources of copper contamination that may be present. It is understandable that this development diminishes the ligand concentration at which a stability constant is determined tremendously.

Secondly, other methods use relatively large amounts of ion exchange resin in order to have a surplus of exchange sites present to fulfil the assumption that the amount of metal ions exchanged by the resin is linearly dependent on the metal ion concentration in solution. In other words, it is assumed that the rate of \( \frac{\text{metal ions in solution}}{\text{metal ions adsorbed per g resin}} \)
is constant and equal to some resin constant, $K_R$. However, this assumption is only true when few exchange sites are occupied.

MnO$_2$ has a rather straightforward ion exchange capability. It is stable and negatively charged over the pH range of natural waters, pH > 3 (pH of zero point of charge). Adsorption of metal ions on MnO$_2$ can readily be approximated by a Langmuir type of equation which describes the adsorption over the entire metal ion site occupancy. As a result one can theoretically work with a very small amount of MnO$_2$ in the medium. Practically it is indeed possible to add very small quantities of MnO$_2$, 20 µM or less, quantitatively, to the sample to be analysed, by making a fine dispersion in distilled water and diluting it.

As a result of working with a low concentration of ion exchange medium the natural water situation is only slightly modified. Not a very large surface area has been added and the chance that unpleasant side effects, such as the adsorption of organic matter onto the medium, will occur, is minimized.

Thirdly, there is no need to work at low pH to keep a high concentration of metal ions in solution, since the ligand concentration is also very low. The MnO$_2$ can be calibrated over the major part of the pH of importance to
natural waters and there is no problem in working at the original pH of each sample.

5.2 PREPARATION OF MATERIALS

Distilled water, free of organics, is prepared daily by distillation of deionised water in presence of permanganate in a glass distiller with a long column filled with little glass rings.

A stock solution of 0.01 M MnO₂ is prepared by mixing stoichiometric amounts of manganese nitrate, potassium permanganate and potassium hydroxide, at molar ratios of 3:2:4 respectively. A premixed solution of potassium permanganate and potassium hydroxide is pipetted into a neutralised solution of manganese nitrate while vigorously stirring with a magnetic stirrer and while taking care that the pH stays neutral. The precipitate is further purified by centrifugation and redispersion in distilled water, whereby the stability of the dispersion increases due to the decrease in ionic strength.

The concentration of manganese dioxide is measured by reducing a sample with oxalate and measuring manganese by atomic absorption spectrometry. The manganese dioxide prepared
as above is amorphous to X-ray diffraction and remains so after two years storage. Analyses have shown that the average oxidation state of Mn in thus prepared manganese dioxide is Mn$^{3.8+}$ and the oxide is MnO$_{1.94}$ indeed approaching manganese dioxide.

The flocculate becomes finely dispersed by stirring the solution after which reproducible quantities are weighed out for adsorption experiments.

A stock solution of $10^{-2}$ M copper is prepared by dissolving copper wire in concentrated nitric acid and diluting with distilled water.

The pH is read in mV and the electrode is calibrated by Gran titration of a carbonate free $10^{-3}$ M potassium hydroxide solution to determine its specific potentials at the ionic strength and temperature of the experiment.

5.3 CALIBRATION OF MnO$_2$

For calibration 500 ml of 100 μM manganese dioxide is titrated with copper at a fixed pH and constant temperature (25°C) and ionic strength (0.01 M KNO$_3$), in a 500 ml flask. The dispersion is continuously stirred with a teflon coated magnetic stirring bar and purged with prepurified
nitrogen to remove carbonate ions from solution. Traces of CO₂ are removed out of the nitrogen by filtering the gas through a column with ascarite interlayered with a drying agent.

Copper additions are made by micropipet, and the pH is kept constant manually by dropwise additions of dilute potassium hydroxide or nitric acid. The solution pH is monitored continuously. After each copper addition one hour equilibrium time is allowed before a subsample of approximately 35 ml is taken, the remaining volume is determined by weighing the flask and the next copper addition is made. Thus the copper concentration is increased in a stepwise manner in a diminishing volume of solution. Copper additions range from 10-100 μM. Of each subsample approximately 15 ml are used to rinse a 0.45 μM Millipore filter, the remaining 20 ml are filtered, acidified with 0.2 ml concentrated HNO₃, and stored overnight. Total dissolved copper is then determined by d.p.a.s.v., plating potential -300 mV, plating time 2.5 minutes, scanning rate 5 mV/sec, pulses with 0.5 sec intervals. D.p.a.s.v. has been chosen since it gives very accurate results and linear response in the range of importance: 0.01 to 10 μM of copper. Three copper measurements are performed on each subsample and the difference is typically ±<1%. Only for the lowest copper concentration
of 0.01 μM, the variation is larger.

A plot of \([\text{Cu}^{2+}]\) vs. \(\frac{[\text{Cu}^{2+}]}{\Gamma_{\text{ads}}}\) will provide the constants, \(\Gamma_{\text{max}}\) and B, which are derived in equation (18) and which are pH specific.

By repetition of the above procedure at different pHs \(\Gamma_{\text{max}}\) and B can be obtained as a function of pH. At pH greater than 7, however, copper becomes increasingly insoluble. Although the free Cu\(^{2+}\) concentration may be high enough to ensure proper coverage of the MnO\(_2\), it becomes too low in solution to be determined without quite large uncertainty and chance of contamination. A 35 μM solution of glycine has been used to increase the dissolved copper concentration at these pHs. A trial at pH 6.7 with and without glycine, showed that the glycine-copper complex does not adsorb on the manganese dioxide and that its stability constant can be accurately reproduced. The calculated Cu\(^{2+}\) concentration has been corrected for the formation of CuOH\(^+\) using \(\log K_{\text{CuOH}^+} = 6.3\) (Smith and Martell 1976).

It appeared that B and \(\Gamma_{\text{max}}\) changed during the first few days, and possibly weeks, after preparation of the oxide, but then became stable for the period of 2 years or more.
5.4 TITRATION OF NATURAL WATER SAMPLES

For the titration of samples or ligand solutions an analogous procedure is applied. Firstly the water is filtered through an acid cleansed 0.45 µm Millipore filter as soon as possible after sampling. Then the sample is stored cool and in the dark. Preferably the titration with copper ions should be done immediately after sampling to avoid loss of ligands by adsorption on container walls.

To 442.5 ml sample 4.5 ml 1 M KNO₃ is added to give 0.01 M KNO₃, and 3 g of a 6.3 x 10⁻³ M MnO₂ dispersion is weighed to give 42 µM MnO₂. The flask is placed in a waterbath at 25°C and is continuously stirred. The flask is covered from the light by aluminium foil to prevent algal growth. The dispersion is purged with nitrogen to eliminate carbonate complexation of copper. Otherwise the correction for the formation of CuCO₃(aq) at pH > 6.5 would involve subtraction of two large numbers to obtain the CuL concentration. Conveniently the pH of the sample is lowered to pH ∼ 5, before purging with nitrogen, to more efficiently remove the carbonates.

When feasible the titration was performed at the equilibrium pH, of the sample, with air. At pHs smaller
than 6, however, both the conditional stability constants, $K'$, of natural ligands and the bindings constant, $B$, and capacity of the ion exchange medium become greatly reduced so that determinations become rather inaccurate due to increased solubility of $Cu^{2+}$ and only partial complexation by the ligands. In such cases the titration was performed at a suitable higher pH. For sample to sample comparability and for convenience a pH of 7.6 was chosen for most samples. When the ligand concentration exceeds 5 μM the sample is diluted. Otherwise interferences occur. For example, high concentrations of organic matter form foam during purging with nitrogen and adsorb on the mercury drop during the polarographical measurements; also the fraction of organic matter that might possibly adsorb on the MnO$_2$ surface, could increase, since this adsorption is a function of the organic matter concentration.

For one complete analysis it was usually sufficient to vary the total copper concentration in ten approximately equal steps from 2 to 20 μM. The pH was kept constant, and between each consecutive copper addition and 35 ml subsample one hour equilibration time was allowed.

The $Cu^{2+}$ concentration is calculated from equation (18), the amount of copper adsorbed, $Γ_{ads}$, and from the appropriate
constants, $B$ and $\Gamma_{\text{max}}$, calibrated for the same pH and ionic strength. Mass balance from the measured total dissolved copper concentration and $[\text{Cu}^{2+}]$ gives the complexed copper concentration, $[\text{CuL}]$. The total ligand concentration, $L$, and the conditional stability constant, $K'$, for the formation of $\text{CuL}$, are then calculated by plotting $[\text{Cu}^{2+}]$ vs. $\frac{[\text{Cu}^{2+}]}{[\text{CuL}]}$ and by using equation (21).

In samples where high concentrations of competing cations are present, it is advisable, for optimal determination of $K'$, to calibrate the $\text{MnO}_2$ in the actual sample, with the ligand removed by filtration or destructed by UV irradiation. Thus, the competing effects of $\text{Ca}^{2+}$ and other cations for sites on the $\text{MnO}_2$ can be evaluated by obtaining a conditional $B'$ and $\Gamma_{\text{max}}'$ for $\text{Cu}^{2+}$ on $\text{MnO}_2$. At high pH, where $\text{MnO}_2$ is calibrated in presence of glycine, the competition of $\text{Ca}^{2+}$ or its affinity for $\text{MnO}_2$, can still be determined by titrating $\text{MnO}_2$ at high $[\text{Ca}^{2+}]$, in presence of glycine. The $K'$ for glycine has to be corrected for $\text{Ca}^{2+}$ competition; then the shift in $B$, relative to $B$ determined without $\text{Ca}^{2+}$, is related to the affinity of $\text{Ca}^{2+}$ for $\text{MnO}_2$, i.e. $\text{MnO}_2$ is then calibrated for use in media containing $\text{Ca}^{2+}$ as competing ions.
5.5 STATISTICAL TREATMENT OF THE DATA

A linear regression to give a and b in \( y = a + bx \) is performed on the data in the form of \([\text{Cu}^{2+}] \) vs. \([\text{CuL}]\), or \( X \) vs. \( Y \) respectively. Then the variance about the regression is calculated, for \( n-2 \) degrees of freedom, since 2 degrees have been used for the calculation of the regression. The following development is based upon the approach of Davies and Goldsmith (1972).

\[
s^2 = \frac{\sum(y-y)^2}{n-2}
\]

where \( Y \) = predicted value from regression
\( y \) = determined value.

For easy calculation this variance simplifies to

\[
s^2 = \frac{S_{yy} - S_{xy}^2/S_{xx}}{n-2}
\]

The standard error in the slope, \( b \), is

\[
S.E.(b) = S/\sqrt{S_{xx}} \quad \text{and}
\]

\[
S_{xx} = \frac{\sum x^2 - (\sum x)^2}{n} = \sum(x-x)^2.
\]

(\( S_{yy} \) and \( S_{xy} \) have similar meaning)
Thus \( b \pm \frac{t_{a} \cdot s}{\sqrt{S_{xx}}} \) where for \( n-2 \) degrees of freedom, and

the variance in \( b \): \( \text{V}(b) = \frac{s^{2}}{S_{xx}} \).

The standard error of the estimate from the regression line for a given value of

\[
x = x_{o} \quad \text{S.E.}(Y_{o}) = s \sqrt{\left(\frac{1}{n} + \frac{x_{o} - x}{S_{xx}}\right)}
\]

where \( \bar{x} \) = mean of \( x \)-values.

For the \( Y \)-intercept \( x_{o} = 0 \), so

\[
\text{S.E.}(Y_{\text{int}}) = s \left(\frac{1}{n} + \frac{x}{S_{xx}}\right) \quad \text{or} \quad a \pm t_{a} \cdot s \left(\frac{1}{n} + \frac{x}{S_{xx}}\right)^{1/2}
\]

and

\[
\text{V}(a) = (\text{S.E.}(Y_{\text{int}}))^{2} = s^{2} \left(\frac{1}{n} + \frac{x}{S_{xx}}\right) = \frac{s^{2} \sum x^{2}}{n \sum (x-\bar{x})^{2}}
\]

\[
= \frac{s^{2} \sum x^{2}}{nS_{xx}}, \quad \text{which is now in a}
\]

form easy to be calculated with a programmable desk calculator.

For the calculation of \( K' \) it is necessary to divide the slope by the \( Y \)-intercept:
\[ S.E.(b/a) = \frac{1}{a} \sqrt{V(b) + \frac{b^2}{a^2} V(a)} \]

and

\[ K' = \frac{b}{a} \pm t_{\alpha, n-2} \cdot S.E.(b/a). \]

The total ligand concentration, \( L \), is calculated from \( \frac{1}{b} \). Calculation of the standard error of \( \frac{1}{b} \) would involve considerable approximations, which were thought to be unwarranted at this stage.

5.6 SAMPLE DESCRIPTION

Samples, usually 2 liters, were taken in plastic containers, filtered as soon as possible, through acid leached and distilled water rinsed 0.45 \( \mu \)m Millipore filters, and stored dark, under refrigeration.

Samples were collected from the following freshwater environments:

- dystrophic waters: low pH, pH 4.6, brown colored waters, containing much organic matter flushed out of soils.
  The rivers Dickie 5, 6, and 10, Red Chalk 3 and 4, and Dickie Lake belong to this group.

- medium alkalinity, \( \approx 0.5 \) meq/L, low productivity, non-polluted: Windy Lake.
- high alkalinity, ~2 meq/L, non-polluted: Lake Huron.
- high alkalinity, ~2 meq/L, medium high algal productivity, but no bloom: Gloucester Pool, Lake Ontario, Onaping River (flows out of Lake Onaping).
- high alkalinity, ~2 meq/L, algal bloom: Bay of Quinte, in early summer.
- rather heavily polluted with metals, low alkalinity, ~0.1 meq/L, and having a medium high production: Whitewater Lake, near Sudbury.
- heavily polluted with metals, acidified lake: Lake Whitson, near Sudbury.
- fulvic acid: supplied by Schnitzer, extracted from soil and dried (Schnitzer and Skinner 1968).

5.7 SUMMARY

Preparation and calibration are described of an aged dispersion of MnO₂. The MnO₂ will be used as ion exchange medium for the determination of stability constants for weak metal complexes.

At pH < 7 calibration of the constants in the Langmuir equation, Γₘₐₓ and B, takes place by a simple titration with
Cu$^{2+}$ at fixed pH. At pH > 7 a weak ligand (glycine) is added to act as a metal buffer to keep copper in solution and to increase the dissolved copper concentration. Total dissolved copper is measured by anodic stripping voltammetry of the MnO$_2$ free, filtered dispersion.

For analysis of ligands in natural waters MnO$_2$ is added in a very small quantity only (~40 µM) so as to disturb the natural water situation as little as possible. Carbonate ions are normally removed out of solution by purging with nitrogen, but measurement can be performed in presence of carbonate ions as well.

A statistical treatment of the data in linearized form is provided, and the different sample localities are described. The samples are from lakes and rivers varying in composition from dystrophic to highly alkaline (~2 meq/L).
The absence of alternatives clears the mind marvelously.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 CALIBRATION OF MnO₂

The constants, B and Φ, in the Langmuir equation (18), have been calibrated over a range of pHs between pH 5.5 and 8.5. Each data point in Figure 6.1 is the result of a complete titration at that pH. A smooth curve has been drawn through the data points. There is indeed some justification for drawing this curve. In some previous experiments the pH was varied while keeping the copper concentration constant. An average ion exchange constant, K_R, can then be calculated for the equilibrium between dissolved and adsorbed copper ions. The data points, describing K_R as a function of pH (Figure 6.2) followed the shape of B (Figure 6.1) quite closely but deviated downward at pH < 6. This is due to the fact that K_R is only constant when MnO₂ is barely covered by Cu²⁺ ions. At higher coverage K_R becomes a decreasing function of the Cu²⁺ concentration. When the pH
Figure 6.1 Calibration of bindings constant, $B$, and cation exchange capacity, $\Gamma_{\text{max}}$, of MnO$_2$ as function of pH, for Cu$^{2+}$.

The curves are graphical approximations of the data. The 2 $\sigma$ variation of log $B$ is between $\pm 0.03$ and $\pm 0.10$. 
Figure 6.2  Conditional stability constant, $\log K_R'$, for the adsorption of copper on $\text{MnO}_2$, as a function of pH.

$[\text{MnO}_2] = 10^{-4} \text{ M}$, $[\text{Cu}_T] = 2 \times 10^{-5} \text{ M}$,

$\mu = 0.01 \text{ M KNO}_3$; 25°C, $K_R = \frac{r_{ads}}{[\text{Cu}']}$

$[\text{Cu}'] = [\text{Cu}_{\text{dissolved}}] = [\text{Cu}^{2+}] + [\text{CuOH}^+]$
is lowered $\Gamma_{\text{max}}$ decreases (Figure 6.1) and additional copper ions are released. The constant $B$ really represents $K_R/\Gamma_{\text{max}}$. The variation of $K_R$ with the pH is obtained more readily than that of $B$, since only a single acid/base titration is required. Therefore more $K_R$ data are available to characterize the variation with pH. Because $K_R$ showed a continuously curved relationship with the pH, I felt justified in drawing a similar curve through the more scanty data relating $B$ and $\Gamma_{\text{max}}$ with the pH. From this curve $B$ and $\Gamma_{\text{max}}$ were chosen at the appropriate pH of titrated samples.

$\log B$ increases with the pH with a slope of 1.0 when $\text{pH} > 7.5$ and seems to level off at $\text{pH} < 6.5$. $\Gamma_{\text{max}}$ increases over most of the pH, but seems to level off at $\text{pH} > 8$. Extrapolation to higher pH provides a terminal adsorption capacity of almost 1 mole Cu/mole MnO$_2$. Such a high capacity indicates that this MnO$_2$ is extremely porous; copper ions presumably are able to penetrate between the rather loose framework that the MnO$_2$ molecules form.

The magnitude of $\log B$, $\log B > 6$ and increasing with pH on a 1:1 basis when $\text{pH} > 7$, fits very conveniently with the magnitude of stability of natural ligands in the environment as was discussed in Chapter 3. At pH 8, a pH commonly found in waters of medium to high alkalinity, $\log B \sim 7.4$ and $\log K' \sim 7.5$ (section 3.6). This very
similar magnitude ensures an even distribution of $Cu^{2+}$ over $MnO_2$ and ligands. For stronger ligands an increase in $MnO_2$ concentration will restore this equilibrium. Furthermore, the linear increase of $\log B$ with $pH$, when $pH > 7$, corresponds very conveniently with the linear increase of $\log K'$ for monoprotonated ligands (section 2.2). So $K'$ of the same monoprotonated ligand can be determined at any $pH > 7$ with the same ratio of $\frac{B}{K'}$. Due to the variation in adsorption capacity, however, more copper should be added at higher $pH$ to obtain the same $Cu^{2+}$ concentration, or conversely, a slightly smaller amount of $MnO_2$ will do the job.

6.2 COMPARISON OF ADSORPTION MODELS

For the calculation of $B$ it was assumed that only $Cu^{2+}$ ions adsorb. $Cu^{2+}$ ions have a low entropy when in solution and the high charge induces a strong Coulombic effect. Such factors ought to favor $Cu^{2+}$ in the adsorbed phase. However, there are some adsorption models in existence which explain metal adsorption in terms of the adsorption of hydroxylated ions (section 1.4). Hydroxylated ions are then strongly favored over free ions in the
adsorbed phase.

I have attempted to calculate the effect that such a model would have on the Langmuir constant of adsorption, B. It appears (Figure 6.3) that log B strongly decreases with the pH when pH < 7, and evens out after that. Log $K_R$ has been drawn in the same Figure and shows a bit more erratic behavior due to the increase in $\Gamma_{\text{max}}$ with the pH. The behavior of log B does not seem to have a rational explanation. One expects indeed B to increase with pH since charge difference between Cu$^{2+}$ and CuOH$^+$ ions on one side and MnO$_2$ on the other side, increases. So from this rational point of view one would conclude that adsorption of copper on MnO$_2$ is not mainly governed by hydroxylated ions.

For comparison a second case has been calculated assuming the adsorption of both Cu$^{2+}$ and CuOH$^+$ ions on MnO$_2$ with equal affinity. Log B in Figure 6.3 is now similar to log B in Figure 6.1, where it represents solely Cu$^{2+}$ adsorption. But the increase in log B, representing both Cu$^{2+}$ and CuOH$^+$ (Figure 6.3), with pH tapers off partially when pH > 8. Still, such a model cannot be simply dismissed. Log B does indeed increase over the pH range which was investigated. It should also be considered that B, calculated as it is here from titrations of MnO$_2$ with copper, is not completely thermodynamically valid. B should have been
Figure 6.3 Comparison of adsorption models for copper on MnO₂:

1: adsorption depends on CuOH⁺

2: adsorption depends on Cu²⁺ and CuOH⁺

B: exchange constant in Langmuir equation

$K_R: B \times \Gamma_{max}$

$\Gamma_{max}$: maximum adsorption capacity for copper
calculated for a surface of constant charge. And presumably the negative charge on the MnO$_2$ will decrease when it is titrated with copper. Yet, this charge can be considered to be a statistical effect. The charge per site does not really change, or not as much as the statistical charge of the complete surface. So, the error in my calculation of B may not be all that large and will not affect the general trend.

Conclusion

A model describing adsorption of copper on MnO$_2$ as the adsorption of CuOH$^+$ ions solely, does not appear to be a reasonable assumption; adsorption can best be thought of as of Cu$^{2+}$ alone, possibly combined with its hydroxylated form, CuOH$^+$. 

6.3 TEST OF THE MnO$_2$ METHOD ON SOME KNOWN LIGANDS

The correctness of the basic assumptions of the MnO$_2$ method was tested by determinations on some known ligands. Conditional stability constants were determined of a very weak ligand, glycine, two stronger ligands, 8-hydroxyl-quinoline (8-OH-Q), nitrilo-tri-acetate (NTA), and of a familiar inorganic ligand, CO$_3^{2-}$ (Table 6.1). The glycine
Table 6.1 Log conditional stability constants as determined with the 
MnO<sub>2</sub> method, compared with literature values, corrected for 
the pH. All determinations in 0.01 M KNO<sub>3</sub>, 25°C; 2σ 
confidence limits as indicated.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>log K&lt;sup&gt;t&lt;/sup&gt; by MnO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>log K&lt;sup&gt;t&lt;/sup&gt; literature</th>
<th>pH</th>
<th>concentration (µM)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycine</td>
<td>4.84±0.09</td>
<td>4.78±0.06</td>
<td>6.0</td>
<td>10-100</td>
<td>Martell and Smith 1974</td>
</tr>
<tr>
<td>carbonate</td>
<td>K 6.8</td>
<td>6.77</td>
<td>7.7</td>
<td>air</td>
<td>Stumm and Morgan 1970</td>
</tr>
<tr>
<td>8-hydroxyquinoline</td>
<td>K&lt;sub&gt;1&lt;/sub&gt; 9.9±0.3</td>
<td></td>
<td>7.6</td>
<td>0.23</td>
<td>Sillen and Martell 1964</td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;1&lt;/sub&gt; 9.4±0.35</td>
<td>9.0-10.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;1&lt;/sub&gt; 8.5±0.25</td>
<td></td>
<td></td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;1&lt;/sub&gt; 9.3±0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;0&lt;/sub&gt; 8.8±0.3</td>
<td></td>
<td></td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;1&lt;/sub&gt; 9.25±0.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTA</td>
<td>8.55±0.18</td>
<td>9.9</td>
<td>7.2</td>
<td>1.2</td>
<td>Ibid.</td>
</tr>
<tr>
<td></td>
<td>8.9±0.4</td>
<td>10.3</td>
<td>7.6</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.2±0.4</td>
<td>10.9</td>
<td>8.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>NTA in Ontario water</td>
<td>8.10±0.13</td>
<td>9.9</td>
<td>7.2</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.2±0.2</td>
<td>11.1</td>
<td>8.4</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>
concentration was varied from 10 to 100 μM, while the copper concentration was kept constant at 3 μM. The conditional stability constant of glycine is very close to the pH-corrected value from the data of Martell and Smith (1974) and does in fact coincide within the 2 σ confidence limits. Glycine is at this pH a very weak ligand, and even at its highest concentration, 100 μM, only part of the copper is complexed. Yet K' has been very accurately determined, which shows the effectiveness of this method for weak ligands. The conditional stability constant of 8-OH-Q has been determined at a very low ligand concentration, 0.2 μM, due to its low solubility. It is capable of forming 2:1 (CuL₂) complexes and K₂' is only a bit smaller than K', about one order of magnitude (Sillen and Martell 1964). A clear knick is visible in the plot of [Cu²⁺] vs. [Cu₂⁺] in [CuL] Figure 6.4. There were not enough data points after the knick for calculation of K₂'. Three titrations of 8-OH-Q produced essentially the same value for K': log K' is 9.4, 9.3 and 9.25 respectively. An average constant, K₀', using all the data points, also those after the knick in the curve, is also given. The literature values (Table 6.1) for 8-OH-Q vary greatly, which is at least partially due to variation in ionic strength.
Figure 6.4 Titration of 8-hydroxyquinoline (8-OH-Q) with Cu$^{2+}$.

$\mu = 0.01$, $25^\circ C$, pH 7.6; 60 $\mu$M MnO$_2$, 0.14 $\mu$M 8-OH-Q.

Conditional stability constants:

$\log K'_O = 8.5 \pm 0.25$ (overall constant)

$\log K'_1 = 9.3 \pm 0.2$
NTA was treated as an unknown ligand and was analysed at several pHs in distilled water and in filtered Lake Ontario water. The sample from Lake Ontario was collected in the winter and the ligand concentration was very low, 0.1 μM, so as not to have a significant effect. The literature values were adapted for these pHs, but at a 10x higher ionic strength. The conditional stability constants analysed in distilled water are slightly smaller than the literature values at all pHs, but they do show the same strong increase in magnitude with the pH, since one proton is involved in exchange. When the same experiment was performed on Lake Ontario water, lower values were found for K'. It is tempting to explain this decrease by competition by other cations, such as Ca^{2+}, Mg^{2+} and heavy metal ions (very low concentrations). It should be taken into account that the 2 σ error was quite large, up to ±0.4 at the higher pH determinations, where K' is quite large, much larger than the MnO₂ exchange constant, B. Probably NTA is too strong a ligand, the Cu^{2+} balance is too much on the NTA side, and as a result large subtraction errors arise in the determinations.

Any decrease of K' as a result of competition by other cations diminishes with the pH because the conditional stability constants of the competing ions decrease with the pH. This shows in the NTA experiments: at pH 8.2 the.
the difference between the values determined in Lake Ontario water and distilled water is 1.0, at pH 7.2 it is 0.45. The exact effect of Ca\(^{2+}\) for example on K' for NTA and Cu\(^{2+}\) cannot be evaluated from these experimental data since MnO\(_2\) had not been calibrated for these conditions.

The carbonate constant was determined by bubbling the MnO\(_2\) dispersion in the presence of Cu with air. The CO\(_{3}^{2-}\) concentration was then calculated from the equilibrium with CO\(_2\). The result is very close to the literature value. Lower limit on the ligand concentration: it should be noted that in those cases where the dissolved copper concentration, [Cu\(^{2+}\)] + [CuL], was smaller than 0.1 µM, the determined copper concentrations usually varied rather erratically. Contamination, especially from the filtration step, is probably the cause. These contamination effects put lower limits on the ligand concentration that can be determined.

**Summary**

Determinations of known ligands show that the MnO\(_2\) method reproduces conditional stability constants accurately (log K' ± 0.1) when the ligand concentration is larger than 0.15 µM and when the conditional stability constant is not too much different from the exchange constant, B, of MnO\(_2\), i.e. when 6 < log K' < 10 at pH 8.
6.4 APPLICATION OF THE MnO₂ METHOD TO SAMPLES OF LAKES AND RIVERS

The MnO₂ method has been applied to a number of lakes and rivers. The effect on the method of the removal of carbonate ions, of changes in the pH and of variations in the ligand concentration has been tested. The results are compared with analyses of fulvic acid (FA) from soil and of siltrates of algal cultures. The ligand concentrations found in natural waters using the MnO₂ method, are compared to the light absorption of the sample at specific wavelengths.

6.5 COMPLEXING CAPACITIES AND CONDITIONAL STABILITY CONSTANTS OF NATURAL WATERS

The rivers Dickie 5, 6, 10, Red Chalk 3, 4 and Dickie Lake have high complexing capacities varying from 2.2 μM for Dickie Lake to 33 μM for Dickie 5 (Table 6.2). All these waters are brown/yellow colored, as rather light tea, and have a low pH, pH 4.6. The colored component of these waters very probably is derived from neighbouring soils and is composed of FAs, HAs and breakdown products of
<table>
<thead>
<tr>
<th></th>
<th>pH of analysis</th>
<th>L (μM)</th>
<th>log K'±2 σ</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Ontario</td>
<td>8.4</td>
<td>0.25</td>
<td>9.5±0.3</td>
<td>concentrated sample</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>0.33</td>
<td>9.5±0.3</td>
<td>concentrated sample</td>
</tr>
<tr>
<td></td>
<td>7.4 (8.4)</td>
<td>0.34</td>
<td>8.6±0.3</td>
<td></td>
</tr>
<tr>
<td>Bay of Quinte</td>
<td>7.6 (8.4)</td>
<td>2.79</td>
<td>7.62±0.10</td>
<td></td>
</tr>
<tr>
<td>Hamilton Harbour (air)</td>
<td>8.5</td>
<td>2.04</td>
<td>7.72±0.19</td>
<td>uncorrected for CO₃⁻</td>
</tr>
<tr>
<td>plus 10⁻²M NaAc buffer</td>
<td>5.9</td>
<td>4.2</td>
<td>5.50±0.30</td>
<td>uncorrected for Ac⁻</td>
</tr>
<tr>
<td>Lake Huron (air)</td>
<td>8.3</td>
<td>0.4</td>
<td>9.9±1.1</td>
<td>corrected for CO₃⁻</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>8.0±0.14</td>
<td></td>
<td>uncorrected</td>
</tr>
<tr>
<td>Whitewater (air)</td>
<td>8.0</td>
<td>0.6</td>
<td>9.3±0.5</td>
<td>corrected for CO₃⁻</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td>8.77±0.16</td>
<td></td>
<td>uncorrected</td>
</tr>
<tr>
<td>Gloucester Pool (air)</td>
<td>8.4</td>
<td>0.7</td>
<td>9.3±0.4</td>
<td>corrected for CO₃⁻</td>
</tr>
<tr>
<td></td>
<td>1.21</td>
<td>8.68±0.14</td>
<td></td>
<td>uncorrected</td>
</tr>
<tr>
<td>Onaping River (air)</td>
<td>7.8</td>
<td>0.51</td>
<td>8.50±0.27</td>
<td>corrected for CO₃⁻</td>
</tr>
<tr>
<td></td>
<td>0.82</td>
<td>8.17±0.19</td>
<td></td>
<td>uncorrected</td>
</tr>
<tr>
<td>Windy (air)</td>
<td>6.64</td>
<td>0.62</td>
<td>6.71±0.31</td>
<td>corrected for CO₃⁻</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>6.63±0.31</td>
<td></td>
<td>uncorrected</td>
</tr>
<tr>
<td>Whitson</td>
<td>8.2 (4.7)</td>
<td>&lt;0.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dickie 5</td>
<td>7.6 (4.6)</td>
<td>2.47 (33)</td>
<td>7.75±0.13</td>
<td>diluted sample</td>
</tr>
<tr>
<td></td>
<td>11.5 (33)</td>
<td>7.47±0.20</td>
<td></td>
<td>diluted sample</td>
</tr>
<tr>
<td></td>
<td>8.4 (4.6)</td>
<td>5.36 (33)</td>
<td>8.49±0.10</td>
<td>diluted sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>---</td>
<td>---</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Dickie 6</td>
<td>7.6 (4.6)</td>
<td>5.75</td>
<td>7.82±0.13</td>
<td></td>
</tr>
<tr>
<td>Dickie 10</td>
<td>7.6 (4.9)</td>
<td>4.95 (10.9)</td>
<td>7.77±0.09</td>
<td>diluted sample</td>
</tr>
<tr>
<td>Lake Dickie</td>
<td>7.6 (4.6)</td>
<td>2.19</td>
<td>7.76±0.08</td>
<td></td>
</tr>
<tr>
<td>Red Chalk 3</td>
<td>7.6 (6.3)</td>
<td>3.35</td>
<td>7.68±0.09</td>
<td></td>
</tr>
<tr>
<td>Red Chalk 4</td>
<td>7.6 (4.7)</td>
<td>4.20</td>
<td>7.57±0.10</td>
<td>Kᵩ</td>
</tr>
<tr>
<td></td>
<td>2.42</td>
<td>7.94±0.11</td>
<td>Kᵢ</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.10</td>
<td>7.6±0.7</td>
<td>Kᵢ</td>
<td></td>
</tr>
<tr>
<td>FA, soil</td>
<td>7.6 (4.6)</td>
<td>7.56</td>
<td>7.95±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.24</td>
<td>7.83±0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- in brackets: the original sample equilibrium (with air) pH and the original ligand concentration when different from the analysed pH or concentration.

- Kᵩ represents an average stability constant ignoring the presence of a knick in the plot, using all the data and assuming that only one site is present.

- the samples which have been titrated in presence of air (containing CO₂) have been indicated by (air) behind the sample name.
bacteria. In all but one case, only one exchange site was found in the water samples since the plot of $[\text{Cu}^{2+}]$ vs. $[\text{Cu}^{2+}]_{[\text{CuL}]}$ produced straight lines. The conditional stability constants determined at pH 7.6 showed remarkably little variation: $\log K'$ varied from $7.68 \pm 0.09$ to $7.94 \pm 0.11$ with an average of 7.79. Where more than one dilution had been analysed the result of the lower concentration was chosen as better representing the ligand, since it was noticed that at high ligand concentrations (a) the copper balance is rather much in favor of the ligands, and (b) foam formation during the polarographical analysis, and possibly adsorption effects on the $\text{MnO}_2$, disturb the measurements resulting in larger 2 $\sigma$ errors.

The conditional stability constant of FA at pH 7.6 falls in the same range as the dystrophic waters and is in fact the same as the average of 7.79 within the 2 $\sigma$ deviation. The similarity of the plots in Figure 6.5 indicates that the complexing groups on the molecules may well be the same for ligands in dystrophic waters and for FA, and that the rest-groups of the molecules, if different from each other, do not affect the binding strength appreciably.

Mantoura and Riley (1975) who worked at a pH similar to the present experiments and who also used a system where
Figure 6.5 Titration with Cu$^{2+}$ of FA and dystrophic river water (Dickie 5) in presence of 40 μM MnO$_2$, μ = 0.01 M KNO$_3$, 25°C, pH 7.6. Ligand concentration is 2.24 μM and 2.47 μM for FA and Dickie 5; log K' = 7.83±0.15 for FA, and log K' = 7.75±0.13 for Dickie 5.
ligand and metal-are in equilibrium with each other, found constants for FA quite similar to those presented here. They found log $K' = 8.4$ at pH 8.

The other samples are from lakes and from a river flowing out of a lake. All these waters sustain some sort of primary production varying from very low, in Lake Huron, to very high, in Bay of Quinte, a rather eutrophicated bay of Lake Ontario, sustaining at the time of sampling a blue-green algae bloom. The ligand concentration in these lakes varies from 0.3 $\mu$M in Lake Ontario to 2.8 $\mu$M in the Bay of Quinte. When no bloom is present the ligand concentrations appear to vary between 0.3-0.7 $\mu$M (corrected for $\text{CO}_3^-$), which is between 1 and 2 orders of magnitude less than in dystrophic rivers. It should be noted that the total copper concentration in natural, unpolluted waters generally is an order of magnitude lower again; e.g. the dissolved copper concentration in Lake Ontario is 0.05 $\mu$M. So the ligands are totally undersaturated with respect to copper.

The conditional stability constant, log $K'$, of ligands in lake waters, varies from 6.7 at pH 6.6, to 9.5 at pH 8.4. There does not appear to be the same sort of clustering as displayed by dystrophic waters.

Some of the samples were titrated in equilibrium with
air, so carbonates were present. Both the ligand concentrations and the conditional stability constants have been given as corrected and uncorrected values (Table 6.2). For the uncorrected values it was assumed that Cu\(^{2+}\) was complexed by organic ligands (and OH\(^-\)) only, which results in an overestimation of the complexing capacity and of the conditional stability constant due to carbonate complexation. At high pH, where the carbonate alkalinity is higher, this overestimation is larger than at low pH. The CO\(_3\)\(^-\) corrections were performed by calculating the amount of CuCO\(_3\)\(^{2-}\)(aq) in equilibrium with the then known concentration of Cu\(^{2+}\), and deducting [CuCO\(_3\)\(^{2-}\)(aq)] from the dissolved copper concentration to obtain [CuL]. Especially at high pH such a correction, though necessary, increases the error in the determination. Since it is not easy to keep the partial pressure of CO\(_2\) constant during the experiment and small changes in pCO\(_2\) effect large changes in [CO\(_3\)\(^-\)], quite a large variation in the complexed copper concentration is observed. Such errors become propagated by the deduction, and end up as large errors on small values. For this reason the 2σ errors for the samples analysed in presence of CO\(_3\)\(^-\) are quite large.

The correction for CO\(_3\)\(^-\) shows (Figure 6.6) that by the presence of CO\(_3\)\(^-\) the ligand concentration is overestimated
Figure 6.6 Overestimation of $K'$ and $[L]$ by the presence of $\text{CO}_3^{2-}$ in equilibrium with air-$\text{CO}_2$ in the sample.

$L' = \text{apparent ligand concentration in presence of CO}_3^{2-}$,
$L = \text{ligand concentration corrected for CO}_3^{2-}$, and
$\Delta \log K' = \text{overestimation of log } K' \text{ by the presence of CO}_3^{2-}$. 
by ~ 70% at pH 8.4 to ~ 10% at pH 6.6, and the conditional stability constants are overestimated by a factor of 4 at pH 8.4, to a factor of 1.2 at pH 6.6 (for these estimates the data of Lake Huron have not been used since the corrected value for $K'$ had too large a 2σ error); on the log scale, $K'$ is overestimated by ~ 0.6 units at pH 8.4 and by ~ 0.1 units at pH 6.6. The magnitude of these errors depends on the product of the ligand concentration and its conditional stability constant: $K' \cdot L$. When $K' \cdot L$ is large the effect of carbonate ions will be small since $[Cu^{2+}]$ will be small. Therefore no linear regression has been performed on the data in Figure 6.6; it should be considered a rough sketch, valid only for the conditions of the particular lakes.

The sample of Hamilton Harbour has been measured at pH 8.5 in presence of $CO_3^-$, and at pH 5.9 with a dilute NaAc buffer, 0.01 M NaAc (Table 6.2). The log conditional stability constant of $Ac^-$ for $Cu^{2+}$ is about 2 at this pH so about equal amounts of free $Cu^{2+}$ ions and $CuAc^+$ ions are present. Indeed the apparent ligand concentration determined at pH 5.9 appears to be a factor of 2 higher than when determined in presence of $CO_3^-$, The apparent ligand concentration at pH 8.5 determined in presence of $CO_3^-$ probably is overestimated as well by a factor of 2, and $log K'$ by about 0.7 (an actual correction for $CO_3^-$ complexation
gave too much variation in the data points). Samples of one of the lakes, Lake Windy, were filtered, stepwisely, through Amicon ultrafilters. Though at each filtration step organics were removed (concentrated above the filter), the organics causing complexation were removed only by the UM 05 filter, which has a molecular weight cut off of \( \approx 500 \). These complexing organics were not retained by the PM 10 filter, which cuts off at a molecular weight of \( \approx 10,000 \). So the molecular weight of the complexing ligands in Lake Windy is between 500 and 10,000.

**Summary**

- It does not appear advisable to perform the MnO\(_2\) experiment in presence of CO\(_3^2\) ions, or any other buffer which also complexes Cu\(^{2+}\) ions, since (a) correction for CO\(_3^2\) complexation diminishes the accuracy considerably and (b) both \( K' \) and \( L \) are systematically overestimated when no correction takes place.

- Log \( K' \) values of dystrophic waters and FA cluster around 7.8 at pH 7.6.

- Other lake waters appear to produce various log \( K' \)s.

- Ligands in natural waters are largely undersaturated with respect to copper ions.
6.6 EFFECT OF pH ON CONDITIONAL STABILITY CONSTANTS.

A number of waters have been analysed at their original pH, some have been adjusted to pH 7.6 for inter-comparison, and some have been analysed twice at different pHs. In Figure 6.7 the data have been plotted; lines of slope 1 have been drawn through the data sets to show the suggested variation of log $K'$ with pH.

The dystrophic waters all give very similar values at pH 7.6, and the log constant increases with a slope close to 1 with the pH: for Dickie 5, log $K'$ is 7.75 at pH 7.6, and 8.49 at pH 8.4, relating to a slope of 0.93.

The other samples provided generally higher constants. The $K'$ determined for Lake Ontario is an order of magnitude higher than F3. Again, a shift with the pH with a slope close to 1 was observed: Lake Ontario, log $K'$ is 9.5 at pH 8.4, and 8.6 at pH 7.4, a slope of 0.9.

This pH-dependence confirms at high pH the trend which had been shown to exist at lower pH for humic ligands mainly isolated from soils (section 3.6): log $K'$ of HAs and FAs, and now also of ligands in natural waters, increases with the pH with a slope of 1, from pH 5 to pH 8.5, and probably at pHs higher than pH 8.5 since no definitive decrease has been observed in the slope yet. Apparently
Figure 6.7 Conditional stability constants for copper complexes with ligands in natural waters and with FA, as function of the pH of determination. Straight lines of slope = 1 have been drawn for reference.

o = dystrophic waters
x = lake water
Δ = FA
the ligand in Lake Ontario is still monoprotonated at pH 8.5, so it must have an acidity constant \((pK_a)\) larger than 8.5.

6.7 DETERMINATION OF CONDITIONAL STABILITY CONSTANTS FOR MIXED LIGANDS AND FOR COMPLEXES OTHER THAN 1:1

Schubert's (1952) study of the complexation of cations by an ion exchange technique was commented upon by I. Feldman, who mentioned that the method works only for 1:1 complexes. This is inherent to any method, however, which does not vary the ligand concentration considerably.

In case of a mixture of ligands an average stability constant may be determined (Allen et al. 1976). But the presence of an obvious knick in the plot of 8-hydroxyquinoline (Figure 6.4) shows that the \(\text{MnO}_2\) method is capable of accurately determining two sites or two ligands if these two sites are present in not too much differing concentrations, and if the sites have sufficiently different conditional stability constants relative to discerning slope changes; i.e. if \(K'\cdot L_{2\text{nd ligand}} < K'\cdot L_{1\text{st ligand}}\). In other cases the graphical representation of the titration
will be slightly curved concavey. It is necessary in any case to continue the titration to as high as possible Cu$^{2+}$ concentration to obtain data for the second part of the plot.

Except in one case, the results produced almost straight lines and narrow confidence limits for the slope showing that one site or ligand is at least in a very dominant position. Although a second site or ligand may appear to be present at higher Cu$^{2+}$ concentration, its presence would have negligible influence on the state of copper at the low Cu$^{2+}$ concentrations in natural waters.

6.8 LIGHT ABSORPTION AT 260 nm AND COMPLEXING CAPACITY

The concentration of humic matter in water has been related to measurements of light absorption at different wavelengths, such as 365 nm and 250 nm (de Haan 1975; Hazen 1892). I tried to relate absorption to ligand concentration. Scanning spectrophotometric measurements were performed on some of the samples from 220 to 380 nm. The peak height generally decreases going to higher wavelengths and the peak became rather small to make accurate measurements
Figure 6.8 Adsorption at 260 nm as function of the complexing capacity of natural water samples.
at 365 nm. In most samples, however, a plateau that lasted from 255 to 265 nm, and sometimes from 250 to 270 nm, was found. It was then decided to measure the absorption at the height of this plateau, at 260 nm. The low pH samples were brought to pH 7 by the addition of sodium bicarbonate buffer until $10^{-3}$ M bicarbonate was present. The results are given in Figure 6.8, as absorption vs. complexing capacity. The data scatter rather widely, which suggests that one would have a hard time to accurately predict the complexing capacity from an absorption measurement. One cannot say more than that high absorption might indicate a high complexing capacity. Probably only part of the absorption is caused by complexing material. The rest is caused by other organic material.

6.9 THE USE OF MnO$_2$ WITH OTHER METAL IONS

Previous discussion shows that MnO$_2$ is an extremely useful medium for Cu$^{2+}$. One wonders of course whether it might be of similar use to other metal ions. One has to keep in mind however that a good balance of forces binding the metal ion to the MnO$_2$ and to the ligand is essential. Quite probably the MnO$_2$ and the ligand bind
by different means: MnO₂ by surface hydroxyl groups, ligands in natural waters by the combination of carboxyl groups and hydroxyl groups, a salicylic acid type of complex. It is imaginable that a different metal ion is bound stronger than Cu²⁺ by MnO₂, but at the same time is complexed less strongly by the ligand, because of different specificity of bonding.

A preliminary calibration of MnO₂ has been performed to test its affinity and its capacity to bind Cd²⁺ and Pb²⁺ ions. At pH 7.6 it has been titrated with these ions, similarly to the titrations with Cu²⁺ ions. The symbols have the same meaning as before.

For Cd²⁺: \( \Gamma_{\text{max}} = 0.15 \) and \( \log B = 6.4 \),

Pb²⁺: \( \Gamma_{\text{max}} = 0.57 \) and \( \log B = 8.4 \), compared with

Cu²⁺: \( \Gamma_{\text{max}} = 0.68 \) and \( \log B = 7.04 \).

It appears that while the maximum capacity of MnO₂ for Pb and Cu is about equal, it is considerably less for Cd. Also the affinity for Cd is lowest while for Pb it is 1.4 orders of magnitude larger than for Cu.

I have not attempted to titrate a natural water since these metals are more prone to interference, such as competition by other ions. But comparison with stability
constants with other ligands may give some ground for prediction:

<table>
<thead>
<tr>
<th>Log K:</th>
<th>Cd</th>
<th>Pb</th>
<th>Cu</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>salicylic acid</td>
<td>5.6</td>
<td>-</td>
<td>19.6</td>
<td>Martell and Smith (1977)</td>
</tr>
<tr>
<td>glycine</td>
<td>4.2</td>
<td>(5.5)</td>
<td>8.2</td>
<td>ibid (1974)</td>
</tr>
<tr>
<td>carbonate</td>
<td>3.5</td>
<td>6.1</td>
<td>8.2</td>
<td>ibid (1976), Bilinski et al. (1976)</td>
</tr>
<tr>
<td>HA (pH 7.6)</td>
<td>7.9</td>
<td>9.1</td>
<td>10.3</td>
<td>Takamatsu and Yoshida (1978)</td>
</tr>
</tbody>
</table>

All four ligands have smallest affinity for Cd\(^{2+}\). The affinity of CO\(_3^2\) ions for Pb is slightly less than for Cu, of glycine possibly a lot less for Pb than for Cu. The affinity of HA follows the order Cu > Pb > Cd, an order of magnitude in between each next metal.

Comparison of the affinities of these metals for MnO\(_2\) and for ligands suggests that MnO\(_2\) might possibly be too strong for Pb, but could be in the right range for Cd. For Pb one would have to decrease, for Cd increase the amount of MnO\(_2\), compared with the MnO\(_2\) concentration used in the experiments with Cu.
6.10 MOLECULAR WEIGHT OF FA

FA was supplied by Schnitzer in dried form and extracted from soil (Schnitzer and Skinner 1965). If one assumes the complexation of one copper ion by one FA molecule and then determines the ligand concentration, one can calculate the molecular weight of FA to be 841, which compares well to the value of 950 as found by Schnitzer (Hansen and Schnitzer 1969).

6.11 ADSORPTION OF THE METAL COMPLEX ON MnO₂

Adsorption of the metal-organic complex, or part of it, on the ion exchange medium would affect the determination of the stability constant in that the dissolved copper concentration will be decreased. Previous studies using ion exchange resins observed no adsorption effects (Schubert 1952) or do not mention it. The present results with known ligands show that adsorption, if any, of the complex does not affect the measurement and the results. It was observed, however, that the organic matter has some sort of surface active effect on the MnO₂ dispersion: subsamples, taken during the titration with copper, of waters containing a high
ligand concentration (10 μM) take more time, up to about twice as long to be filtered than samples containing a very low ligand concentration (10 μM). Also, the type of sample seems to affect the filter speed. A sample of Lake Ontario, containing 0.5 μM ligands, has roughly the same effect as a 5 μM FA solution. Apparently the size and complexity of the molecules determines in what way the MnO₂ dispersion will be stabilized.

Adsorption of molecules of the size of FA and smaller is determined for a great part by differences in electrical charge between it and the surface (section 3.8). From a Coulombic-forces point of view one can speculate that when the copper binding ability of the ligand and the MnO₂ is well balanced, the charges will be equal over the entire titration, so that little or no adsorption will occur: when no copper is added both the ligand and the MnO₂ have a negative charge; during the titration with copper, both obtain a neutral to positive charge at the same time.

When the ligand binds copper ions stronger than the MnO₂, the process can be thought to go as follows: (a) no copper added, both negative; (b) some copper added, the ligand saturates firstly and obtains a neutral-positive charge, while the MnO₂ might still have some negative charge; (c)
more Cu added, both are neutral-positive. Some adsorption could be thought to take place in the (b) situation. During the titration of natural samples such an unpleasant behavior was never observed however. The binding strengths always appeared to be well balanced between ligand and MnO₂, and straight lines followed when a plot was made of [Cu²⁺] vs. [CuL], which suggest that no significant adsorption occurred of the ligand or the complexed ligand.

6.12 SUMMARY

The constants in the Langmuir equation, describing adsorption of copper on MnO₂, are calibrated for pHs between 5.5 and 8.5: the maximum adsorption capacity, \( \Gamma_{\text{max}} \), varies from \( \sim 0.1 \) at pH 5.5 to \( \sim 0.9 \) (mole Cu/mole MnO₂) at pH 8.5, the exchange constant, \( \log B \), varies from \( \sim 6 \) at pH 5.5 to \( \sim 8 \) at pH 8.5. Various chemical models which explain adsorption in terms of Cu²⁺, CuOH⁺, or both, are compared by calculating the variation of B with pH for each of those models. For qualitative reasons it is concluded that adsorption of copper on MnO₂ does not mainly depend on its hydroxylated ion, CuOH⁺, but on the free Cu²⁺ ion, possibly in combination (to a minor degree) with CuOH⁺.
Tests of the MnO₂ method on some known ligands show that the method reproduces conditional stability constants accurately (normally log K' ± 0.1) when the ligand concentration is larger than 0.15 µM and when the conditional stability constant is not too much different from β, i.e. when 6 < log K' < 10 at pH 8.

From analyses of a number of lakes and rivers it appears that a good balance is obtained, by regulating the MnO₂ concentration, of Cu²⁺ ions between MnO₂ and natural ligands.

It is observed that the ligand concentration in dystrophic rivers is high: 4-33 µM ligands; in lakes of higher alkalinity the ligand concentration is between 0.3-3 µM. Conditional stability constants, log K', for complexes with Cu²⁺ in dystrophic waters and of FA, cluster around 7.8 at pH 7.6; other lake waters produce various log K's, between 9.1 ± 0.3 and 7.1 ± 0.3 at pH 8.

From various experiments it is concluded furthermore that:

- ligands in natural waters are largely undersaturated with respect to copper;
- complexation analyses should be performed under exclusion of carbonate and other competing anions;
- a proton is released upon the complexation of Cu$^{2+}$ by ligands in natural waters;
- log $K'$ varies linearly with pH on 1:1 basis;
- normally only one complexing site is mainly regulating the concentration of Cu$^{2+}$ in natural waters;
- measurement of light absorption at 260 nm cannot be used to estimate the ligand concentration in a sample accurately;
- MnO$_2$ binds Pb rather too strongly, but Cd with suitable strength to provide a good balance between MnO$_2$, ligands in natural waters, and these metals;
- by assuming one complexing site per FA molecule the MnO$_2$ method provides an accurate estimation of the molecular weight of FA;
- interference of measurements by adsorption of ligands or metal-ligand complexes onto the MnO$_2$ surface does not occur to any significant degree in the tested samples.
Vision is less expensive than a computer model.

Parkinson, East and West (1960)
CHAPTER 7.

MODELS TO ESTIMATE THE IMPACT OF COMPLEXATION ON THE NATURAL ENVIRONMENT

7.1 INTRODUCTION

By means of the MnO₂ method it has now been established that commonly Cu²⁺ ions are complexed by ligands in natural waters, and that these ligands are considerably undersaturated with respect to Cu²⁺ ions. What is now the effect of such ligands on the concentration of Cu²⁺ ions, and what is the effect on complexation of a change in pH, for example by acidification of a lake by acid precipitation? It is important to know this, because the availability, toxicity, and transport of metals depends on their ionic state.

It will now be attempted to answer these questions.
7.2 COMPLEXATION OF Cu BY SOME KNOWN LIGANDS AND NATURALLY OCCURRING LIGANDS AT pH 8.3

Many organic compounds are known to occur in natural waters. Frequently these are called FAs, but amino acids form an important fraction in them. It is interesting to compare the stability constants measured for ligands in lake waters with the constants of some known ligands, and to calculate the effect that such ligands have in lowering the free metal ion concentration. The result of such a comparison is shown in Figure 7.1. I have chosen a low concentration of $10^{-7}$ M of each ligand and of $10^{-8}$ M of total dissolved copper. The ligand concentration is similar to values found in open ocean water which has about 1 gm/L of organic carbon (Le Williams 1975); if the organic carbon occurred as a compound with the molecular weight of FA, it would represent a molar concentration of $10^{-6}$ M ligands, but because of the very long residence time the molecules probably are more complex and heavier than FA.

The free copper ion concentration is calculated for equilibrium with each ligand as if only this particular ligand and copper ions are present. Cumulative complexing effects of these ligands are not apparent from this model. The effect of a change of pH on the complexing ability of such ligands will be discussed in section 7.3.
Figure 7.1 The effect of complexation, at pH of 8.3 by naturally occurring ligands and some artificial ligands, on $[Cu^{2+}]$, shown against the conditional stability constant, $K'$, at this pH. Conditions for the plot are total ligand concentration $[L] = 10^{-7}$M, total copper concentration $Cu_T = 10^{-8}$ M, 25°C, $\mu = 0.01$. 
The equilibria have been calculated at pH 8.3, as in seawater, and for 25°C and 0.01 M ionic strength. The stability constants of the known ligands have been corrected for the pH using their acidity constants as found in Sillen and Martell (1964): For FA and Lake Ontario the constants have been taken from Table 6.2 and adjusted for pH 8.3.

Histidine, which has been reported to occur at levels of $10^{-8}$ M (Parks 1975) to $10^{-7}$ (Clark et al. 1972), would bring the free copper ion down to a very low level of $10^{-10.8}$ M. NTA which might enter the sea as a result of pollution, would bring it down to $10^{-12}$ M, but it is biodegradable and is not produced in situ. Organic matter occurring in Lake Ontario would produce a $p[Cu^{2+}]$ of 10.5. Anderson and Morel (1978) calculated with the REDEQL computer model a $p[Cu^{2+}]$ of 9.6 by inorganic complexation. The presence of only $10^{-7}$ M organic complexing ligands may well be sufficient to diminish the free ion concentration by an order of magnitude. An inorganic particulate such as MnO$_2$ would not be a successful competitor for cupric ions under these circumstances.
7.3 COMPLEXATION OF Cu AT VARYING pH

In order to compare the effects of complexation by lake organics at different pHs, the fraction (α) of total dissolved copper (Cu$_T$) was calculated which is in the form of the free metal ion Cu$^{2+}$:

$$[\text{Cu}^{2+}] = \alpha \cdot [\text{Cu}_T] \quad \text{and} \quad \alpha = \frac{1}{K'[\text{L}]+1}$$

From Figure 7.2 one can see that until about pH 9, organics are much more important in complexing copper than are carbonate and hydroxyl ions. At a lower pH of 6, and with organics with a stability constant as found in Lake Ontario, 90% of the copper present is complexed by $10^{-6}$ M organics, whereas at a pH of 8, 99.9% of the copper is complexed.

7.4 SUMMARY

The complexation of Cu$^{2+}$ is modeled as a function of pH and of the conditional stability constant, log K'. By using the previously observed, linear variation of log K' with the pH it is calculated that:
Figure 7.2 Model for complexation of copper by lake-organics.
\[ [\text{Ligand}_{\text{total}}] = 10^{-6} \text{ M}, \log K_{\text{FA}}' = 7.8 \text{ and} \]
\[ \log K_{\text{Ontario}}' = 8.8 \text{ at pH 7.6, } \log K_{\text{CuOH}^+} = 6.0, \]
\[ \log K_{\text{CuCO}_3^2} = 6.8, p\text{CO}_2 = 10^{-3.5}, \mu = 0.01, 25^\circ\text{C}. \]
\[ [\text{Cu}^{2+}] = \alpha [\text{Cu}_T], \alpha = \frac{1}{K_L \cdot [L] + 1}, [\text{Cu}_T] = 2 \times 10^{-7} \text{ M}. \]
\[ 1:L = \text{OH}^-, 2:L = \text{CO}_3^{2-}, 3:L = \text{FA}, 4:L = \text{Ligand in Lake Ontario}. \]
- organic matter in natural waters forms complexes with copper in such a way that the Cu$^{2+}$ concentration is lowered considerably; a ligand as in Lake Ontario, at its natural concentration, lowers the Cu$^{2+}$ concentration by more than 2 orders of magnitude;

- upon change in pH, natural organics determine the state of copper when $5.5 < \text{pH} < 9$; at higher pH carbonate ions are expected to take over, at lower pH Cu$^{2+}$ loses its competition with H$^+$ ions.
Pollution is not a long term problem. Its cause, or better, the lack of its cause in the near future, is our largest problem.
CHAPTER 8

MEASUREMENT OF COMPLEXING MATERIALS EXCRETED
FROM ALGAE AND THEIR ABILITY TO AMELIORATE
COPPER TOXICITY

8.1 INTRODUCTION

There are several suggestions in the literature that at least some phytoplanktonic organisms excrete organic material. This organic material may be composed of waste products or it may be excreted to have a function in influencing the environment. Floating algae generally are so small that there really is not much space left over for storing enzymes or food reserves inside the cell. Possibly the environment is used as storage. Alternatively one can imagine the potential of an algal species that limits the trace metal supply to other species by excreting strong ligands. Some data on algal excretion products have been collected and are shown in Table 3.4.

The MnO₂ method is developed as a good tool for analysing complexing ligands in natural waters. The method
can also be used to test whether algae may be responsible for the origin of these ligands. Additionally the ability of such excretions in altering toxicity of copper ions is examined.

Firstly some effects that dissolved organic matter (DOM) has on its inhabitants, the phytoplanktonic organisms, are described, then how the organisms affect their environment, using a chemical model of the algal cell and of toxicity, and finally the experimental results are presented.

8.2 THE EFFECTS OF DISSOLVED ORGANIC MATTER ON PLANKTONIC ORGANISMS

The average concentration of dissolved organic matter (DOM) in ocean water amounts to about 1.5-1.8 mg/L (Duursma 1974; Clark et al. 1972; le Williams 1975). It is estimated as 300 (Krogh 1934) to 2000 (data of le Williams 1975) times the amount of organic matter present in planktonic organisms. It represents a storage equivalent to about 30 years total primary production (data of le Williams 1975). By whatever means this DOM arrives into the water, by excretion or autolysis, it seems plausible that its final concentration represents a steady state balance between
decomposition, release, uptake and sedimentation.

One wonders what are the effects of the DOM on the planktonic composition in natural waters. Krogh (1934) suggested that the concentration is too low to be of any use to heterotrophic organisms and that it is a total loss from the biomass. By using radiotracers, however, it has been shown that heterotrophic organisms are quite well capable of absorbing DOM. It is harvested by bacteria and converted into particulate organic matter (POM) (Paerl 1978). Indeed, some sort of symbiosis may be possible of bacteria and algae, algae producing oxygen and DOM, the bacteria using both and producing CO₂ and other products of mineralisation. Also normally autotrophic organisms can supply part of their nutrients by absorbing, for example, glycollic acid (Miller et al. 1971) or amino acids (Wheeler et al. 1974). Alternatively, considerable amounts of DOM are converted into small particles by adsorption onto bubbles which travel through the water column (Johnson 1976; Sutcliffe et al. 1963). These concentrated forms of organics with adsorbed nutrients may well be easily accessible for microorganisms.

Besides the immediate use of DOM as nutrient supply there are a number of indirect ways along which it may affect the plankton composition. Chelating compounds can make nutrients available, or unavailable, or protect against
toxic metals. It has been suggested that chelators of the hydroxymate type make Fe available by dissolving iron hydroxides or oxides. Enrichment with Fe, chelated Fe, or chelators alone, has a growth promoting effect (Sakamoto 1971); similarly the addition of the Fe-EDTA complex induced a 4 times larger growth in a marl lake (Schelske et al. 1962). The opposite effect was observed by Goldberg (1957): the marine diatom Asterionella japonica utilised only particulate and/or colloidal iron as a growth nutrient, whereas ionically complexed ferric ion such as the citrate, ascorbate, or artificial humate were not available for uptake (Goldberg 1952).

One should realise that independent of the way (as a complex or as the free uncomplexed ion) which a metal enters the cell, the organism will still be dependent on the free metal ion concentration to be able to use it: if Fe enters the cell as a complex, the organism still has to release Fe$^{3+}$ to incorporate it, for example into a protein as ferredoxin. The addition of a chelator, strong enough to be able to compete successfully for Fe$^{3+}$ with iron hydroxides and oxides, will indeed increase the concentration of total dissolved iron, but decrease the free metal ion concentration. The solubility of Fe$^{3+}$, at pH 8.0, in equilibrium with its hydroxide is about $10^{-21}$ M and about
$10^{-24}$ M in equilibrium with goethite ($K_{Fe(OH)}^{3(s)} = 10^{-38.8}$ and $K_{FeOOH}^{3(s)} = 10^{-41.5}$, Smith and Martell 1976).

This means a concentration of only 1-1000 Fe$^{3+}$ ions per liter. Fortunately, microorganisms are known to have very strong chelators at their disposal in the form of hydroxamates (Neilands 1967). At pH 8 conditional stability constants for complexes of Fe with hydroxamates such as deferriferrichrome and deferriferrichrome A vary from $10^{28}$ to $10^{31}$ (values for $K$ and $K_a$ from Neilands, 1967). This is strong enough to bind free metal ions. The problem may be the speed with which metal ions can be obtained. The only way the necessary flux can be reached is by the presence of solid Fe-hydroxides near the cell wall, as has been suggested by Goldberg (1957) or the presence of Fe-complexes. In addition to hydroxyl ions, organic chelators may thus play a role in increasing the flux of iron to the cell, even though they lower the free metal ion concentration.

**Vice versa** one can imagine that reduction of free metal ion availability by organic chelators may protect against an otherwise toxic effect. Addition of 1 µg Cu to a medium without chelators depressed the growth of *Chlorella pyrenoidosa* (Steeman Nielsen and Wium-Andersen 1970). They mention that the copper concentration in sea
water is high enough to suppress algae growth, but chelating agents counteract the poisonous influence. By varying chelator concentrations, Sunda and Guillard (1976) showed that copper ions were toxic from $10^{-8.3}$ to $10^{-10.5}$ M, completely inhibiting an estuarine diatom, *Thalassiosira*. The dinoflagellate *Gonyaulax tamarensis* is inhibited for 50% at $10^{-10.4}$ M Cu$^{2+}$ (Anderson and Morel 1978). A linear response has been correlated with a log Cu$^{2+}$ concentration of -12 to -8 M (Jackson and Morgan 1978).

8.3 EXCRETION OF ORGANIC MATTER BY PLANKTONIC ORGANISMS

An important consideration in toxicity is the excretion of complexing ligands by planktonic organisms. This consideration is exemplified by a remark made by Steeman Nielsen and Wium-Andersen (1970) that it appeared possible for their *Chlorella* culture to counteract the poisonous effect of copper. Ordinary growth rate was observed after a delay of 24 hours, for 1 μg, and 48 hours, for 5 μg Cu. Especially the *Nitzchia palea* appeared to become very leaky and lost considerable amounts of organic matter; up to 22% of the synthesized organic matter was
excreted.

After a period in which it seemed generally accepted that algae excrete organic material (Aaronson et al. 1971; Aaronson et al. 1977; Anderson and Zeutschel 1970; Billmire and Aaronson 1976; Fogg et al. 1965; Penhale and Smith 1977; Thomas 1971) recently, discussion started (Sharp 1977) concerning the significance of excretion. Sharp (1977) explains almost all excretions to experimental errors, autolysis or breakage of cells, cultural shocks, and maybe some leakage due to diffusion. In contrast to this article, Fogg (1977) states that there are many cases where excretion is limited to certain compounds, which precludes autolysis or breakage of cells, and that cultural shocks may not be so unnatural after all. Similar comments were given in a more recent article (Aaronson 1978) where also the possibility is mentioned of functional excretion: the excretion serves a purpose, such as the solubilisation of Fe, chelation of heavy metals, or the removal of waste products.

Phytoplankton tends to spend a large part of its life in the same environment. A planktonic alga may have some movement due to sinking, but all excretions will affect its own environment or that of its neighbours. Also microorganisms tend to be hampered by a lack of space.
Unnecessary proteins cannot be stored. To make up for this space problem the organisms are extremely adaptable: 10% of the cell volume is made up of induced enzymes (Schlegel 1974). So, all inhibited or blocked enzymes ought to be removed by excretion.

Sometimes excreted organic matter inhibits competing organisms (Pratt 1966); the bloom sequence over a 3 year period could be traced back by effects of the cell free filtrates of the dominant blue-green algae (Keating 1977). Deprivation of Fe induced the production of hydroxamate chelators by Anabaena (Murphy et al. 1976), which at the same time suppressed other algae; phosphatase is excreted by Chlamydomonas depending on whether the cells are phosphate starved or not (Patni et al. 1977).

The excretion of low molecular weight organics by Ochromonas seems to be related to waste disposal (Jüttner and Friz 1974). The excretion of glycollic acid on the other hand has been related to extra cellular storage to sustain algal growth under unfavourable conditions (Pogg et al. 1965). So, less is excreted by Chlorella at high cell density, because of a tendency towards the establishment of equilibrium with extracellular substances in the medium (Nalewajko 1966). It is therefore necessary that these
organisms are capable of absorbing glycollic acid, which has indeed been shown (Miller et al. 1971).

8.4 ALGAE AND METAL TOXICITY: A CHEMICAL MODEL

It becomes quite tempting to imagine a feed-back mechanism that induces algae to excrete complexing ligands to ameliorate metal toxicity or for competitive purposes. To be sure one has to analyse the algae in a cultured situation. In algal cultures very little accumulation of excretion products takes place, however. So one needs an accurate method to determine DOM. The \( \text{MnO}_2 \) method was applied to this problem, to analyse filtrates of algal cultures of three different algal species.

Algal cells can be simply described as "just" membranes filled with a mixture of proteins and lipids. In the case of green algae there is also a supporting cell wall around the membrane, but this does not seem to influence the permeability of the organism for inorganic and organic substances. This is regulated by the cell membrane. This membrane consists of lipids, with proteins sticking through, as if to provide holes for transport of metal ions. It
may even be the case that proteins travel out of the cell, catch a metal ion, which changes its water- and fat-solubility, and travel back into the cell. With either active transport or passive transport because of concentration gradients, the final concentration of metals in the cell will still be dependent on the strength with which complexing substances inside the cell can hold the metal. The free metal concentration will be very low with strong complexing agents inside the cell, and the metal gradient will be towards the cell. In this model of the algal cell the means of transport are suitable only to speed equilibrium up or slow it down. In the case of heavy metal pollution the cell may be trying to hold the metals out, until, at least, it has reproduced; and it may have to spend a lot of energy on re-acquiring the proteins and phospho-lipids, which it loses because of complexation with metals.

When metal ions are added to the environment, the algae will become affected as soon as one, or more, essential complexing ligands in the cell are occupied by metal ions. From then on it is just a matter of time until the algal cell cannot function any more. Keeping this in mind we could just as well depict toxicity to algae as the titration of some ligand. Therefore, when the free metal ion concentration reaches the value of its conditional stability constant,
it has had it, since \( [\text{Me}^{2+}] K' = \frac{[\text{MeL}]}{[L']} \).

In support of the assumptions that are made here, I should point out that algae have been modelled before quite successfully as complexing ligands (Sunda and Guillard 1976). It is even possible, by working carefully, to determine the conditional stability constant of EDTA by the shift in toxicity (Gächter et al. 1978).

The availability of copper ions to algal cells is described theoretically. Firstly, I assume the presence of complexing ligands strong enough to complex most copper present, following a process whereby one proton is exchanged per \( \text{Cu}^{2+} \) ion bound:

\[
\frac{[\text{CuL}]}{[\text{Cu}^{2+}][L']} = K' \quad \text{(symbols have their usual meaning)}.\n\]

Combination with mass balance equations

\[
L = [L'] + [\text{CuL}],
\]

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

gives

\[
[\text{Cu}^{2+}] = \frac{(\text{Cu} - [\text{Cu}^{2+}])}{(L - \text{Cu} + [\text{Cu}^{2+}]) K'}.
\]
Conversion:
\[
\frac{1}{[\text{Cu}^{2+}]} = K' \left( \frac{L}{\text{Cu} - [\text{Cu}^{2+}]} - 1 \right).
\]

So
\[
p[\text{Cu}^{2+}] = \log K' + \log \left( \frac{L}{\text{Cu} - [\text{Cu}^{2+}]} - 1 \right).
\]

When in the natural environment \(L \geq \text{Cu}\) and \(L' \leq K'\) most Cu is complexed, so approximately
\[
p[\text{Cu}^{2+}] = \log K' + \log \frac{L}{\text{Cu}}.
\]

Now the Cu\(^{2+}\) concentration depends on \(K'\), the ligand concentration, the copper concentration, and the pH since \(K'\) depends on the pH (section 2.2). Substitution of
\[
K' = K \cdot \frac{K_a}{H} \quad (6) \text{ for } K' \text{ gives}
\]
\[
p[\text{Cu}^{2+}] = \log K + \log K_a + \text{pH} + \log \frac{L}{\text{Cu}} \quad (42),
\]
which shows the dependence of \([\text{Cu}^{2+}]\) on the pH, other parameters being constant.

The toxicity/availability of Cu\(^{2+}\) to algae depends on some algal stability constant, \(K_{\text{algae}}\). Similarly to the previous development:
\[
\frac{[\text{Cu Algae}]}{[\text{Cu}^{2+}][\text{Algae}']} = K'_{\text{algae}}
\]

where \([\text{Cu Algae}]\) = concentration of algal ligands occupied by \(\text{Cu}^{2+}\),

\([\text{Algae}']\) = the remaining concentration of algal ligands, partially occupied by protons.

Then
\[
\frac{[\text{Cu Algae}]}{[\text{Algae}']} = K'_{\text{algae}} \cdot [\text{Cu}^{2+}].
\]

When
\[
\frac{[\text{Cu Algae}]}{[\text{Algae}']} > 1 \text{ the majority of algal ligands is occupied by } \text{Cu}^{2+}.
\]

So this ratio represents a toxic effect. \(K'_{\text{algae}}\) depends on the pH just like \(K'\):

\[
K'_{\text{algae}} = K_{\text{algae}} \cdot \frac{K_{a,\text{algae}}}{[H^+]}.
\]

and substitution gives

\[
\log \frac{[\text{Cu Algae}]}{[\text{Algae}']} = \log K_{\text{algae}} + \log K_{a,\text{algae}} + \text{pH} + \log [\text{Cu}^{2+}] + \log \frac{L}{\text{Cu}}.
\]

Substitution of \(p[\text{Cu}^{2+}] \) (42) gives then

\[
\log \frac{[\text{Cu Algae}]}{[\text{Algae}']} = \log K_{\text{algae}} + \log K_{a,\text{algae}} - \log K - \log K_{a}
\]

\[
- \log \frac{L}{\text{Cu}} \quad \text{(43)}
\]
So the availability/toxicity of Cu$^{2+}$ to algae depends on the relative magnitudes of algal and dissolved ligand stability constants and acidity constants, and on the ligand concentration and the copper concentration. It does not depend on the pH.

This result is understandable since both the dissolved ligand and the algal ligand complex Cu$^{2+}$ by exchanging a proton so they are equally dependent on the pH. The result also shows the importance of determining both the ligand concentration and its stability constant for evaluation of possible effects of toxicity in natural waters.

**Conclusions**

- Assuming that: complexation of copper ions in the natural environment is a process whereby a proton is exchanged, and algal cells can be modelled as complexing ligands, the toxicity/availability of Cu$^{2+}$ ions to algae is not dependent on the pH, but depends on the relative magnitudes of stability constants and the ligand concentration.

- For evaluation of toxicity of metal ions in natural waters it is necessary to determine both the conditional stability constant and the ligand concentration.
8.5 EXCRETION OF COMPLEXING LIGANDS BY DIFFERENT ALGAL SPECIES AND THEIR ABILITY TO AMELIORATE COPPER TOXICITY

Experimental

The complexing capacity and the conditional stability constants of the copper complexes of algal excretion products were measured by the MnO₂ method. It is effective at very low ligand concentrations so that the measurement is carried out directly on the cell-free filtrate of the algal culture.

Three axenic algal cultures (grown by P.T.S. Wong at C.C.I.W.) were used: *Anabaena cylindrica* (Texas 144), *Scenedesmus quadricuada* (Carolina Biological Supply Co.) and *Navicula pelliculosa* (Indiana 668). They were each grown in one litre of CHU-10 medium (Chu 1942) which contains no chelators. No trace elements were added to the medium since sufficient amounts were already present as contaminants in the chemical used. Only the major nutrients, nitrate and phosphate, were present and some bicarbonate to buffer the solution to pH 8. The algae were allowed to grow for 10 days at 20°C on a rotary shaker (100 rpm) under conditions of 18 h of light (5000 lx) and 6 h of darkness. It is thought that the algae would then be well past their exponential growth periods. The cells were separated from the
medium by filtration under mild vacuum (~15 cm Hg), through 0.45 µm Millipore filters previously cleaned with dilute nitric acid (0.1 mol/L) and alkali permanganate distilled water. Filters were frequently renewed to prevent clogging and pressure build-up.

An aliquot (200 mL) was used for dry weight determination (by P.T.S. Wong) of the cells; another aliquot (800 mL) was filtered for the analysis of the complexing capacity and for toxicity experiments.

Experiments to determine the ability of the algal filtrates to ameliorate the copper toxicity on algae were carried out with Chlorella vulgaris (Carolina Biological Supply Co.). Logarithmically growing algal cells (0.3 mL) were added to 13 mL of the filtrates of which the complexing capacities have been determined. An aliquot (1.5 mL) of a 10× concentrated CHU-10 medium was added to supplement the nutrients. Copper sulfate at concentrations from 0 to 200 µg Cu/L was added. A 0.1 mL aliquot of 14C-sodium carbonate (2.8 µc/mL, Amersham) was used as algal primary production indicator. The volume of the test system was made up to 15 mL with glass distilled water. A short incubation of 4 h was adopted to avoid the excessive excretion of ligands from Chlorella vulgaris during the experiments.
8.6 RESULTS AND DISCUSSION

For the conditional stability constants, ligand concentrations (Table 8.1) and total copper concentrations, the cupric ion concentrations have been calculated at pH 8, the pH at which the algae were grown (Table 8.2). The K' values have been adjusted for pH 8 assuming competition by H\(^+\) evaluated empirically from natural water samples (van den Berg and Kramer 1979b). The equations, other constants and K' values which were used to calculate [Cu\(^{2+}\)] are given in Table 8.3. An average total copper concentration of 5 \times 10^{-8} \text{ mole/L}, measured in the algal media after 10 days growth, was used for calculation.

All three algal species produced complexing ligands (Table 8.1) in varying quantities, with *Anabaena* producing the most (6.73 \text{ \mu mol/L}) and *Scenedesmus* the least (0.66 \text{ \mu mol/L}), which were equivalent to 0.11 \text{ \mu mol/mg dry wt} and 0.01 \text{ \mu mol/mg dry wt}, respectively. The differences are significant. Using a different method of potentiometric titrations with an ion electrode with detection limit of 1 \text{ \mu mol/L} complexing agent, Swallow et al. (1978) found that only one of eight marine algae studied produced copper complexing materials. The discrepancies could possibly be due to differences in sensitivity of methods or differences
Table 8.1 Production of complexing ligands by three phytoplankton species and the conditional stability constants of the copper complexes of the exudates and of natural waters

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry wt. mg/L</th>
<th>Ligands produced µmol/L</th>
<th>Ligands per mg dry wt. µmol/mg</th>
<th>Con. Stab. Const. ( \log K' )</th>
<th>pH of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabaena</td>
<td>61</td>
<td>6.73</td>
<td>0.11</td>
<td>7.7±0.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Navicula</td>
<td>80</td>
<td>2.86</td>
<td>0.036</td>
<td>8.1±0.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>64</td>
<td>0.66</td>
<td>0.010</td>
<td>8.6±0.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Bay of Quinte</td>
<td></td>
<td>2.79</td>
<td></td>
<td>7.6±0.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Lake Ontario(^1)</td>
<td></td>
<td>0.33</td>
<td></td>
<td>8.7±0.3</td>
<td>7.6</td>
</tr>
</tbody>
</table>

\(^1\) Van den Berg and Kramer 1979b.

\(^2\) \(K'\) values were calculated with 95% confidence limits.
in physiology between marine and freshwater algal species.

The conditional stability constants at pH 7.6 for the copper complexes of these exudates also varied from $10^{8.6}$ for *Scenedesmus* to $10^{7.7}$ for *Anabaena*. The constants for water of Lake Ontario and Bay of Quinte were $10^{8.7}$ and $10^{7.6}$, respectively, at the same pH. The similarity in the conditional stability constants of the copper complexes suggests, but without affirmative proof, that natural chelators in lake water can possibly be derived from biological excretion.

It should be noted that these determinations of conditional stability constants are empirical. It serves the purpose, in that one may now calculate the effect of such ligands on the free copper ion concentration. It is still not known, however, what kind of molecule the ligand is. One may get some idea by comparing stability constants of known ligands. At pH 7.6 the log conditional stability constants of amino acids such as arginine, histidine and glycine are 10.6, 9.0 and 6.4, respectively for 1:1 complexes with Cu$^{2+}$ (values for $K$ and $K_a$ from Sillen and Martell 1964; Chaberek and Martell 1959). So amino acids could possibly function as complexing ligands, something which is denied occasionally (Swallow et al. 1978).

From the data summarized in Table 8.1, it is of
Table 8.2  Conditional stability constants of the copper complexes of algal exudates (after 10 days' growth) and of lake waters, and the calculated free copper ion concentrations. Calculations based on pH 8.0, pCO$_2$ = 10$^{-3.5}$ (the copper concentration in the algal cultures was 5 x 10$^{-8}$ M).

<table>
<thead>
<tr>
<th></th>
<th>log K' at pH 8</th>
<th>p Cu$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabaena cylindrica</td>
<td>8.1±0.1</td>
<td>10.3±0.1</td>
</tr>
<tr>
<td>Navicula pelliculosa</td>
<td>8.5±0.2</td>
<td>10.3±0.2</td>
</tr>
<tr>
<td>Scenedesmus quadricauda</td>
<td>9.0±0.2</td>
<td>10.2±0.2</td>
</tr>
<tr>
<td>Bay of Quinte</td>
<td>8.0±0.1</td>
<td>9.8±0.1</td>
</tr>
<tr>
<td>Lake Ontario</td>
<td>9.1±0.3</td>
<td>9.9±0.2</td>
</tr>
<tr>
<td>Whitewater</td>
<td>9.3±0.5</td>
<td>10.0±0.5</td>
</tr>
<tr>
<td>Gloucester Pool</td>
<td>8.9±0.4</td>
<td>10.2±0.5</td>
</tr>
<tr>
<td>Lake Windy</td>
<td>8.1±0.3</td>
<td>9.3±0.3</td>
</tr>
</tbody>
</table>
interest that the greatest stability constant is associated with the ligands which are excreted in the lowest concentration. The net result of this variation may result in a similar effect on the free copper ion concentration in the different algal exudates. Indeed, when the final copper ion concentration is calculated for each algal exudate after 10 days growth at pH 8 (Table 8.2), the values appear to be at the same level (10^{-10.3} \text{ mol/L}) within 95% confidence limits of the measurements. This concentration level (10^{-10.3} \text{ mol/L}) may be the threshold concentration that algal species are trying to maintain by excreting complexing materials to prevent the metal ions from being toxic to their growth. This concentration of copper ions is in agreement with and in fact, falls in the range of, 10^{-12} - 10^{-8} \text{ mol/L} (Jackson and Morgan 1978) and coincides with 10^{-10.4} \text{ mol/L} (Anderson and Morel 1978) for having inhibitory effects on some marine phytoplankton species.

p[Cu^{2+}] has been calculated for the lake data obtained previously (Table 8.2), for comparison with the situation in lakes, where equilibrium between algae and their exudates can be expected. The lakes give similar but slightly lower p[Cu^{2+}] values. Lake Windy is a lake with low productivity and high Cu^{2+} concentration. Beside a different ecology of
Table 8.3  Equations and stability constants used to calculate the free copper ion concentration in equilibrium with complexing ligands.

<table>
<thead>
<tr>
<th>Equilibria</th>
<th>Constant</th>
<th>Log K</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu + L = CuL</td>
<td>$K'_L$</td>
<td>this dissertation</td>
<td></td>
</tr>
<tr>
<td>Cu + OH = CuOH</td>
<td>$K_1$</td>
<td>6.3</td>
<td>Smith and Martell 1976</td>
</tr>
<tr>
<td>Cu + CO$_3$ = CuCO$_3$(aq)</td>
<td>$K_1$</td>
<td>6.75</td>
<td>Smith and Martell 1976</td>
</tr>
<tr>
<td>Cu + 2CO$_3$ = Cu(CO$_3$)$_2$</td>
<td></td>
<td>9.92</td>
<td>Smith and Martell 1976</td>
</tr>
</tbody>
</table>

For simplicity, charges have been omitted.

Mass Balance:

$$\begin{align*}
\text{Cu}_T & = \text{CuL} + \text{CuOH} + \text{CuCO}_3 + \text{Cu(CO}_3)_2 + \text{Cu} \\
\text{L}_T & = \text{CuL} + \text{L}
\end{align*}$$

Substitution:


in which $a = 10^{6.3}.[OH] + 10^{6.75}.CO_3 + 10^{9.92}.(CO_3)_2 + 1$

- $L_T$ - total ligand conc.
- $Cu_T$ - total copper conc.
- $Cu$ - free copper conc.
- $L$ - free ligand conc.
- $K'_L$ - conditional stability constant of complex CuL
phytoplankton (it has a lower pH) slight contamination of the filtrate with Cu could be the cause of this anomaly.

To compare the effects of different complexing agents in reducing the toxicity of metal ions to algal growth, it is necessary to eliminate as much as possible other factors that might influence the growth of the test biota. In a biological system, this may be difficult to achieve. The complexing agent itself may be used as a source of nutrient or it may interfere with the uptake of other nutrients. In the present experiment where Chlorella was grown in algal exudates of other species, the effect of extraneous substances on the primary production of Chlorella is difficult to assess.

At zero copper addition, the primary production of Chlorella was highest in the Anabaena filtrate and lowest in the Scenedesmus filtrate (Figure 8.1). It is possible that Anabaena filtrate is more stimulatory than Navicula and Scenedesmus. In the range of copper additions from 10 to 200 μg/L, the primary production of Chlorella in Anabaena filtrate only decreased about 8%, whereas in Navicula and in Scenedesmus filtrates the primary production decreased 45% and 36%, respectively, from their original values. Such observations are also in accord with the
Figure 8.1 $^{14}$C primary production of *Chlorella vulgaris* in exudates of other algae in various levels of total copper at 20°C and pH 8.

A - *Anabaena cylindrica*;
B - *Navicula pelliculosa*;
C - *Scenedesmus quadricauda*.
complexing capacities of the three filtrates (Table 8.1). Anabaena filtrate has the highest complexing capacity (6.73 μmol/L), which protects Chlorella against copper toxicity, even at 200 μg/L. In our control experiments without the addition of algal exudates, copper toxicity was more pronounced. For example, the additions of 50 and 200 μg/L to CHU-10 medium reduced the primary productivity of Chlorella by 60% and 83%, respectively.

The copper ion concentrations in each experiment were calculated using the conditional stability constants obtained and the complexing capacity values as concentrations of total ligands. It should be noted that due to the contribution of copper by the filters, the experiments did not start at the same total copper concentration. Such contaminations have been measured (the copper contamination from the filters for Scenedesmus was 3.8 μg/L, for Navicula 10.2 μg/L and for Anabaena 0.6 μg/L) and incorporated in the calculations of the copper ion concentrations. If the effect on primary production is due solely to copper ion concentrations, all the curves in Figure 8.2 should be superimposable. However, linear regression of these curves gave slopes varying from 0.95 ± 6.3 for Anabaena, 9.0 ± 4.1 for Scenedesmus to 19 ± 3.3 for Navicula. The deviations
Figure 8.2. $^{14}C$ primary production of *Chlorella vulgaris* in exudates of other algae as function of the calculated free copper ion concentration at 20°C and pH 8.

A - *Anabaena cylindrica*;
B - *Navicula pelliculosa*;
C - *Scenedesmus quadricauda*. 
from ideal situations in Figure 8.2 could well be due to the multiple binding sites in the algal excretions, as indicated by Chlorella grown on Scenedesmus and Anabaena filtrates. The decrease in primary production of Chlorella in Navicula filtrate with the increasing logarithmic concentration of copper ion showed very little deviation from linearity. Such relationship may suggest that there is only one binding site available in the complexing material. It is not surprising that some other physiological effects exist in growing one species of algae in the excretion of other species. The quantitative effect is beyond the scope of the present study.

8.4 SUMMARY

Three algae species are grown in a special medium which contains no chelating compounds. After 10 days growth, analysis of the filtrates of these cultures (with the MnO$_2$ method) revealed the presence of complexing ligands excreted by the algae. These ligands are similar to the ligands observed previously in lakes.

From calculation of the Cu$^{2+}$ concentration it appeared
that the Cu\(^{2+}\) concentration in all three filtrates is practically equal, although the three ligands are of different bonding strength and were excreted in various concentrations.

If these findings are generally applicable, it means that ligands in lakes are derived from planktonic algae and that the algae make use of their ability to complex Cu\(^{2+}\) (and other metal ions) by actually regulating the Cu\(^{2+}\) concentration to a favorite level.

The observations of this study are:

- *Anabaena cylindrica*, *Navicula pelliculosa* and *Scenedesmus quadricauda* excreted 6.73, 2.86 and 0.66 \(\mu\text{mol/L}\) of complexing ligands, respectively, with conditional stability constants of \(10^{7.7}\), \(10^{8.1}\) and \(10^{8.6}\), respectively, for their corresponding copper complexes.

- Their ability to ameliorate copper toxicity towards the primary production of *Chlorella vulgaris* is related to the conditional stability constants which regulate the ionic copper concentration.

- When these conditional stability constants were applied in calculations, a free copper ion concentration of \(10^{-10.3}\) mol/L was found in each exudate.

- The conditional stability constants of the excreted ligands are similar to the values observed in lake waters.
"Love not to talk
Love not to boast
Grief comes to him
Who brags the most"

Cape Breton Schoolboy Poem
CHAPTER 9

SUMMARY AND IMPLICATIONS FROM THIS STUDY

9.1 INTRODUCTION

A geochemical approach is very useful in explaining the behavior of metal ions in the natural environment, but such an approach on its own is limited. The abundance of organisms present in water and on land is bound to have large implications for cycles of many elements (nutrients) in the thin surface layer of the earth, the layer of such great importance to human beings. So, it is necessary to include biological considerations in the study of reactions which may seem to be purely chemical at first sight.

Most environmental scientists are familiar with the idea that some metals, notably copper, are present in a complexed form in natural waters: the free metal ion, Cu$^{2+}$, is bound by another inorganic ion of opposite or apparently neutral charge. The form in which the metal is present
determines its transport, its dissolved concentration in equilibrium with sediments, and its availability or toxicity to aquatic organisms. The effect of organic complexing ligands (ligands) in water on the state of metal ions in the dilute situation of a natural water, was until recently fairly uncertain. There were, however, several indications that metal ions are indeed complexed by organics and that it has significant toxicity lowering effects.

In this thesis a method (the MnO₂ method) has been developed, theoretically and experimentally, to determine copper complexation for weak complexes with ligands present in low concentration. The method is suitable for natural waters but it has not yet been tested on seawater. The MnO₂ method has a sound theoretical basis, functions in an equilibrium situation with metal ions and ligands, no preconcentration of the ligands is necessary, and the natural water sample is disturbed as little as possible.

But, really this thesis presents an idea; the idea of using a weak ion exchange medium, in small amounts so as to disturb the natural water situation as little as possible, to measure metal complexation.
9.2 IMPLICATIONS OF ANALYSES WITH THE MnO₂ METHOD OF NATURAL WATERS:

Analysis of various aquatic environments revealed that:

- in all water samples that have been analysed a surplus of complexing ligands is present;
- dystrophic waters, of low pH and ionic strength contain high concentrations of fulvic acid (FA)-type ligands;
- medium to highly alkaline lakes supporting algal growth contain lower concentrations of more powerful ligands; FA-type ligands are not present, and
- algae in culture produce potent complexing ligands similar to those found in lakes.

From the conditional stability constants, K', and ligand concentrations (both determined by the MnO₂ method) the Cu²⁺ concentration in equilibrium with these ligands has been calculated. From such calculations it is now apparent that the Cu²⁺ concentration in natural waters is determined predominantly by organic ligands. In lakes these ligands are normally produced by algae.

These observations have important implications for our understanding of the complexation of Cu²⁺ in natural waters. Since algae determine the ligand type and concentration
in lakes, they have the opportunity to actually regulate the Cu\(^{2+}\) concentration and keep it at a favorite level. Indeed, three algal species in culture produced equal Cu\(^{2+}\) concentrations, similar or slightly lower than the concentrations observed in lakes. A fourth species appeared to be protected against copper toxicity when grown in the filtrate of these cultures.

These considerations should be taken into account when water standards for pollution are set, when metal toxicity experiments are planned, or when metal complexation is modelled. Pollution standards should be related to the free metal ion concentration rather than to the total metal concentration. The maximum allowable flux (from pollution) of metals into a lake could possibly be related to the rate with which algae excrete complexing ligands; this flux may have to be lower in winter than in summer due to decreased photosynthetic activity in winter.

Theoretical considerations show that (in presence of complexing ligands) a change of pH does not affect the copper toxicity, when the pH is between 5.5 and 9. This conclusion should be of interest to toxicologists. Experiments with algae should be performed preferably over as short a period as possible to avoid excretion of ligands by the algae; or, it is necessary to correct for the metal
complexation as a result of this excretion.

If the observation that the Cu$^{2+}$ concentration in natural waters is basically kept constant (at fixed pH) by algae, is generally true for most freshwaters, then modelling of metal ion concentrations is certainly greatly simplified.

These conclusions confirm the importance of the biomass in determining the chemistry of the natural environment. When we pollute the environment we will affect the biomass, and damage to the biomass will in turn again affect our environment; we should be aware of cumulative effects, since "the world can handle only so much pollution".
REFERENCES


Addenda
