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The Complexation of Iron by Marine Humic Acid

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THE COMPLEXATION OF IRON BY MARINE HUMIC ACID

BY

GERALD L. PICARD

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

Cation exchange capacity measurements, performed before and after removal of the humic acid fraction from various Narragansett Bay sediments, indicate that low concentrations of these organic substances strongly influence the ability of the sediment to react with metal ions. Atomic absorption and spectrophotometric methods have been developed which allow quantitative determinations of the extent of reactions between a naturally occurring humic acid and iron in artificial seawater. The results indicate that different humic acid-iron complexes are formed with varying solubilities depending on the humic acid-iron ratio used in the experiment. This study suggests that humic acid is a transport substance for trace metals in Narragansett Bay.

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PREFACE

This thesis has been prepared according to the manuscript thesis plan. The manuscript is in the format of the journal, Geochimica et Cosmochimica Acta.

MANUSCRIPT

THE COMPLEXATION OF IRON BY MARINE HUMIC ACID

THE COMPLEXATION OF IRON BY MARINE HUMIC ACID

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ABSTRACT

Cation exchange capacity measurements, performed before and after removal of the humic acid fraction from various Narragansett Bay sediments, indicate that low concentrations of these organic substances strongly influence the ability of the sediment to react with metal ions. Atomic absorption and spectrophotometric methods have been developed which allow quantitative determinations of the extent of reactions between a naturally occurring humic acid and iron in artificial seawater. The results indicate that different humic acid-iron complexes are formed with varying solubilities depending on the humic acid-iron ratio used in the experiment. This study suggests that humic acid is a transport substance for trace metals in Narragansett Bay.

INTRODUCTION

The nature of iron in seawater has been the subject of considerable speculation in recent years (Siegel, 1971). Part of the discrepancy between the high concentrations of 'soluble' iron found in near shore waters and the concentrations predicted on the basis of the solubility product of iron hydroxide has been attributed to the presence of natural organic chelators (Duursma and Sevenhuysen, 1966).

Scant data are available on the complexing ability of the organic materials present in seawater (Rashid, 1971, 1972; Rashid and Leonard, 1973). Studies have indicated that these materials resemble humic substances found in soils and fresh water (Hair and Bassett, 1973; Schnidler, et al., 1972). The similarity of these humic substances found in all natural systems, their abundance, and their ability to form complexes with metals (Schnitzer and Khan, 1972) make marine humic materials of prime concern in the transport, availability, and accumulation of iron and other trace elements in marine environments.

We have investigated the iron complexing properties of a marine humic acid (H.A.) isolated from a Narragansett Bay sediment. A model has been developed which yields some insight into the role of this organic chelator in the transport and accumulation of iron in sedimentary deposits.

METHODS AND MATERIALS

A. Isolation and Purification of Humic Acid

The humic acid isolated for use in the iron complexation experiments was extracted from sediment samples obtained with an Eckman Dredge from a location in Narragansett Bay, R.I., designated station F.P. by Farrington and Quinn (1973). This location is about 100 yards downstream from the municipal sewage outfall for the city of Providence, R.I. The sediment was kept frozen until use, at which time it was air dried, ground to pass a 2 mm sieve, and oven dried at 100°C for 24 hours.

The method of extraction and purification of the humic acid from the sediment samples is a modification of the procedure commonly used by humic acid chemists and has been described in detail by Pierce and Felbeck (1974). The method consists of 0.5 N NaOH extractions of the sediment samples with subsequent precipitation of the humic acid from the sodium hydroxide solution by addition of 6 N HCl to pH 2.0, followed by centrifugation. The humic acid is then redissolved in 0.5 N NaOH, the alkaline solution of humic acid is dialyzed for 5 days against distilled water, and the resulting humic acid solution is centrifuged at 20,000 X g for 30 min to remove clays and other impurities. This humic acid solution is used in the complexation experiments.

The ammonium acetate method (Peech, et al., 1947) was used to determine cation exchange capacities (C.E.C.) of the sediment samples from stations F.P., D, E-2 and B before and after removal of the humic substances with 0.5 N NaOH. Cation exchange capacity measurements were performed on the station F.P. humic acid using the barium acetate method of Chandler (1939). Organic carbon analyses of the sediment

samples were performed using an F and M Model 185 Carbon, Nitrogen, Hydrogen Analyzer.

B. Complexation Studies

The complexation studies were performed in the following manner. Ten ml of a concentrated artificial seawater solution containing 175 ppt NaCl, 2.0 ppt Ca, and 6.0 ppt Mg were added from a buret to 100 ml beakers containing various amounts of the humic acid stock solution, ranging from 0 μg to 7500 μg of humic acid. Fifty μg of iron as ferric chloride from Baker Dilut-It Standards were added slowly, drop-wise from a separate buret. The mixture was constantly readjusted to pH 8.0 with NaOH and stirred using a Teflon-coated magnetic stirring bar during the addition of iron. The volume of the mixture at this point was about 40 ml. Time studies indicated that 18 hours was sufficient time for equilibrium to be attained. The mixture was then readjusted to pH 8.0 and diluted to 50 ml in volumetric flasks.

At this point, the mixture contained the following concentrations of materials: 35 ppt NaCl, 400 ppm Ca, 1200 ppm Mg, 1 ppm Fe and concentrations of humic acid ranging from 0 ppm to 150 ppm.

The 50 ml of solution was centrifuged at 40,000 X g in polycarbonate centrifuge tubes for 30 minutes and the supernatant solution was decanted from the precipitate. Four to seven replicates were performed for each experimental point corresponding to a particular ratio of humic acid to iron in these experiments. A flow diagram describing the analytical procedures used in the complexation experiments is presented in Figure 1.

C. Analysis of the Soluble Fraction

The concentration of humic acid in the supernatant solutions was determined at 350 nm using a Beckman DU Spectrophotometer equipped with 1 cm glass cuvettes. Standards were prepared from the same artificial seawater solution and from the humic acid stock solution which were used to prepare the samples. The standards were adjusted to pH 8.0 with 0.05 N NaOH.

A possible source of error in this measurement was that the humic acid-iron complexes could have different absorption properties than the uncomplexed humic acid in the standards. This possible source of error was investigated by preparing a standard humic acid solution at exactly the same concentration of the humic acid in the sample solutions measured by the described technique, and comparing these two solutions on a double beam spectrophotometer. This experiment yielded a straight base line relationship from 600 nm to 320 nm indicating no detectable differential absorption of light by the humic acid-iron complexes in the sample compared to the uncomplexed humic acid in the standards.

Iron in the supernatant solutions was analyzed using a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer equipped with a three-slot burner head, a manual readout system and an air-acetylene flame. The standards for the iron analysis were prepared from the same seawater solution as the samples but contained no humic acid.

There are two possible corrections that need to be made in this iron measurement. One correction involved the presence of residual iron remaining after isolation and purification of the humic acid; this residual iron in the humic acid was taken into consideration in the

mass balance calculations performed on the system. The second source of error was the possibility of an enhancement of the iron signals due to the presence of humic acid in the samples. This enhancement has been reported by Yuan and Breland (1969) to be on the order of 10%. We were unable to detect the enhancement effect in our system and therefore included no factor for enhancement in our mass balance calculations for iron.

D. Analysis of the Precipitate

The precipitate was dissolved in 4 ml of a 0.026 N EDTA solution (pH = 12). This solution was diluted to 10 ml or 25 ml depending on the nature of the particular experiment performed. The humic acid and iron were analyzed using the instrumentation already discussed.

Standards were prepared from the same EDTA solution used to prepare the samples. Standards for iron analysis contained a fixed amount of humic acid and varying amounts of iron in the EDTA medium. Standards for humic acid analysis contained the same concentration of iron as the samples for that particular set of experiments and varying amounts of humic acid.

E. Blanks

The blanks for the iron analyses consisted of the humic acid in the artificial seawater medium and were treated in the same manner as the samples except for the addition of iron. The iron in the blanks was never more than 10 percent of the iron in the samples. This iron in the blanks was most likely due to the residual iron in the humic acid remaining after purification and, as already mentioned, was taken

into consideration in the mass balance calculations. The blanks for the humic acid analysis consisted of iron in the artificial seawater medium and were treated in the same manner as the samples except for the addition of humic acid. The absorption of light at 350 nm by these blanks was essentially zero.

F. Statistics

All results are reported as an average of replicates with its uncertainty calculated at the 90 percent confidence limit using the t-test based on range developed by Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

Cation exchange capacity measurements performed before and after removal of the humic fraction from various Narragansett Bay sediments indicated that extraction of the humic fraction significantly lowered the cation exchange capacity of the sediment (Table 1). There is a positive correlation between cation exchange capacity and percent organic carbon. The removal of the humic fraction decreased the cation exchange capacity by as much as 55% (sediment E-2). This indicated that the humic fraction strongly influences the ability of the sediment to interact with metal ions.

This capacity of the humic fraction to complex metal ions was investigated in our model complexation experiments. The humic acid used in these experiments was obtained from the sodium hydroxide extract of sediment from station F.P.

The results of the iron analyses in the complexation experiments are presented in Figure 2. As the amount of humic acid used in the experiment was increased from 0 mg to 7.50 mg, the amount of iron 'solubilized' increased and the quantity of iron found in the precipitate decreased. The mass balance for each set of experimental conditions is also presented (Figure 2).

The results of these complexation experiments indicate that humic acid could be involved in the transport and accumulation of iron in Narragansett Bay. This conclusion is in agreement with the findings of Rashid (1971, 1972), and Koshy, et al., (1969) in their work on the solubilization of metal salts by marine humic substances. Moreover, iron could be one of the trace metals responsible for the precipitation

of humic acid in estuaries, thus implicating these reactions as mechanisms for the accumulation of iron-rich organic matter deposits common in near-shore sediments (Swanson and Palacas, 1965).

The humic acid-iron ratios used in our model experiments regulated the solubilization and precipitation equilibria. High humic acid-iron ratios increase the solubility of iron in the medium.

The results of the humic acid analyses in the model experiments are presented in Table 2. As the amount of total humic acid used is increased, the amount and the percent of soluble humic acid are also increased. Above the humic acid-iron ratio of 50/1 w/w, the amount of humic acid found in the precipitate decreases (Figure 3). This increase in the solubility of humic acid-iron complexes is due to a different distribution of the limited quantity of iron on the humic colloids, thus making them less susceptible to precipitation by the iron. The mass balance for each humic acid analysis is also included in Figure 3.

The results of these experiments can be visualized if one considers that humic acid consists of fragments ranging in molecular weight from a few hundred to several million (Schnitzer and Khan, 1972). Rashid (1971) has demonstrated that the lower molecular weight fragments of marine humic acid are more efficient in solubilizing iron than the higher molecular weight fragments. Since the higher molecular weight fragments are less efficient in solubilizing iron due to a smaller charge to mass ratio, these will be precipitated out upon reaction with a given number of ferric ions (x), while the lower molecular weight fragments will solubilize the x ferric ions (Figure 4). As the humic acid-iron ratio is experimentally increased, the iron becomes limiting

and each humic acid fragment becomes associated with fewer than x atoms of ferric iron. Hence, the negative charge on each fragment is maintained and as a consequence some of the higher molecular weight fragments, present as a gradation of molecular weight sizes, are more efficiently hydrated and are thus more soluble (Ong and Bisque, 1968). Increasing the humic acid-iron ratio further will lead to the solubilization of all the iron and humic acid used in experiment.

This rationale attempts to explain the increase in the iron and humic acid in the soluble phase as the humic acid-iron ratio is experimentally increased. It also accounts for the increase in humic acid as precipitate for humic acid-iron ratios up to 50/1 w/w, and the subsequent decrease in precipitate as the humic acid-iron ratio is increased.

Other observations lending plausibility to this model are summarized in Figure 5. A decrease in the percentage of 'soluble' iron and soluble humic acid results when increasing quantities of iron are added to a constant amount of humic acid. We would predict from our model that the humic acid fragments eventually would become so saturated with iron that all the complexes would become insoluble, due to charge reduction on the polymers, regardless of the size of the humic acid fragment.

An additional line of evidence to substantiate this concept is presented in Table 3. By calculating the cation exchange sites available on the humic acid in both phases of the system and using the equivalent weight of iron along with the assumptions listed below, we were able to calculate the percent of saturation of the humic acid in

each phase of the system at the various experimental points. The cation exchange capacity measurements were performed on the humic acid using the $\text{Ba}(\text{Ac})_2$ method of Chandler (1939). This yielded a cation exchange of 141 ± 42 meq/100 g of humic acid. This figure, along with the measured quantity of humic acid found in each phase at each experimental point, was used to calculate the sites available for reaction on the humic acid. The amount of iron found in each phase along with its equivalent weight was used to calculate the sites occupied by iron on the humic acid fragments. The three assumptions we made in making this calculation were:

1. The primary inorganic iron species reacting with the humic acid in the medium was $\text{Fe}(\text{OH})_2^{+1}$. Thermodynamic calculations indicate this to be the main form of iron at the pH and ionic strength used in these experiments (Kester and Byrne, 1972). Also, Schnitzer and Khan (1972) present data suggesting that at pH 8 humic acid forms iron hydroxy complexes.

2. Iron is preferentially chelated over calcium, magnesium and sodium.

3. The precipitated humic acid in our complexation experiments has the same cation exchange capacity as the humic acid measured as a whole. This assumption may not be entirely valid because the exchange capacity of the precipitated humic acid may be lower than the exchange capacity of the bulk humic acid.

The results of these calculations are presented in Table 3. The data indicate that more of the available sites are occupied by $\text{Fe}(\text{OH})_2^{+1}$ on the precipitated humic acid than on the soluble humic acid. Although there are great variations in the percent saturation of the

humic acid, particularly in the precipitate, this calculation does illustrate that the negative sites on the humic acid in the precipitate exhibit a saturation effect and hence are insoluble in the medium as defined by our centrifugation technique.

The results of an experiment indicating the stability of these 'soluble' iron humates are presented in Table 4. After centrifugation and analysis, the soluble phase of a complexation experiment was refrigerated and reanalyzed after 60 days. A t-test performed at the 10% level indicates that there is no significant difference between the means of the two populations in the iron or humic acid data. This indicates that the 'soluble' iron humates are capable of resisting hydrolysis to the insoluble iron hydroxide.

These reactions between iron and humic acid may be important in the solubilization and precipitation of these materials in natural water systems, particularly in areas where municipal and domestic sewage dumping promote high organic matter and trace metal concentrations. The formation and transport of soluble metal complexes from these areas, with subsequent deposition as the complexes become more saturated with iron and other metals or clays, may be an important mechanism in the accumulation of iron-organic matter complexes in sedimentary deposits.

The possibility also exists that these soluble and insoluble complexes are capable of undergoing ion exchange or adsorption reactions with toxic metal ions, thus implicating this mechanism in the scavenging of trace metals from natural waters and accumulating these metals in the organic fraction of sedimentary deposits and into the food web via deposit and filter feeders.

TABLE 1. EFFECT OF HUMIC SUBSTANCES ON CATION EXCHANGE CAPACITY OF SOME NARRAGANSETT BAY SEDIMENTS

SEDIMENT SAMPLE	C.E.C. Meq./100g	% ORGANIC CARBON	C.E.C. Meq./100g AFTER 0.5 N NaOH EXT.	% DECREASE IN C.E.C. DUE TO 0.5 N NaOH EXT.
F.P.	22.1 ± 0.8	3.4 ± 0.3	15.6 ± 0.5	29%
D.	15.4 ± 0.5	2.5 ± 0.8	12.9 ± 0.7	16%
E-2.	7.6 ± 0.4	1.7 ± 0.3	3.4 ± 0.1	55%
B.	5.2 ± 0.2	1.0 ± 0.1	2.4 ± 0.1	54%

TABLE 2. EFFECT OF ADDED HUMIC ACID ON SOLUBLE HUMATE IN THE SOLUBLE FRACTION OF THE COMPLEXATION EXPERIMENTS

H.A. ADDED TO EXPERIMENT	H.A. SOLUBLE	H.A. % SOLUBLE
0 μ g	- -	- -
750	400 \pm 31 μ g	53%
1250	873 \pm 30	70%
1750	1175 \pm 47	67%
2500	1738 \pm 53	70%
5000	4514 \pm 105	90%
7500	7150 \pm 66	95%

TABLE 3. PERCENT SATURATION OF THE AVAILABLE EXCHANGE SITES ON THE HUMIC COLLOIDS WITH $\text{Fe}(\text{OH})_2^{+1}$

H.A. USED IN EXPERIMENT	% OF AVAILABLE SITES OCCUPIED ON H.A. IN SOLUBLE FRACTION	% OF AVAILABLE SITES OCCUPIED ON H.A. IN PRECIPITATE
750 μg	$30 \pm 9.6 \%$	$148 \pm 50 \%$
1250	24 ± 8	127 ± 38
1750	22 ± 8	80 ± 25
2500	18 ± 7	56 ± 18
5000	10 ± 3	62 ± 20
7500	8 ± 3	100 ± 43

TABLE 4. STABILITY DATA ON SOLUBLE IRON-HUMATES
2500 μg H.A. USED IN EXPERIMENT 50 μg IRON ADDED

TIME (DAYS)	SOLUBLE IRON	SOLUBLE HUMIC ACID
0	24 \pm 4 μg	1750 \pm 50 μg
60	19 \pm 3 μg	1950 \pm 100 μg

Fig. 1. Flow diagram of the analytical procedure used in the H.A. complexation studies.

ARTIFICIAL SEAWATER

35 PPT NaCl, 400 PPM Ca, 1200 PPM Mg, pH 8.0

PLUS 1 PPM Fe, H.A. (0-150 PPM)

CENTRIFUGED AT 40,000Xg

PRECIPITATE

DISSOLVED IN NaOH-EDTA

ANALYZED FOR:

Fe BY ATOMIC ABSORPTION

H.A. USING A BECKMAN DU

SPECTROPHOTOMETER

SOLUBLE FRACTION

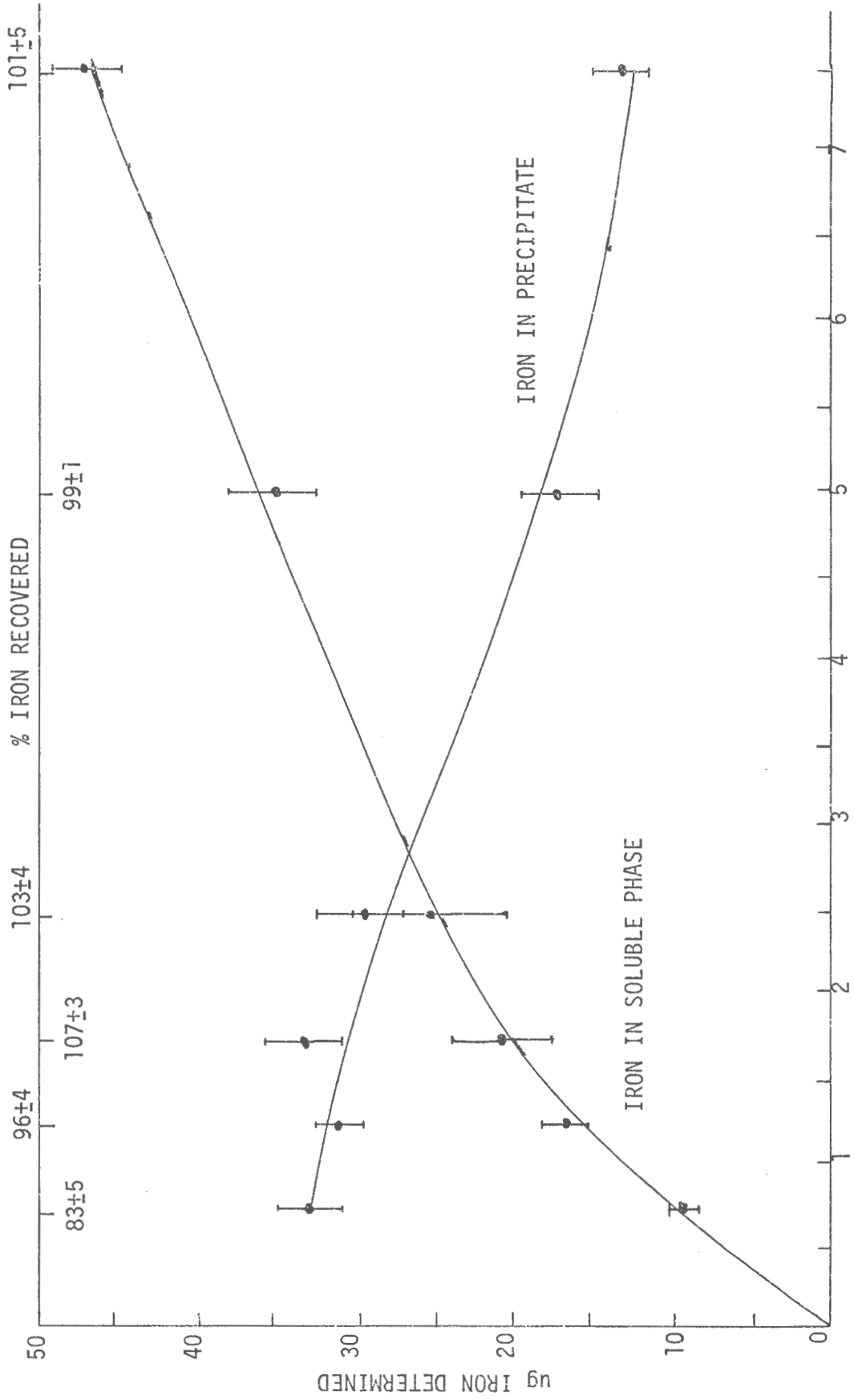
ANALYZED FOR:

Fe BY ATOMIC ABSORPTION

H.A. USING A BECKMAN DU

SPECTROPHOTOMETER

Fig. 2. Effect of humic acid on iron solubility in the complexation experiments. (constant amount of iron added = 50 ug)



mg H.A. USED IN EXPERIMENT

ug IRON DETERMINED

Fig. 3. Effect of added humic acid on precipitated humate in the complexation experiments. (constant amount of iron added = 50 ug)

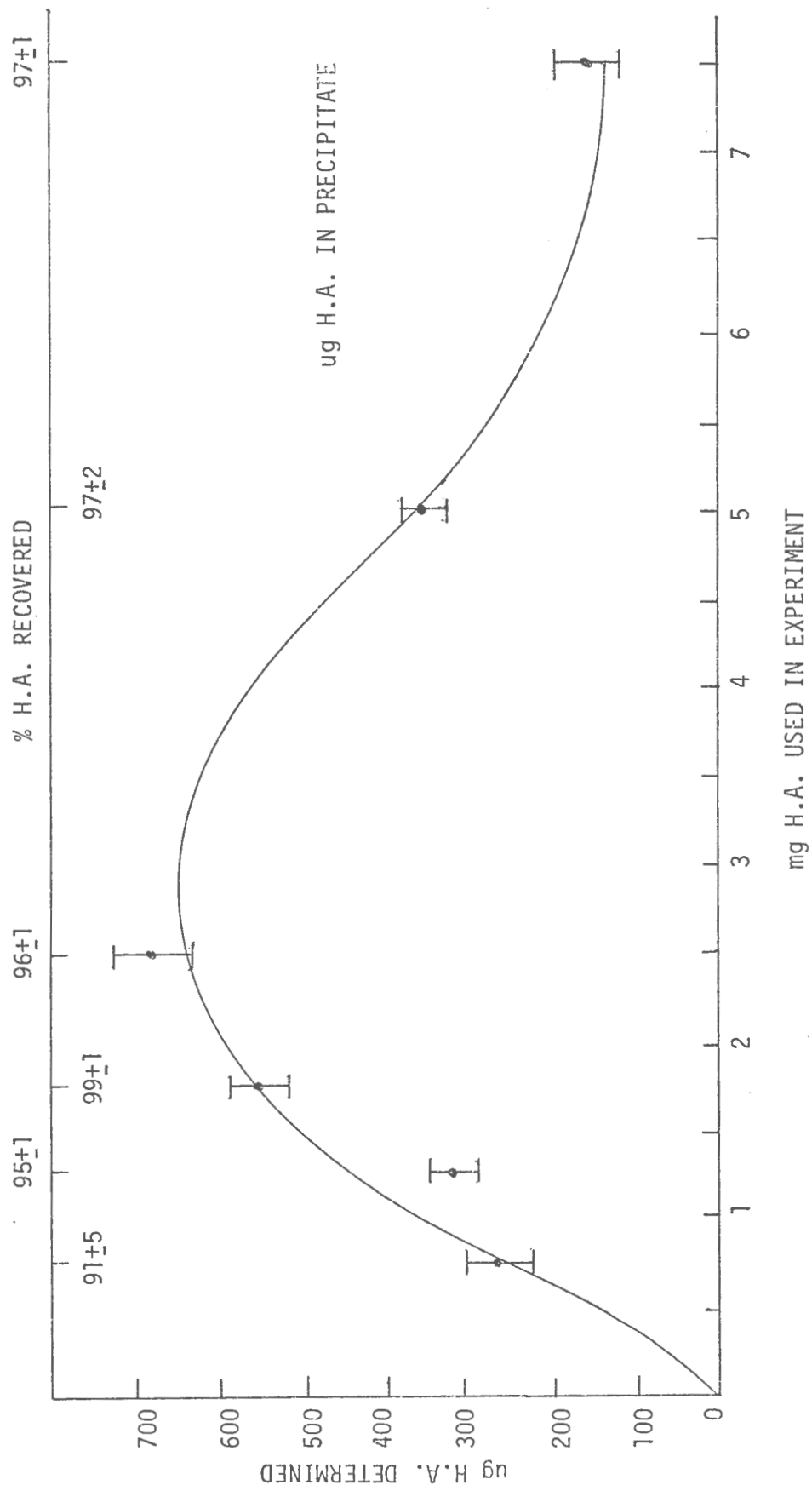


Fig. 4. Schematic representation of the interaction of iron with humic with increasing humic acid to iron ratios.

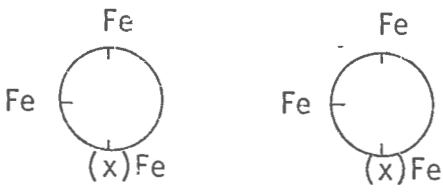
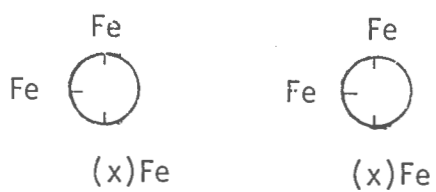
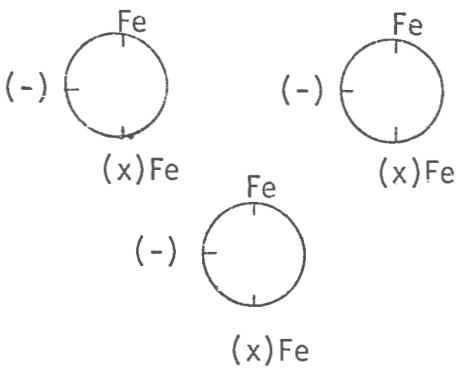
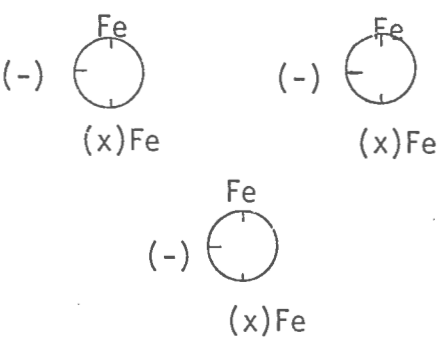
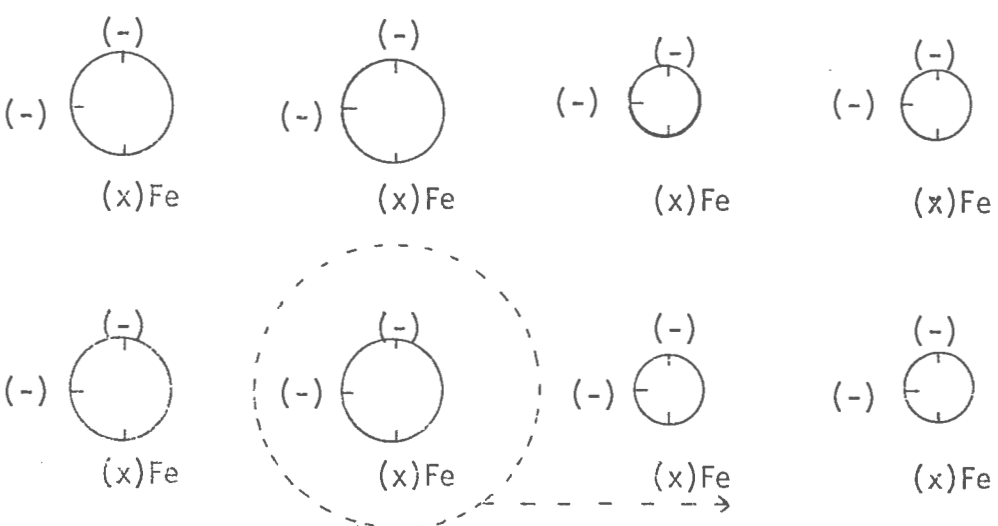
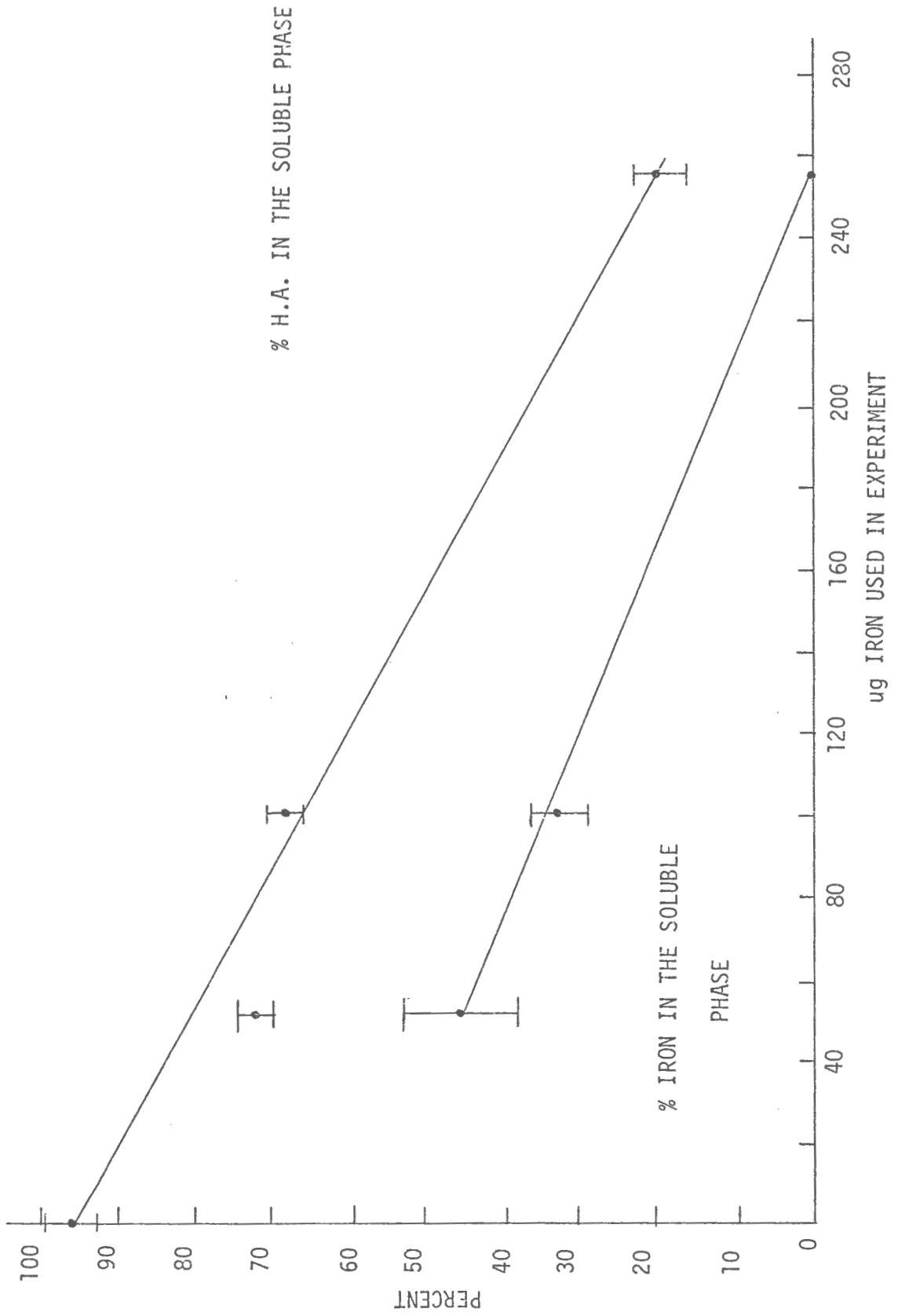
HIGH MOLECULAR WEIGHT FRAGMENTS ASSOCIATED WITH IRON - 'INSOLUBLE'	LOW MOLECULAR WEIGHT FRAGMENTS ASSOCIATED WITH IRON - 'SOLUBLE'
	
	
<p style="text-align: center;">INCREASING H.A. ADDED</p> 	

Fig. 5. Effect of added iron on percent materials in the soluble phase of the complexation experiments. (constant amount of humic acid added = 2500 ug)



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APPENDIX A

LITERATURE REVIEW

Introduction to the Nature and Complexation of
Iron in Seawater

The nature of iron in seawater has been the subject of considerable speculation in recent years (Siegel, 1971). Part of the discrepancy between the high concentrations of 'soluble' iron found in seawater and the concentrations predicted on the basis of the solubility product of iron hydroxide has been attributed to the presence of natural organic chelators (Duursma and Sevenhuysen, 1966).

These chelators can change the solubility characteristics and the properties of iron in aqueous systems (Corcoran, 1967). Iron, like many other metallic ions in seawater, becomes coordinated with water molecules. These water molecules may be replaced or displaced to varying extents by other groups, including hydroxyl ions. Chelation resembles coordination except that the molecular linkage is a ring structure (Johnston, 1964).

Experiments conducted by Laevastu and Thompson (1957) demonstrated that up to 75% of the 'soluble' iron present in coastal seawater was present in the form of iron organic complexes. They concluded that these organic chelators play an important role in regulating the concentrations of 'soluble' iron in seawater. Corcoran (1967) found iron to exist in two principal forms in tropical seawater, namely particulate and as a soluble organic complex. He states that while runoff controls the supply of iron to the sea, the content in seawater is regulated by water movements, solubility, biological activity and the ability of iron to form organic complexes.

Soil Humic Substances and Iron Complexation

Scant data are available on natural organic substances in seawater (Rashid, 1971). However, studies indicate that these organics resemble humic substances found in soils and fresh water (Schnidler, et al., 1972). Although the actual structure of soil humic substances is poorly understood, much is known about the ability of these soil organics to chelate metal ions.

The formal distinction between complex and chelate is often arbitrary for metal humates and fulvates. It is difficult to differentiate between the two, especially when the same kind of donor groups on the organic molecules are involved (Schnitzer and Khan, 1972). Hence, these terms will be used interchangeably in referring to metal organic compounds in the course of this discussion.

Humic substances are a group of amorphous, dark-colored, polymeric materials operationally defined as an acid soluble, base soluble fulvic acid and a base soluble, acid insoluble humic acid. The molecular weights of these substances have been reported to range from a few hundred to several millions and are probably the most widely distributed natural products on the earth's surface, occurring in lakes, soils, rivers and the sea (Schnitzer and Khan, 1972).

The great variation in molecular weights of these substances could effect the solubilities of the metal complexes formed (Rashid, 1971). Superimposed on this solubility relationship, due to the size of the molecular weight fraction in these complexation reactions, is the fact that more than one type of complex can be formed between metal ion and complexing agent. Schnitzer and Skinner (1963) demonstrated that

soil humic acid is capable of forming a range of molar complexes with iron and aluminum ranging from 1:1 to 6:1 metal to humic acid complexes and becoming increasingly water insoluble as more metal is complexed.

The Fuoss theory can be used to understand the coagulation of humic acid by metal ions (Ong and Bisque, 1968). In the presence of cations, the charge on the humic acid is reduced. This reduction of the charge decreases the amount of water of hydration that can be held by the colloids. Consequently, the macromolecule takes on the properties of a hydrophobic sol and precipitates out of solution. Ong and Bisque (1968) investigated the coagulation of soil humic acid with metal ions and concluded that the greater the charge on the metal ion, the less metal required to precipitate the humic acid, in agreement with the Schultz Hardy Rule. Also, for monovalent or divalent metal ions, the coagulation power increases as the radius of the hydrated ion decreases.

Schnitzer and Skinner (1965, 1966) have been able to identify the functional groups of soil humic acids involved in the solubilization and precipitation of metal ions. Using methylation and acetylation techniques, they concluded that two types of reactions must be involved in the retention of iron and other metallic ions by humic acid: one involving simultaneously both acidic carboxyl and phenolic hydroxyl groups and a minor reaction in which only carboxyl groups participate. In another study by Schnitzer and Skinner (1964), infrared spectra of iron humic acid complexes indicated the formation of electrovalent metal carboxylate bonds.

Other investigators have demonstrated that iron can react chemically with humic acid. Van Dijk (1971) showed iron to be firmly bound to the soil humic acid molecules, judging from the magnitude of

the pH decrease on addition of the inorganic iron salt. Potentiometric titrations of soil humic acid in the presence of iron III indicated the formation of metal humic acid complexes (Khan, 1969). Drozdova (1968) has even suggested that iron can act as a catalyst in the formation of humic acid polymers.

Hall and Packham (1965) performed coagulation experiments on soil fulvic acid with iron and followed the precipitation of fulvic acid spectrophotometrically. The fact that maximum removal of the humic acid occurs at or near the pH corresponding to the maximum zeta potential suggests the formation of basic salts rather than occlusions of humic acid in iron hydroxide sols.

Aquatic Humic Substances and Iron Transport

The ability of humic acids to form complexes with metal ions and their widespread distribution in the environment has led investigators to consider these materials as a means of transport of otherwise insoluble metal ions in aquatic systems. Studies of organic color from fresh water lakes indicate that the nature of these substances and their possible reactions with iron are similar to those of soil humic substances (Christman, 1968). Shapiro (1964) investigated the phenomena of solubilization of iron by yellow organic acids isolated from lake water. He found increased iron holding capacity of the yellow organic acids under alkaline conditions. Shapiro proposed that the solubilization of iron is due to chelation and dispersion of iron hydroxide coated with organic acids. He ascribed the iron holding capacity of these acids to carboxyl and phenolic hydroxy groups. The results

suggest the large quantities of iron held in apparent solution in highly colored lake waters may be in the form of a protected colloid. Although there is some evidence for chelation, it seems to play a minor role unless there is a high organic matter to iron ratio.

Christman (1968) has separated concentrates of organic color isolated from lake water on Sephadex and found iron to be associated with all size molecular weight fractions. A strong chelating agent was not able to remove all the iron from the colored macromolecules and was least efficient in removing iron from the largest size molecular weight fractions.

Martin, Doig and Pierce (1971) have suggested that there is a linear correlation between humic acid and soluble iron in west coast Florida streams.

Swanson and Palacas (1965) analyzed the metal content of natural humates isolated from river water and found high iron and aluminum concentrations and varying amounts of trace metals. They suggest that trace metals can be adsorbed during flocculation of the iron or aluminum humate. They implicate this as a possible mechanism for concentrating trace elements in organic deposits.

Marine Humic Substances and the Solubilization of Iron

A few studies have been conducted which demonstrate the ability of marine humic substances to effect the migration and accumulation of various metals. Rashid (1971) determined the ability of humic acids of marine origin (sediments) to solubilize metal ions. He found low molecular weight fractions of humic acid to be considerably more

effective in solubilizing various metals, including iron, than high molecular weight fractions. Rashid (1972) has demonstrated the importance of quinone groups in complexing studies with marine humic acids isolated from sediment samples and the ability of these humic acids to solubilize metal carbonates. Koshy, Desai and Ganguly (1969), using radioactive tracer techniques, demonstrated that the dissolved organic matter from a sediment extract was capable of solubilizing iron.

Significance of Iron Complexation in Natural Waters

The phenomena of chelation and the availability of iron to organisms in aquatic systems has been discussed by many investigators. Saunders (1957) states that organic compounds present in seawater may effectively keep iron in a soluble state in the photic zone thus keeping this essential element available to organisms. Johnston (1964) concludes that in seawater there is a very small margin of safety in the supply of iron to algae and that there is reason for concern over the mechanism of sustaining this supply. He states that the chelation of iron in seawater increases the concentration of soluble iron, presents exchangeable iron to the cell membrane and affects the oxidation-reduction equilibrium of the trace metals. Johnston's experiments with mixed cultures confirms the importance of chelation by demonstrating that there was an optimum level of chelation for growth and that in unfiltered sea water no addition of metals need be supplied as enough are already present in colloidal or particulate form. He concludes that the solubilization of trace metals is a very important aspect of seawater fertility in the presence of adequate nutrients.

Schelske's (1962) experiments on a Marl lake water indicated that nutrients did not increase primary productivity of phytoplankton greatly unless chelated iron was also added.

Prakash and Rashid (1968) have demonstrated that humic substances exert stimulatory effect on marine dinoflagellates that is reflected in increased yield, growth rate and carbon-14 uptake. However, they concluded that the positive effect of humic substances on phytoplankton growth cannot be attributed entirely to chelation processes.

Burk (1931) concluded that natural humic acid increases growth primarily, if not entirely, by virtue of the iron it contains.

Barsdate and Matson (1966) state that an excess of a strong complexing agent like humic acid would certainly alter the cycling of trace metals in aquatic systems. They also conclude that the availability of the metals involved would be governed largely by ability of the organisms to assimilate the complex.

Schnidler, et al. (1972), have stated that studies of naturally occurring organics in aquatic systems have not kept pace with their counterparts in soils and drawing parallels between the behavior of soil humics and aquatic humics is questionable. They conclude that more information on the chemical composition and behavior of this organic material, as well as the metal complexes, must be obtained for an understanding of the role of organic material in the metal transport process.

APPENDIX B

METHOD OF ISOLATION AND PURIFICATION OF HUMIC ACID

Method of Isolation and Purification of Humic Acid

The method of extraction and purification used to obtain the stock solution of humic acid was one commonly used by humic acid chemists. The method consists of twenty-four hour exhaustive extractions of 100 g of sediment with 0.5 N NaOH and centrifugations at 800 X gravity for 20 min until the supernatant liquid obtained was colorless. The combined supernatant solutions are acidified with concentrated hydrochloric acid to pH 2 and centrifuged at 800 X gravity for 20 min. The supernatant fulvic acid was discarded and the precipitated humic acid redissolved in 0.5 N NaOH and reprecipitated with concentrated hydrochloric acid at pH 1. The solid humic acid was then redissolved in a minimum of 0.5 N NaOH, centrifuged at 20,000 X gravity in a Sorvall Superspeed RC 2-B for twenty minutes to remove clays and other impurities and then dialyzed against distilled water for five days. The resulting soluble humic acid was centrifuged again at 20,000 X gravity and the humic acid concentration of the solution determined by drying aliquots of the solution at 100°C for 24 hrs in platinum crucibles. The remaining solution was diluted to a volume which was calculated from the humic acid determinations to yield a 250 ppm humic acid stock solution.

APPENDIX C

THE ARTIFICIAL SEAWATER MEDIUM

The Artificial Seawater Medium

The artificial seawater solution was made by adding a sufficient amount of concentrated hydrochloric acid to dissolve 12.3 grams of magnesium metal and 10.1 grams of calcium carbonate. Three hundred and fifty grams of A.C.S. Certified sodium chloride was added and the solution was adjusted to pH 8.0 with NaOH. The iron impurities in the reagents, now in the form of iron hydroxide, were removed by repeatedly filtering through a double layer of Whatman #42 filter paper in a porcelain Buchner funnel under suction and the filtrate was diluted to two liters with distilled water. This was used as the concentrated seawater solution in all the complexation experiments.

APPENDIX D

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