METAL COMPLEXATION BY
SOIL HUMIC SUBSTANCES

by

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# INTRODUCTION

It is will known that the humic substances play an important part in the soil chemistry of transition metals. Studies of the chemical nature of the humic substances indicate that they are capable of forming coordination complexes with transition metals having stabilities greater than found in purely electrostatic ion exchange.

The phenomena of metal complexation by humic substances has been of interest for many years since it is important in processes of metal translocation through the soil in cases where the humic substances are mobile, and in accumulation of metals in soil horizons containing significant amounts of relatively immobile humic substances. Because these substances are clearly involved in the various equilibria involving transition metals in the soil, it is important to determine the extent to which humic substances can complex the transition metals and perhaps measure the stability of the complexes that may form.

It was the purpose of this particular study to investigate certain aspects of metal complexation by humic substances. In the broadest of terms, the intention of the study was to evaluate the range in stabilities of metal-humic substance complexes that may form in the soil and attempt to measure this range by measuring the "stability constants" of metal-humic substance complexes involving humic substances that had been fractionated on the basis of their metal complexing stability.

# LITERATURE REVIEW

## THE NATURE AND PROPERTIES OF SOIL, SEWAGE SLUDGE, AND SURFACE WATER HUMIC SUBSTANCES.

Let us begin by putting the nature of humic substances into perspective by briefly summarizing the current ideas concerning their acidic and metal complexing properties. Bremner (65) describes humic substances from soils as polymeric products of amino acids and lignin residues. There are probably other components in these polymeric compounds, including monosaccharide and polysaccharide units, but certainly the alteration products of lignin are major constituents. Gamble et al. (70) described the coordination sites on the humic substances as "chemically similar but inherently non-identical." Perhaps this characteristic, more than any other, will restrict the interpretations we can draw from measurements of complex stabilities.

Sposito and Holtzclaw (77) describe the humic substance extract as a "heterogeneous assembly of organic molecules that is characterized by wide ranges in chemical composition, molecular weight, and functional group acidities of its constituents." They postulate that it is possible for the humic substances to co-associate and self-associate through H-bonding and salt bridges. The degree of association depends on the pH, temperature, and salt concentration. Furthermore, the acidities of the functional groups in these polyacids cover a range such that distinct equivalence points

are often missing or are nothing more than faint inflections in a titration curve.

MacCarthy and Mark (76) describe the soil humic substances as being "composed of a highly complicated mixture of organic constituents (to which) one cannot apply the term stoichiometry to its complexes in the normal chemical sense." The functional groups that can coordinate metals in the humic substances have acid dissociation constants and metal complexing stabilities falling within a range of values.

Subtle differences in structure and configuration can affect the acidity and metal complexing properties of a given functional group.  It is reasonable to expect that when this mixture of compounds form complexes with transition metals there will be complexes with varying stoichiometries, including both mono- and polynuclear complexes. The views summarized above have been proposed by several workers in addition   to those already cited (Adhikari and Hazra, 72; MacCarthy and O'Cinneide, 74; Sposito et al., 78; and Zunino and Martin, 77).

## PURIFICATION OF THE HUMIC SUBSTANCES.

In general, the method for extracting and preparing a sample for analysis should not involve modification or alteration of the sample, yet when measuring the metal complexing properties of the humic substances a special treatment is necessary.  This is the removal of those metals that

are co-extracted with the humic substances and are associated with these substances in their natural state. Granted, this does not leave the humic substances as they existed in a natural state, but if we wish to measure the degree of complexation of specific metals by the humic substances the co-extracted metals will effect the results and obscure the complexing reactions we want to follow.

Bremner et al. (46) observed that "on the whole, compounds that are good polyvalent-metal extractants are also good organic matter extractants." Thus, it was known early on that in the process of extraction there were many metals that were co-extracted with the humic substances.

The most common extractant for studies of soil humic substances is NaOH in concentrations ranging from 0.1 to 1.0 M. It is a popular extractant because it extracts large quantities of organic materials from soils. Concern that concentrated strong base solutions could catalyze oxidation of humic substances during extraction has led most workers using NaOH to extract under a $N_2$ atmosphere as a precaution.

Still, as pointed out by Dubach et al. (64), the removal of the extracting electrolyte becomes a major problem since it is commonly present in concentrations exceeding the concentration of the humic substances in the extract. Some used milder extractants such as $Na_2CO_3$ (eg., Adhikari and Hazra, 70, 72; Adhikari et al., 72, 73; and Ong et al., 68). Removal of the electrolyte remains a problem even if the concern about chemical alteration is disregarded.

Removal of the extracting electrolyte may be done by dialysis (eg., Elgala et al., 76; Khan, 69; Kribek et al., 77; Schnitzer and Skinner, 65; and Verloo and Cottenie, 72) or electrodialysis (eg., Adhikari and Hazra, 70, 72 and Adhikari et al., 72, 73), in which the dialysis is carried out under an electrical potential. Geering and Hodgson (69) dialyzed against 0.001 M HCl followed by dialysis against water. This has the advantage of combining ion exchange (protons from the acid with cations associated with the humic substances) and dialysis.

The dialysis methods have been criticized by Holtzclaw et al. (76) after they found that low molecular weight fractions were lost by dialysis. Their chemical and IR analyses show that the compounds lost by dialysis were significantly different from those retained. Dialysis against water will only remove the bulk extracting electrolyte and leave the metals associated with the humic substances.

Several workers have used ion exchange resins to remove both the bulk extracting electrolyte and the co-extracted metals. Commonly, a $H^+$-saturated sulfonyl cation exchange resin was used (eg., MacCarthy and O'Cinneide, 74; Ong and Bisque, 68; Schnitzer and Skinner, 63; Sequi et al., 75; and Weber and Wilson, 75) although Shapiro (64) used a weak acid carboxylic cation exchange resin. Others have combined cation exchange with dialysis (Schnitzer and Desjardins, 62) or cation exchange with anion exchange (Hori and Okuda, 61; Matsuda and Ito, 70; and Van Dijk, 71).

This last variation using anion exchangers is probably not advisable. Hori and Okuda (61) reported irreversible adsorption of humic substances on their anion exchanger. Mantoura and Riley (75) used anion exchangers to remove humic substances from lake water by adsorption on the anion exchange resin.

There is a special cation exchange resin with imminodiacetate functional groups on a cross-linked polystyrene resin that has a high affinity for divalent cations and transition metals, with the latter forming stable coordination complexes. Mantoura and Riley (75) used ammonium saturated chelate resin (the imminodiacetate ion exchange resin) to remove metals from the humic substances they extracted from lake water. Sposito and Holtzclaw (77, 79) and Sposito et al. (77, 78) combined strong acid cation exchange followed by chelate exchange to remove divalent alkaline earth metals and transition metals from sewage sludge-soil humic substance extracts.

Several workers have reported that removal of metals from humic substances improves the solubility of these compounds considerably (eg., Bremner et al.,46 and Hori and Okuda, 61). An investigation of the effects of the co-extracted metals on the solubility of the humic substances was done by Sequi et al. (75). They found that treatment of soil extracts with strong acid H-resins increased the solubility of these substances at low pH's. Thus, if one used acid precipitation as the means of separating humic

acids from fulvic acids as is traditionally done, the amount
and nature of the co-extracted cations will strongly influence
the solubility and hence the separation that actually occurs.

## CHROMATOGRAPHY BASED ON METAL COMPLEXING PROPERTIES.

As mentioned above in the section on the purification
of the humic substances, the majority of workers who frac-
tionate the humic substances have traditionally followed
the convention begun by Kononova (61) in which the base ex-
tracted soil humic substances are acidified with an inorganic
acid to a pH between 2 and 3. The substances that precipi-
tate are called collectively the humic acids while the
substances remaining in solution at this low pH are called
the fulvic acids.

Although this convention is simple and widely used, it
is not necessarily reliable (eg., Sequi et al., 75). If
we are interested in the metal complexing properties of
the humic substances, then fractionation on the basis of
pH dependent solubility, although perhaps correlated to the
metal complexing ability of the fraction, is not entirely
the most suitable method of separation.

Schnitzer and Khan (72, 78) and Flaig et al. (75),
as well as numerous other reviewers discuss the chromato-
graphic techniques that have been used for separating humic
substances. Amoung these are gel filtration (based on
molecular volume under ideal conditions) and adsorption
chromatography on a variety of supports (activated charcoal,

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alumina, silica gel, starch, and cellulose). Dialysis, although not a chromatographic technique, has also been used to achieve a rough separation based on molecular volume (eg., Bloomfield et al., 76 and Khanna and Stevenson, 62).

When the property of interest is metal complexing stability the best chromatographic support would be one that interacted with the humic substances and separated the mixture of compounds on the basis of metal complexing stability. Ligand-exchange chromatography was first described by Helfferich (61). Early methods saturated sulfonyl resins with a transition metal such as $Ni^{2+}$ and equilibrated them with ammonium solutions. A disadvantage in using sulfonyl resins was that most transition metals would "bleed" off the column as the result of ion exchange with the ammonium solution used to elute the samples. This restricted choice of metals to $Ni^{2+}$ which forms a reasonably stable complex with the sulfonyl group (Shimomura et al., 67). With the availability of the chelate resins with the iminodiacetate groups it was possible to prepare a column support that, once saturated with the metal of choice, the metal did not "bleed" off the resin during chromatography.

The methods of ligand-exchange chromatography are discussed by Shimomura et al. (67) and Goldstein (67). From Goldstein (67) we have: "Even when attached to the resin, the metal ion retains its ability to coordinate ligands so that complexing agents can be sorbed on the column and then eluted by displacement with another ligand. Separations

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occur because of differences in the stabilities of the metal-ligand complexes." In both of the studies cited above the columns were equilibrated with ammonium solutions whether they were $Ni^{2+}$ or $Cu^{2+}$ saturated resins.

Others, working with surface waters, have used metal saturated chelate resins to remove dissolved amino acids from the water samples (Clark and Jackson, 72 and Siegel and Degens, 66), displacing the amino acids with 2 M $NH_4OH$ solutions. Siegel and Degens (66) suggest that the imino nitrogen is also involved in coordinating the chelated metal in addition to the two acetate groups giving a tri-dentate site "similar to those found on ethylenediamine-tetraacetate." Shimomura et al. (67) found that there were fewer coordination sites on the $Ni^{2+}$ held by an iminodi-acetate group on a chelate resin than when it is held by a sulfonyl group. As a result, the elution volume for a ligand is smaller for a $Ni^{2+}$-saturated chelate column than when passed through a $Ni^{2+}$-saturated sulfonyl column.

## TECHNIQUES FOR THE MEASUREMENT OF MOLECULAR WEIGHTS.

There are several ways for expressing the concentra-tion of a solution of humic substances. Some forms are more desirable than others since they carry more information about the system. Concentrations of solutions of humic substances have been reported variously as: weight, "max-imum complexing capacity", equivalents, and moles per unit volume of solution.

Weight concentration carries the least amount of information and is the least useful. "Maximum complexing capacity" and related forms of expressing concentrations will be discussed in a later section dealing with the measurement of complexing stabilities.

Determining the equivalent concentration, or perhaps more accurately the moles of titratable protons per unit volume, is straight forward involving simply titration of the humic substance solution with a strong base in a solution where the ionic strength is held constant through the use of a background electrolyte. Special considerations involved in the titration of these solutions will be discussed more fully in the section on base titration of humic substances.

Considering the availability of simple techniques for determining molecular weights it seems that the molar concentration should be determined and used when measuring the stability of metal-humic substance complexes. This, combined with the number of equivalents of titratable protons per unit volume of solution gives us considerable useful information about the system.

Gel filtration has been used by some (Ghassemi and Christmen, 68 and Kribek et al., 77) for the study of metal-humic substance complexes. This method (discussed by Schnitzer and Khan, 72 & 78) while attractive for its simplicity should be used with caution.

The two most common "gels" used in this method are dextran and polyacrylamide. Dextran is simply a highly

cross-linked polysaccharide consisting of D-glucopyranose units. Since cellulose and starch, both polysaccharides, have been used as solid supports for adsorption chromatography separations of soil humic substances (Schnitzer and Khan, 78 and Flaig et al., 75) it seems quite possible that humic substance compounds may be adsorbed on dextran gels thereby rendering molecular weight separations impossible.

Kribek et al. (77) report gel filtration chromatography of humic acids in solutions without metals. Peaks corresponding to the elution of each fraction shifted when the pH of the buffer solution increased. They report "a very substantial shift in the direction of the high molecular weight fraction. . .with increasing pH of the buffer." Ghassemi and Christman (68) found that the "apparent molecular weight" increased as the pH increased. This increase was gradual in the pH range 4 to 7, but increased "sharply" in the pH range 8 to 10. They suggest that adsorption of humic substances by the dextran gel may occur. They also report precipitation of humic substances at the top of their gel column.

The other common gel material, polyacrylamide, is chemically similar to the polyamide supports that are suggested for adsorption chromatography of phenolic compounds. Since there is general agreement that phenols are common in humic substances it seems likely this material would not be suitable for determining molecular weights just as the dextran gel.

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Dialysis and electrodialysis do not give molecular
weights but ranges of weights determined by the porosity
of the membrane (Adhikari and Hazra, 70, 72; Adhikari et al.,
73, 77; and Khanna and Stevenson, 62). These values will
not be useful in determining molar concentration.

There are several methods for determining the number-
average molecular weights using the colligative properties of
solutions. Bonner et al. (58) discusses the meaning of
number-average molecular weights: "All actual samples to be
tested are to some extent mixtures, or may be considered
mixtures in order to set limits to the experimental errors
of measurement. As a result, it is necessary nearly always
when dealing with actual samples to think in terms of the
average molecular weight of the sample. When molecular
weight measurements are made on mixtures, the kind of aver-
age result obtained depends on the method used. The term
'number-average' denotes a method in which every molecule
present, regardless of its size, gives rise to the same
response." Considering the nature of the humic substances,
it seems reasonable this approach to measuring molar concen-
tration will give us the best results.

The cryoscopic methods are one class of methods for
measuring number-average molecular weights. It has been
used by Schnitzer and Desjardins (62) to obtain molecular
weights for humic substances extracted from a Podzol and
purified by ion exchange and dialysis. The humic substances
from the $A_0$ horizon were reported to have a molecular weight

of 1648 and the substances from the $B_h$ horizon were reported to have a number-average molecular weight of 669. In a later study Schnitzer and Skinner (65) used the cryoscopic method to obtain a molecular weight of 670 for soil fulvic acids.

Wilson and Weber (77) report molecular weights <u>corrected for dissociation of weak acid groups</u> for samples of soil and water humic substances using a cryoscopic osmometer. This method involves supercooling the solution to a temperature below its freezing point then intitating crystallization by agitation and measuring the temperature during crystallization. They report corrected molecular weights of 644 (soil humic substances) and 626 (surface water humic substances).

Shapiro (64) used isothermal distillation to measure the molecular weights of humic substances from surface water that had been purified by ion exchange. Measured molecular weights for several different samples were in the range of 180 to 426 with an average of 322.

There is yet another number-average method that is both reliable and simple. This method is based on the fact that a dissolved solute will lower the vapor pressure of the solvent in which it is dissolved. A detailed discussion of the theory behind this method appears in appendix I. This method has been widely used in polymer science (Wachter and Simon, 69) and recently has been used in humic substance studies.

Schnitzer and Khan (72) report molecular weights for two fractions, separated using gel filtration chromatography, measured using vapor pressure osmometry (VPO). The values were, for the low molecular weight fraction: 300, and for the high molecular weight fraction: 1000. Hansen and Schnitzer (69) describe a technique for correcting the apparent molecular weight by VPO. This method is discussed along with the one given by Wilson and Weber (77) in appendix II.

In work that followed this, Schnitzer and Hansen (70) report a molecular weight of 945 for humic substances from the soil using VPO and corrected for dissociation. Sposito et al. (78) report a corrected molecular weight measured by VPO of 243 for humic substances extracted from a sewage sludge-soil sample. Geering and Hodgson (69) obtained a value of 907 by VPO for the molecular weight of the humic substances in their study, but there is no mention whether this is corrected for dissociation.

## EVIDENCE FOR THE FORMATION OF HUMIC SUBSTANCE COMPLEXES OF TRANSITION METAL IONS.

There are many reports verifying the existance of metal-humic substance complexes (Allison, 73; Gieseking, 75; and Schnitzer and Khan, 72, 78). Beyond this, there have been attempts to show that chelate structures exist in the complexes involving soil or surface water humic substances and transition metals. Although this is an interesting question, the exact nature of the complex; i.e.,

whether or not a chelate forms; has no direct value when we are interested in the complexing stabilities of a mixture that contains a variety of complexed forms existing simultaneously. In the absence of techniques that give definitive information on the nature of the complex and distinguish between mono- and polydentate complexes within a mixture; it is more important to characterize the stability of the coordinated metal.

Bremner et al. (46) reported that when a solution of humic substances containing metals was dialyzed against pyrophosphate or malate solutions the metal content of the humic substances decreased but dialysis against water did not remove metals associated with the humic substances. Thus it seems that removal of the metals required species capable of complexing the metals in the humic substances

Bloomfield et al. (76) reported that the dialyzable humic substances from composted lucerne prevented the precipitation of Cu, Co, Zn, Cd, Ni, and Mn as the pH of solutions containing these species increased. These metals precipitate as hydroxyls at high pH in the absence of the humic substances. Several workers have titrated metal solutions and metal-humic substance solutions with base and report that inflections associated with titration of coordinated water observed in metal solutions were not observed or occurred at high pH in solutions where humic substances were present (Adhikari et al., 72; Khan, 69; Khanna and Stevenson, 62; Schnitzer and Skinner, 63a, 63b; Stevenson, 77;

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and Van Dijk, 71).

Many workers have interpreted the release of $H^+$ upon the addition of the metal to the solution of humic substances as evidence for the formation of a complex and seek to relate the magnitude of the decrease in pH to the stability of the complex that forms. Schnitzer and Khan (72) try to relate the number of protons released to the equivalents of metal being complexed. However, Khanna and Stevenson (62) and Khan (69) found that the number of protons released were less than the number of equivalents of metal added.

Beckwith (59) reports: "Chelation reaction can occur with, for example, copper without the demonstrated release of two hydrogen ions per metal atom." Furthermore: "When oxalic acid is titrated with alkali, the addition of even the equivalent amount of copper does not displace the end-point of the titration curve. On the acid side of the end-point the extra alkali consumed, in the presence of copper, is less than the stoichiometric amount and differs at differ-ent pH values." The copper was added as a neutral salt. Thus it seems that titration of solutions of metals and humic substances, although giving evidence of the formation of complexes, will not yield the stoichiometry of the complexes. An exception to this is the method reported by Irving and Rossotti (54) for determining the average number of ligands around the metal center and involves several types of titra-tions.

Stevenson (77) and Van Dijk (71) suggest that some of

the protons titrated result from the titration of coordin-
ated water on partially aquated metal complexes. Schnitzer
and Skinner (63a) observed $Fe^{3+}$ complexes of fulvic acids
titrated identically to the $Fe^{3+}$ in the absence of the fulvic
acids at pH's above 8 and interpret this to mean that above
this pH the fulvic acid complex of $Fe^{3+}$ no longer existed.

Senesi et al. (77) present ESR and Mössbauer spectra
that show some of the $Fe^{3+}$ complexed by the humic substances
they were using was resistant to ligand substitution by
thiocyanate and reduction by electron transfer involving
hydrazine. Shapiro (64) reports that surface water humic
substances change color when $Fe^{3+}$ was added. This color
change was not observed when $Fe^{2+}$ was added although the
color change did occur when $H_2O_2$ was added to oxidize the
ferrous to ferric. MacCarthy and O'Cinneide (74) also
observed that "the organic matter solutions darkened consid-
erably in colour on addition of the ferric ions." Such
color changes are characteristic of transition metal complex
formation.

A number of workers have shown that blocking of carbox-
ylic and phenolic functional groups in humic substances
results in a marked reduction in the complexing capacity
of the substances (Davies et al., 69; Himes and Barber, 57;
and Schnitzer and Skinner, 63b, 65). Schnitzer and Skinner
(65) present evidence from their blocking experiments which
suggests the carboxylic and phenolic groups in their humic
substance sample do not act independently when complexing.

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Schnitzer and Skinner (63b, 65) interpret their results as evidence that the major coordination site in humic substances is a bidentate chelate site having a carboxylic group ortho to a hydroxyl on an aromatic ring. They consider salicylate (o-hydroxybenzoate) as modeling the complexing behavior of humic substances. The modeling of metal-humic substance complexes with o-hydroxybenzoate will be discussed more fully in appendix III.

## BASE TITRATION OF HUMIC SUBSTANCES.

Base titrations of solutions of humic substances follow the behavior we would expect for the titration of a complex mixture of acids and again emphasizes the fact that we are dealing with a heterogeneous system. There are many references in the literature to the type I and type II acidic groups proposed by Schnitzer and Skinner (63b, 65) and cited briefly above. Type I groups are carboxyls ortho to a hydroxyl on an aromatic ring. It is felt by sone that when a metal is coordinated in such a bidentate site it displaces the proton on the phenol (it is assumed that the carboxyl is already dissociated). The type II sites are simply the remaining "less acidic carboxyls." From Gamble (70): "One must assume, as the most general case, that no two carboxyl groups are inherently chemically identical." Furthermore, Gamble (70) suggests that "as neutralization advances to more weakly acidic carboxyl groups, the electrostatic charge accumulating on the polymer molecules will make the carboxyl groups

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even more weakly acidic." Wilson and Kinney (77) describe
the acidic behavior of humic substances from surface waters
in the same manner.

Sposito and Holtzclaw (77) describe the humic substances
extracted from soil-sewage sludge mixtures as polynuclear
polyacids. The functional groups responsible for the acidity
of these compounds are ionized from below pH 2 to above pH 10.
On the basis of elemental analysis of different samples with
varying composition and acidity, these authors concluded
that the groups ionized below pH 2 were sulfonyl groups.
They consider all other acidic groups to be carboxylic,
phenolic, and nitrogen-containing groups.

In later work Sposito et al. (77) propose modeling the
acidic behavior of humic substances using classes of acids.
"The assembly as a whole possesses average properties that
are classifiable and describable in terms of a few macroscopic
parameters. For example, the acidic functional groups
fall into just four classes, each describable in principle
by a certain number of equivalents of dissociable protons
and a protonation equilibrium constant, although on the mole-
cular level it is probable that no two acidic functional
groups are chemically the same."

They develop an expression for a "conditional proton-
ation constant" that is defined in terms of the proton
concentration and the extent of dissociation of the partic-
ular class of acidic groups. On this basis they could model
the titration curve for their humic substance sample using

the following dissociation constants for the pH range 2-10:
$\log K_{II}$= -3.9, $\log K_{III}$= -6.5, and $\log K_{IV}$= -9.4; these being
"conditional protonation constants" for the classes II, III,
and IV; class I is the sulfonyl group.

Gamble (70, 72) reports the existence of two classes
of acidic groups in his humic substance samples and lists
the dissociation constants for these two groups as: $\log K_I$=
-2.05 and $\log K_{II}$= -4.00. These dissociation constants were
determined numerically since the titration data reported
in these papers by Gamble did not reveal any inflections
that could be interpreted as equivalence points.

Although Sposito and Holtzclaw (77) and Gamble (70, 72)
can model the <u>titration curves</u> with acids having the disso-
ciation constants listed above in appropriate equivalent
amounts, this does not mean that a mixture of two or four
acids (as the case might be) having specific acid dissociation
constants will accurately model the humic substances them-
selves in terms of their acidic behavior, buffering charact-
eristics, or metal complexing properties.

Geering and Hodgson (69) report the titration curves
for two fractions, designated simply dialyzable (D) and
non-dialyzable (ND). The ND fraction had only one equival-
ence point at pH 7. At pH 9 the titration curve still had
not plateaued although it seemed to approach an end-point.
The D fraction had an equivalence point at pH 7 and an in-
flection point at pH 3.2-3.7 (it is indistinct). This
inflection, if considered an equivalence point, would give

a $pK_a$ of about 2.5, while the major equivalence point at pH 7 would give a second $pK_a$ (assuming the inflection is an equivalence point) of about 4.3.

Stevenson (76) reports titration curves; however the reported curves do not cover the full pH range and, unfortunately, those given did not include the end-points of the titrations. Stevenson performed the titrations at differing ionic strengths using KCl as the background electrolyte. He found an inflection appearing in the titration curve, which diminished as the ionic strength increased from 0.001 M to 0.01 M and disappeared completely when the titrations were done in solutions that had ionic strengths 0.025 M and above. Although there is an inflection in the titration curves, the inflection is not well defined and it would be difficult to assign a $pK_a$.

In a system where the polyfunctional acid is characterized by groups whose dissociation constants are very similar to one another it is often very difficult if not impossible to distinguish the titration curve for such an acid from a monoprotic acid. In a very interesting paper, Sturrock (68) showed that, for a monoprotic acid:

$$pH_{3/4} - pH_{1/4} = \Delta pH = 0.954$$

where $pH_{3/4}$ and $pH_{1/4}$ are the pH at three quarters and one quarter through the titration of all titratable protons respectively. This holds provided the solution is relatively dilute and the $pK_a$ of the acid is neither very large or very

small.

When a diprotic acid is titrated we will detect no
equivalence point corresponding to the dissociation of the
first group if, for the two dissociation constants $K_{a1}$ and
$K_{a2}$, we have: $K_{a1}/K_{a2} \leq 16$. When this is the case we would
not distinguish the diprotic acid in question from a mono-
protic acid on the basis of the presence of inflection
points in the titration curve alone. Statistically, all
real diprotic acids will have a ratio of the first dissoci-
ation constant to the second greater than 4. Sturrock shows
that for the limiting case hypothetical acid with $K_{a1}/K_{a2} = 4$,
where the conditions allowing use of "$\Delta$pH" hold, we have:

$$\Delta pH = 0.954 .$$

This is the same value for "$\Delta$pH" for a monoprotic acid. For
all _real_ diprotic acids then:

$$\Delta pH \geq 0.954 .$$

As in the case for the diprotic acid, the acid dissoci-
ation constants must meet a minimum requirement concerning
the ratios of successive dissociation constants for triprotic
acids. The statistically limiting case for triprotic acids is:

$$K_{a1}/K_{a2} = K_{a2}/K_{a3} = 3 .$$

Sturrock takes this statistically limiting case and shows
that:

$$\Delta pH = 0.954 .$$

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All real triprotic acids have a "$\Delta$rH" greater than 0.954.

Campbell and Meites (74) discuss the titration curves for generalized polyfunctional acids and show that "$\Delta$pH" for real acids is a function of both the number of dissociable protons and the quotient for each pair of successive dissociation constants for a given acid. It is assumed that: $K_i > K_{i+1}$. Their treatment and the discussion by Sturrock (68) were attempts to determine whether a previously uncharacterized acid was polyfunctional on the basis of information comming from the titration curves alone.

If we compute the ratio of the first "dissociation constant" to the second "dissociation constant" for the two dissociation constants reported by Gamble (70, 72) for his type I and type II acid groups we find that:

$$K_I/K_{II} = 89 \ .$$

Sturrock (68) calculated the titration curves for a series of hypothetical diprotic acids where $K_{a1}/K_{a2}$ varied from 16 to 100. An inflection point corresponding to the most acidic proton was first detectable when $K_{a1}/K_{a2} = 50$, and was somewhat more clearly defined when $K_{a1}/K_{a2} = 100$. Examination of these curves leads to the conclusion that even with careful techniques an inflection corresponding to the type I acids described by Gamble would be very faint.

If, instead of a polyfunctional acid, we were to consider a mixture of acids upon which there is no restriction concerning successive dissociation constants we might well

expect complex behavior.  If we apply the concepts developed
by Sturrock (68) to an equimolar mixture of the acids listed
in Table 1 we would detect only two "equivalence points".  If
we can apply that term to the titration behavior of a mixture.
The first "equivalence point" would be rather broad since
the carboxylic acids dissociate in a range from below pH 2.9
to a pH above 5.0.  The second "equivalence point" would
be somewhat sharper since the phenols do not dissociate over
such a wide range.  Needless to say, the titration curve
would give us little information beyond the extent of dissoci-
ation of the mixture.

TABLE 1

REPRESENTATIVE CARBOXYLIC AND PHENOLIC ACIDS.  (Weast, 75)

| ACID | $pK_{a1}$ | $pK_{a2}$ |
|------|-----------|-----------|
| o-phthalic | 2.89 | 5.51 |
| o-hydroxybenzoic | 2.97 | 13.4 |
| p-phthalic | 3.51 | 4.02 |
| m-phthalic | 3.54 | 4.60 |
| 1-naphthoic | 3.70 | |
| m-hydroxybenzoic | 4.06 | 9.92 |
| 2-napthoic | 4.17 | |
| benzoic | 4.20 | |
| p-hydroxybenzoic | 4.48 | 9.92 |
| acetic | 4.76 | |
| octanoic | 4.89 | |

Acid titration would not, in general distingish between groups that could form complexes of high stability; eg., o-phthalic acid; and groups that we might expect to form complexes of low stability; eg., p-hydroxybenzoic acid and octanoic acid. Association of structural classes; i.e., o-phthalate type structures; with inflections in the titration curve of a complex mixture of acids such as we have with the humic substances is extremely tenuous at best and should be considered highly unreliable until solid, specific evidence supporting such an association is available.

Base titration of mixtures of acids may detect groups that differ significantly in acidity and may give information concerning the magnitude of the difference as well as some estimate of the number of equivalents that can be associated with each broad group, but little information about the mixture beyond this can be obtained. We do not know what effect the distribution and range in the acidities has on the form of the titration curve so that it may not be possible to characterize the nature of the mixture statistically. This would be useful information to know.

Up to this point we have reviewed research that supports a view of the humic substances in which the properties of the sample cover a range of values whether we are interested in molecular weights, functional group acidities, or (as we may conclude) metal complex stabilities. The variablity occurs both within a given molecule and amoung different molecules in the mixture.

## MEASURING THE STABILITY OF THE COMPLEXES CF TRANSITION METALS WITH HUMIC SUBSTANCES: ION EXCHANGE METHODS.

An ion exchange method for measuring the stability constants of metal complexes was first presented by Schubert (48). In the method described by Schubert, it is assumed the stoichiometry of the complex is known since this method does not provide a means of determining the stoichiometry. Schubert's method is not limited to mononuclear complexes, but it does require a specific complex to exist and be the predominant complexed form of the metal in the solution.

This method expresses free metal by a distribution of the metal between that held on the ion exchange resin and in bulk solution.

$$\lambda_o = \frac{(MR)}{(M)}$$

(MR) = the concentration of adsorbed metal for the given conditions at equilibrium.

(M) = free metal concentration in solution for the given conditions at equilibrium.

This distribution, which Schubert calls the "adsorption isotherm", is constant over a fairly large range of metal concentrations.

If metal complexes do not become exchanged on the resin and free metal is the only specie exchanged on the resin, then the distribution of free metal between solution and resin will be the same in the presence and absence of a ligand in the solution even though the total metal in solution

in the presence of the ligand will be higher provided a complex forms. Thus, using the "adsorption isotherm" for free metal on the cation exchange resin, determined in the absence of the ligand, and the metal concentration on the exchange resin one can determine the free metal concentration in solution in the presence of the ligand.

By requiring as a condition of the experiment that the ligand concentration be much greater than the metal concentration, which permits the free ligand concentration to be approximated by using the initial ligand concentration, we will know the concentration of all important species directly except the complex concentration. Since it is assumed that the stoichiometry of the complex is known, the concentration of the complex is simple to calculate.

It is at this point that Schubert makes an error. He states: "It is seen that $[M_xA_y^c]$ is equal to the total counts remaining in solution at equilibrium less the counts of $M^{+a}$, the value of the latter being simply $[MR]/\lambda_o$." Schubert speaks of "counts" because he used a radioactive tracer to determine the metal concentration. The superscript on the complex: $M_xA_y^c$, "c", denotes the charge on the complex. Schubert requires "c" to be less than or equal to zero. By failing to divide the concentration of the complex by "x", the number of moles of metal per mole of complex, Schubert fails to account for the stoichiometry of the complex.

The explicit assumptions made by Schubert are: (1) "The complex-forming ions are 'swamped' by excess neutral salt;

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the ionic strength remains nearly constant," (2) "the con-
centration of $M^{+a}$ is negligible as compared to that of the
complex-forming anion, $A^{-b}$; actually, $M^{+a}$ is present in
radiochemical concentrations ($\approx 10^{-9}$ M)," (3) "all pairs
of solutions which are compared have the same pH, volume of
solution, and weight of absorbent," (4) "the exchanger
used has been previously saturated with the cation compon-
ent of the bulk electrolyte," and (5) "no adsorption of the
complex-forming anion or of the complex ion takes place."
In assumption (3) Schubert is refering to metal-resin sys-
tems with and without the ligand.

To the list of assumptions give above, we can add the
implicit assumptions made by Schubert: (1) the stoichio-
metry of the complex is known, (2) there is only one type
of ligand in the solution (no mixtures), and (3) one complex
is found in the solution which predominates; all other
complexes are so insignificant they can be neglected (again,
no mixtures of types of complexes with the same ligand).

Schnitzer (69) suggests the method proposed by Schubert
may be an "over simplified treatment of the (humic substance)
system." Later, Schnitzer and Hansen (70) state that Schubert's
method works only for mononuclear complexes. It is true
Schubert's method is not suited for the metal-humic substan-
ce system, but not because it is limited to mononuclear
complexes, which it is not. The reason it fails for the
system containing metal-humic substance complexes is its
violation of all of the implied assumptions upon which

Schubert's method is based.

We will again reiterate the important characteristics of the humic substances relating to measurement of complex stabilities. From Gamble and Schnitzer (73): "It must be remembered that fulvic acid is primarily a polyelectrolyte, and that it is even more a mixture than are most polymers. Since, in fact, one cannot assume that any two of the ionizable functional groups are chemically identical, it follows necessarily that an experimentally observed equilibrium function is a weighted average function." We cannot assume that we know the stoichiometry of the complexes in this mixture, that there is one ligand in the solution, or that only one complex specie predominates.

Martell and Calvin (52) describe a modification of Schubert's method. In the method by Martell and Calvin, another condition is placed on the ion exchange experiment that must be met if the results are to be valid. Martell and Calvin introduce the condition that the complex which forms and predominates in solution must be mononuclear. If the complex is mononuclear it is then possible to develop a relation that allows us to determine the stoichiometry.

Martell and Calvin show that, given these conditions, if the distribution of the metal between the exchanger and solution in the presence, "$\lambda$", and absence, "$\lambda_o$", of the ligand are determined then a relation can be developed using the equilibrium expression for the formation of the complex. The distribution of metal between the exchanger

and the solution in presence of the ligand includes both free and complexed metal in solution.

$$\lambda_o = \frac{(MR)}{(M)} \quad ; \quad \lambda = \frac{(MR)}{(M) + (MKe_x)} \tag{A}$$

$(MR)$ = moles of uncomplexed metal bound per unit weight of resin at equilibrium.

$(M)$ = moles of free metal in solution per unit volume at equilibrium.

$(MKe_x)$ = moles of the complex, $MKe_x$, in solution per unit volume at equilibrium.

The equilibrium expression is:

$$K = \frac{(MKe_x)}{(M)(Ke)^x} \tag{B}$$

Eliminating $(M)$ from the expressions in (A) yields:

$$(MKe_x) = \frac{(MR)}{\lambda} - \frac{(MR)}{\lambda_o} \tag{C}$$

Substituting the expression for $(MKe_x)$ given by (C) into (B):

$$K = \frac{(\lambda_o/\lambda) - 1}{(Ke)^x} \tag{D}$$

Taking logarithms gives the final expression:

$$\log\left[(\lambda_o/\lambda) - 1\right] = \log K + x \log (Ke) \tag{E}$$

Plotting: $\log\left[(\lambda_o/\lambda) - 1\right]$, which I will call the "Martell-Calvin distribution function", versus: $\log (Ke)$ will give a straight line if the conditions have been met. The slope of the line will give the stoichiometry "x" and the intercept will give the stability constant "K" (provided the conditions for measuring a true stability constant were met).

The Martell-Calvin method uses the same conditions
and is based on the same assumptions as the Schubert method,
except that the assumption the stoichiometry of the complex
is known has been modified to requiring the complex be mono-
nuclear. The Martell-Calvin method, an improvement over
Schubert's method for certain pure systems, fails when applied
to the system we are interested in for the same reasons
Schubert's method fails.

The Martell-Calvin method has been the ion exchange
method used almost exclusively in attempts made to measure
the "stability constants" for the formation of metal-humic
substance complexes. A number of studies have used this method
with a special modification (Courpron, 67; Elgala et al., 76;
Dhillon et al., 75, Matsuda and Ito, 70; Miller and Chlrogge,
58; Randhawa and Broadbent, 65; and Tan et al., 71).

The procedure followed with little variation in all of
these studies was employed primarily because the concentra-
tion of the solution of humic substances was not known. These
workers plotted the logarithm of the "M-C distribution function"
versus the relative ligand concentration for a series of humic
substance solutions. The slope of this plot was taken as
the stoichiometry of the "complex". The intercept, however,
was the logarithm of the product of the "stability constant"
and the proportionality constant relating the relative con-
centration to the "real" concentration of the humic sub-
stances in solution.

These workers then added sufficient metal to a solution of

humic substances to cause them to precipitate. The metal used was not the same in all of the studies. The difference between the amount of metal added to cause the precipitation and the amount remaining in the supernatant solution when the precipitation was complete was assigned to the precipitated compounds. This was considered the complexing capacity of the humic substances. Using the "stoichiometry" of the metal-humic substance "complex" determined earlier, the equivalent concentration was calculated.

The physical meaning of the slope of a plot of the "distribution function" against the relative concentration of the solution of humic substances is open to question when we consider that different ligands exist in the solution as well as complexes of differing stoichiometries. Furthermore, the relation between the "complexing capacity" measured by the method described above and the equivalent or molar concentration is not clear. It is likely that it depends heavily on the extent of dissociation of the acidic functional groups at the pH of the experiment as well as the tendency of the metal used to hydrolyze to form hydroxyls of lower charge.

There have been other studies that have expressed the concentration of the humic substances as moles per unit volume following measurement of the number-average molecular weights of the samples. Adhikari and Hazra (70) report both the concentration of the humic substances and the metals used when they measured the "stability constants"

of metal-humic substance complexes using the Martell-Calvin method. They used essentially equimolar solutions of metal and humic substances, so that even if the humic substances are a mixture of compounds the conditions set by Martell and Calvin were violated. In other work by this group (Adhikari and Hazra, 72 and Adhikari et al. 73, 77) the metal concentrations used were not reported, although the same method for determining the metal concentration using complexometric titration was used, so that we do not know if the restrictions were followed in this later work.

We should note that Adhikari and Hazra (72) use a disclaimer at the end of their paper stating that it is quite possible that the complexes in the solution are not mononuclear and, furthermore, the fact that the solution is a mixture of ligands will have important implications concerning the validity of interpreting the results as true stability constants.

Schnitzer and Skinner (66, 67) report studies where the condition that the ligand concentration must be much greater than the metal concentration was violated, as in the case in the study by Adhikari and Hazra (72). In later work, Schnitzer and Hansen (70) recognize the violations made in the earlier works. Schnitzer and Hansen (70) compare the ion exchange method and Job's method of continuous variations as alternative methods for measuring the "stability constants" of metal-humic substance complexes. They conclude that Job's method is just as accurate as the ion

exchange method of Martell and Calvin and much easier to perform. Job's method of continuous variations will be considered in greater detail later in this section.

Schnitzer and Hansen (70) also show that an error of $\pm$ 3.3% in measuring the metal concentration at the 20 ug/kg level will result in the slope of the plot of the "distribution function" versus the ligand concentration to vary from 0.69 to 1.55. Thus, the stoichiometry as determined by the Martell and Calvin method is very sensitive to error in the determination of the solution metal concentrations.

Clark and Turner (69) are also critical of the application of the Martell-Calvin method to measurements of stability constants" of metal-humic substance complexes since the method is limited to mononuclear complexes. They point out that a true thermodynamic equilibrium constant refers to the formation of a specific specie. Thus, if the stoichiometry of the complex determined by the Martell-Calvin method is not an integer, the results are questionable.

These workers fail to address the central issue. The fact that the stoichiometry of the "complex" is an integer, determined using Martell and Calvin's method, is not sufficient evidence that a specific specie does exist. It is possible to have a slope of "n", where "n" is an integer, without specific "complexes" existing in the solution.

Zunino et al. (72) discuss the ion exchange method as developed by Schubert, but it is clear in their discussion that they misunderstand the conditions set by Schubert.

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Zunino et al. (72) conclude that the ligand should be considered the central group and not the metal. Clark and Turner (69) come to the same conclusion. Zunino et al. employ the ion exchange method but use metal concentrations much greater than the ligand concentration. They fail to see that the ion exchange method was developed to determine free metal concentration in a system: (1) containing complexed metal and (2) where there is no suitable method for determining the free ligand concentration, thereby requiring the approximation of free ligand by the initial ligand concentration.

Verloo and Cottenie (72) report an experiment where the "stability" of metal-humic substance complexes were measured using an ion exchanger. This work was not based on the relations developed by either Schubert or Martell and Calvin. No attempt was made to determine the stability constants. These workers added metal to a solution of humic substances and allowed the complexes to form, then added Na-saturated resin and measured the total metal in solution. They erroneously assumed the ion resin removed all of the free metal from the solution and considered the metal remaining in solution to be completely complexed.

Ardakani and Stevenson (72) developed relations that were designed to extend the Martell-Calvin method to polynuclear complexes while retaining the capacity to determine the stoichiometry of the complexes by the appropriate graphical methods. Although their methods are capable of deter-

mining the stoichiometry of polynuclear complexes in systems
with a single ligand that reacts with the metal to form a
well defined complex which predominates in the solution
under the experimental conditions, it fails when extended
to systems containing a mixture of ligands forming complexes
of varying stoichiometry.

Other ion exchange methods have been developed to correct
the limitations of methods such as those developed by Schubert,
Martell and Calvin, and Ardakani and Stevenson. Froneaus (51)
describes an ion exchange method for evaluating the stepwise
constants as successive ligands coordinate the metal. This
method follows the formation curve of the complex. In deter-
mining the stepwise constants it is not necessary to deter-
mine the stoichiometry since at any given point no complexed form
may be considered to be the only important specie. The method
is much more general than the other methods described earlier,
primarily because it is not necessary for a specific specie
to predominate in solution.

In his derivation, Froneaus made an error in deriving
the expression for the mean number of ligands around the metal
center as a function of the ligand concentration, $\bar{n} = \bar{n}(A^-)$.
This error is covered in appendix IV.

The Froneaus method was not designed to deal with poly-
nuclear complexes, although it may be possible to generalize
the treatment to include polynuclear complexes. This method,
however, is not suitable for complexes involving humic sub-
stances. It does not extend to a mixture in which there

are stepwise formation reactions occuring for mixed ligand complexes. This method will not allow us to assign a physical meaning to the constants determined using the specified procedures.

MacCarthy (77) reports a derivation of an ion exchange method that is designed for systems with mixed ligands, and where complexes of differing stoichiometry co-exist in the solution. This system, however, increases exponentially in complexity as the number of ligands and the different types of complexes increase. Although this method was designed as a means of treating humic substances with several classes of complexing groups, with each group consisting of complexing sites that are similar in their properties. It attempts to assign physical meaning to classes of non-identical ligands whose properties are poorly defined at best.

## MEASURING THE STABILITY OF THE COMPLEXES OF TRANSITION METALS WITH HUMIC SUBSTANCES: POTENTIOMETRIC METHODS.

Another approach to the measurement of complexing capacity involves using ion-selective electrodes (ISE's) to measure the free metal activity in solutions in the presence of complexed metal. This approach eliminates the need of an alternative equilibrium to determine the free metal concentration in a complex solution.

Cheam (73) reports the measurement of Cu-fulvic acid "stability constants" using ISE's and evaluating the data

using a statistical method first used by Cheam et al. (70) in the measurement of vapor phase association of methanol. In this method, Cheam assumed the stoichiometry of the complex was 1:1 on the basis of the work reported by Schnitzer and Hansen (70). Using this assumption and the free metal activity, measured with a $Cu^{2+}$ ISE, along with the known total metal concentration, Cheam (73) calculated the concentration of the complex: (CuL).

He defines the formation expression for the complex:

$$\bar{K} = \frac{(CuL)(H^+)}{(Cu)(HL)} \tag{F}$$

The degree of ionization of a bidentate chelate site, using the o-hydroxybenzoate model, is given as:

$$\alpha = (HL)/[(H_2L) + (HL)] \tag{G}$$

Cheam shows that the following relation exists:

$$\alpha T_{fa} = (HL) + \alpha(CuL) \tag{H}$$

where $T_{fa}$ is the total fulvic acid concentration in all forms.

Given the expression for the formation of the complex (F); given values for (Cu), ($H^+$), and (CuL); for each trial value for $\bar{K}$ we can calculate $(HL)_c$. Substituting $(HL)_c$ into (H) we can calculate $T_{fa,c}$. Thus, for each $\bar{K}_{trial}$ we have a corresponding $T_{fa,c}$. Cheam then computes the root mean standard deviation for the difference between the measured values for " $T_{fa}$" and calculated values for " $T_{fa,c}$". By

varying $\overline{K}_{trial}$, it is possible to minimize the root mean
standard deviation to obtain a best estimate of $\overline{K}$.

In later work, Cheam and Gamble (74) use this method
to measure the "conditional stability constant" for Hg(II),
Cd(II), and Cu(II) complexes of fulvic acid.  Unlike the
proceeding work, where they called the number determined a
stability constant, in this work they recognize the "appar-
ent" or "conditional" nature of the constant for complexes
involving fulvic acid (and for that matter all humic sub-
stances).  The "conditional stability constant" is defined
as:

$$K^* = \frac{(complex)}{(M)(\overline{FA})} \qquad (I)$$

where (M) is the free metal concentration, (FA) is the
unreacted fulvic acid concentration.  Cheam and Gamble
assume that 1:1 complexes form.

Manning and Ramamoorthy (72a) report values for "stab-
ility constants" for mixed complexes involving fulvic acid
ligands and other common organic and inorganic ligands.
In this work the stoichiometry of the complexes was expressed
not as an absolute number; but as "$\overline{n}$", the average number
of ligands around the metal center.  The experiments were
done in solutions that were dilute enough that only mono-
nuclear complexes formed.  Manning and Ramamoorthy used the
method of Irving and Rossotti (53) to determine the stabil-
ity constants.

The value that Manning and Ramamoorthy (72a) report
for the "stability constant" for the Cu-fulvate complex

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for a fulvic acid from a Podzol $B_h$ horizon is: K = 3.37.

In later work, Ramamoorthy and Kushner (75) express their feeling that a true thermodynamic stability constant cannot be determined for the complexes of metals with soil or surface water humic substances. The alternative is to measure a "conditional stability constant." Unlike the earlier work by Manning and Ramamoorthy (72 a,b & c) the mean number of ligands around the metal center was not determined and the authors made the assumption, an "arbitrary" assumption by their own admission, that the complex is of the 1:1 type. The humic substances used in this study come from river water and are neither analyzed for metal content nor purified by removal of associated metals before measurements of the "conditional stability constant" were made. The concentration of the humic substance solution is expressed as a weight concentration.

Stevenson (76, 77) reports studies measuring the stability constants for $Cu^{2+}$, $Pb^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ complexes of humic acids. The humic substance solutions were titrated using a metal solution and the titration followed with a pH meter, the free metal activities were not measured. Stevenson assumes that a proton is released for each equivalent of metal that reacts to form a complex. Beckwith (59) showed that it is not necessary for a proton to be released for each equivalent of metal reacting to form a complex.

Chau et al. (74) reports using differential pulse

anodic stripping voltammetry to measure free metal activities in solutions containing complexed metal. The authors conclude that this method will be reliable for the measurement of complexes with stability constants with values for $K_{formation} \geq 10^{13}$, which is higher than we expect.

## MEASURING THE STABILITY OF THE COMPLEXES OF TRANSITION METALS WITH HUMIC SUBSTANCES: MISCELLANEOUS METHODS.

Hodgson et al. (65) and Geering and Hodgson (69) report using a method involving solvent extraction and distribution of the metal between two immiscible liquids. The essential features of this method, use of competing equilibria, are no different than the ion exchange methods and fails to measure a stability constant for the same reasons.

Gamble et al. (77) report determination of the "stability constant" for Mn(II) complexes using electron spin resonance spectrometry to measure free and complexed metal concentrations in humic substance solutions. The results had a standard deviation of 45%.

Job's method of continuous varitations has been used by many workers (Broadbent and Ott, 57; Gamble and Schnitzer, 70; Schnitzer and Skinner, 63a; and Schnitzer and Hansen, 70) as a method of determining the stoichiometry of metal-humic substance complexes. MacCarthy and Mark (76) show that Job's method is restricted to a system where only one ligand is present and a single complex predominates in the solution. Under these circumstances, the maximum optical density will

be independent of the wavelength at which the measurement is made. MacCarthy and Mark show that for mixtures of ligands the optical density reaches a maximum at different molar ratios of metal to humic substances for different wavelengths. Crosser and Allen (77b) also report data showing that the optical density reaches a maximum at four different molar ratios of metal to humic substances at the four different wavelengths chosen. The ratio of metal to humic substance; that is, metal to ligand; at maximum optical density should be the ratio of metal to ligand in the complex if Job's conditions hold. Thus, Job's method cannot be used to determine the stoichiometry of metal-humic substance complexes since the molar ratio is not unique but dependent on the wavelength chosen.

MEASURING THE STABILITY OF THE COMPLEXES OF TRANSITION METALS WITH HUMIC SUBSTANCES: METHODS MEASURING THE "EXTENT OF COMPLEXATION".

In beginning this section, consider a statement made by Gamble and Schnitzer (73): "The experimentally measured equilibrium function for the reaction of fulvic acid with a cation is a variable. This variable is a function of the weighting factors of the participating functional groups, and of the electrostatic charge accumulated on the polymer molecules. Any attempt to describe such an equilibrium with a simple numerical constant is therefore a fundamental flaw in logic. This mistake may lead to wrong conclusions

about the chemistry of natural waters."

Up to this point we have examined many attempts to measure the complexing behavior by determining a "stability constant" for the metal-humic substance complex. The techniques do not give useful results because the methods were not designed for the sort of system we are concerned with. A method or methods are needed that are less strictly limiting and give more meaningful results. These methods should measure the extent of complexation in systems where a mixture of ligands and complexes of different types co-exist.

We recognize that it is impossible to measure the molecular weight of a fulvic acid or humic acid molecule since these terms are applied to mixtures of compounds, a number-average molecular weight is measured instead. When we perform a base titratinn of a solution of humic substances we are titrating a mixture of polyfunctional compounds that contain groups with dissociable acids whose acidity varies over some range of values. We might expect to find certain broad classes of functional groups with acidities clustering around some mean values. These clusters would probably be associated with the acidities of carboxylic and phenolic groups. The acidities within these classes may range over several orders of magnitude.

In keeping with this approach, it may be better to use the extent of dissociation, defined in terms of the total acidity of the mixture, than attempting to measure dissociation constants using equivalence points that are

poorly defined on a titration curve under the best of conditions and have physical meanings that are equally poorly defined.

Irving and Rossotti (54) use a number that is equivalent to Bjerrum's (41) "degree of formation of the system" for "ligand-proton" complexes, denoting  the <u>average number of protons bound to ligand</u> by "$\bar{n}_H$". This gives us the maximum information we can reasonably expect given the current level of knowledge about the humic substances. By titrating an inorganic acid with base and measuring the pH throughout, followed by titrating a mixture of the inorganic acid and the weak acid being studied at the same ionic strength, the average number of protons bound to the ligand as a function of the amount of base added can then be determined.

Using much the same approach applied to characterizing the acidic properties of humic substances, the metal complexing properties can be evaluated. It will be impossible to define any stoichiometry for a mixture of coordinating sites available to react with the metals in a humic substance solution, but the "degree of formation of the system" or the average number of ligand molecules per metal ion as a function of the ligand concentration is useful information that can, in principle, be measured. It is probably better to use Bjerrum's name for the function; i.e., the degree of formation of the system; keeping in mind that it gives information concerning the progressive formation of complexes of higher coordination in the system.

Irving and Rossotti (54) describe a method for deter-
mining the degree of formation of the system by performing a
base titration of a mixture of inorganic acid and weak acid
ligand; followed by a base titration at the same ionic strength
of a mixture of inorganic acid, weak acid ligand, and the
metal.  The pH is measured throughout the titration.  Rossotti
and Rossotti (61) discuss other methods for determining
"$\bar{n}$", the degree of formation of the system.

There are several methods that express the extent to
which the metal in the system containing a ligand is com-
plexed under a given set of conditions.  Buffle et al. (77)
defines the degree of complexation as:

$$\alpha = \frac{(M)_t}{(M)} \qquad (J)$$

where $(M)_t$ is the total metal in solution and $(M)$ is the
free metal in solution measured using an ISE.  The measured
potential is:

$$E = E_0 + \frac{RT}{zF} \ln (M) \qquad (K)$$

where $E_0$ combines the standard electrode potential, the
reference electrode potential, and the junction potential.
Solving (K) for the free metal concentration (M) and sub-
tituting this into (J) gives:

$$\alpha = (M)_t 10^{(E_0 - E)/p} \qquad (L)$$

where "p" denotes: $[RT/zF]$.

By plotting the measured potential "E" versus the total

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metal in solution "$(M)_t$", measured during the titration of solutions with and without humic substances at the same ionic strength, for the two results it is possible to determine the maximum complexing capacity of the humic substances by observing the point at which the two curves deviate from one another. The authors point out that reliable measurements can be obtained if the following precautions are followed: (1) the potential must be measured to a precision of 0.1 mV because the error arising from the junction potential and systematic errors are of the same magnitude as a 0.1 mV error, (2) the working range of the metal solutions must be $10^{-1}$ to $10^{-6}$ M, and (3) adsorption of humic substances on the electrode surface must be monitored to prevent interferences with electrode response.

Sposito et al. (78) used a "formation function" to express the extent of complex formation. The free metal concentration was measured using an ISE. The "formation function" is given as:

$$\delta n_M = \frac{C_M - (M)}{C_L} \qquad (N)$$

where "$C_M$" is the total metal concentration in solution, (M) is the activity of the free metal in solution measured by ISE, "$C_L$" is the total ligand concentration, and $\delta n_M$ is the "formation function". Sposito et al. (78) found that when the formation function for $Ca^{2+}$ was measured for fulvic acid from sewage sludge-soil mixtures, the formation of the complex remained low up to $(Ca^{2+}) = 10^{-2.4}$ M. Above

that concentration there was a rapid increase in $\delta n_{Ca}$ for increasing $(Ca^{2+})$.

Gamble et al. (70) approached the problem by considering the "complexing equilibrium a function of the degree of ionization of the carboxyl groups at the complexing sites." For each incremental extent of complexation there is a formation constant that is a function of the degree of ionization. The "mass action quotient for chelate formation" is the weighted average of all of the incremental formation constants:

$$\overline{K} = \Sigma[m_{A_i H} \; K_i]/ \; \Sigma[m_{A_i H}] \qquad (0)$$

where $\overline{K}$ is the "mass action quotient for chelate formation", $K_i$ is the incremental formation constant, and $m_{A_i H}$ is the molar concentration of the singly ionized bidentate site. We recall that Gamble et al. (70) use the o-hydroxybenzoate model for the coordination sites on humic substances.

Experimentally, the free metal in solution is expressed as a function of the metal concentration on a cation exchange resin, determined for the system containing the bulk electrolyte but no humic substances. Stock solutions were prepared containing both the metal ion, in this case $Cu^{2+}$, and the humic substances. These stock solutions were combined in differing weight ratios with K-saturated ion exchange resin. The total metal in solution was expressed as a function of the "composition of the experimental sample" expressed as grams of stock solution per grams of resin. The total

metal on the resin for the experiments is also expressed
as a function of the "composition of the experimental sample".
These functional forms were determined by least squares
curve fitting of the experimental results.

This approach permits the calculation of the free metal
in solution in the presence of the humic substances using
the same **type** of relations based on competing equilibria
used in the ion exchange methods described earlier.  It is
then possible to describe the degree of complexation of
the system as the composition of the solution changes since
the total metal and total ligand concentrations are also
known.

## SOILS USED AS THE SOURCE OF HUMIC SUBSTANCES.

The soil samples were taken at the municipal sewage
sludge disposal site for the city of Manhattan, Kansas.
The disposal site was on the flood plain of the Kansas
River. The sampling sites were chosen for their history
of sewage sludge treatment. Liquid sewage sludge was
disposed on these plots by injecting the sludge into the
soil. Field crops were grown on the disposal plots during
periods when sludge was not being disposed on a particular
plot. The history of disposal was: (1) disposal on plot
#1 from November 1976 through January 1977, (2) disposal
on plot #2 from February 1977 through April 1977, (3) dis-
posal on plot #3 from May 1977 through July 1977, and (4)
plot #4 received no treatment. Samples were taken at the
first of August 1977.

The soil in plots #2 and #4 at the sampling site was
Haynie very fine sandy loam (Typic Udifluvent, coarse-silty,
mixed, calcareous, mesic). Plots #1 and #3 were nearer
the Kansas River and the soils on these plots were Haynie
with inclusions of Carr fine sandy loam (Typic Udifluvent,
coarse-loamy, mixed calcareous, mesic).

Surface soil samples were taken in all plots, the soils
air dried, ground to pass a 16-mesh sieve, and dried at $110^\circ$C.
The soils were stored in a dessicator until used.

## METHOD OF EXTRACTING THE HUMIC SUBSTANCES.

Prepare an extracting solution that is 0.2 M in $NH_4(HCO_3)$ and 0.6 M in $NH_4OH$ to give a solution of pH 10. Weigh 10 gm of dried soil into a 100 ml centrifuge tube and add 50 ml of extracting solution. Shake the suspension for 24 hours and centrifuge at 2200 rpm for one hour to separate the extracted humic substances from the soil. Filter the supernatant solution through glass-fiber filter paper (Gelman type A-E) using a Buchner funnel. Combine 300 ml of extracted solution and concentrate to a volume of $\approx$10 ml using rotary evaporation under vacuum, heating the boiling flask with warm tap water.

REMOVAL OF THE CO-EXTRACTED METALS.

Fill a column with the dimensions: 70 cm x 1.67 cm with chelate resin (Bio-Rad Chelex-100) in the ammonium form. Prepare the chelate resin prior to the initial use by washing the resin thoroughly with 1 M NaOH, 1 M $H_2SO_4$, ethanol and distilled-deionized water.

Add the concentrated humic substance solution to the resin and elute slowly with 2 M $NH_4OH$. Elute with a total volume of 250 ml, collecting the solution in a boiling flask. After regenerating the chelate column, as described below, the once purified humic substance solution is re-concentrated to $\approx$10 ml by rotary evaporation and again eluted through the $NH_4$-chelate resin by the same procedure. The twice purified humic substance solution is reconcentrated to $\approx$15 ml and stored in a refrigerator.

The first step for regenerating the chelate resin
is to remove the resin from the column. This is done due
to the substantial volume changes the resin undergoes
during certain phases of regeneration. The chelate resin
is placed in a 1000 ml beaker and $\approx$200 ml of 1 M $H_2SO_4$ is
added. When the change in resin volume is complete it is
returned to the column and eluted slowly with 250 ml of
1 M $H_2SO_4$. This resin is then rinsed rapidly with $\approx$200 ml
of distilled-deionized water and removed from the column
and placed in a 1000 ml beaker. While the resin is in the
beaker, it is thoroughly batch washed with distilled-deion-
ized water until there is no precipitate when the super-
natant solution is teated with $BaCl_2$. The resin is then
washed with 2 M $NH_4OH$ until the change in resin volume is
complete and returned to the column. The column is now ready
to accept samples.

## METHOD FOR THE LIGAND-EXCHANGE CHROMATOGRAPHY OF HUMIC SUBSTANCES.

Adjust the humic substance solutions to a 1% weight
per volume concentration and add 2 ml of the humic sub-
stance to the ligand-exchange column. The column was a
Pharmacia chromatography column with end-adaptors with
the dimensions: 65 cm x 1.67 cm. The end-adaptors allowed
adjustment against the resin in the column eliminating
dead solution space at the ends of the column

The column was eluted with $NH_4OH$ solutions pumped.

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through the column from bottom to top using a peristaltic

pump (Cole-Parmer pump head) at a flow rate of 0.06 ml/min.

The pump was situated at the out-flow end of the column and

the solution was essentially pulled through the column. This

was done to avoid introducting air bubbles into the column.

Between the reservoir and the column were two four-way

valves (Pharmacia). The humic substance solution was in-

jected "in-line" using these valves with a hypodermic syringe.

There was a "bleed-valve" located just at the entrance of

the column so that while the sample was injected, the solu-

tion being displaced could "bleed off" without putting

pressure on the column. Fractions were collected using a

rotary fraction collector that advanced every 15 minutes.

The ligand-exchange column was prepared by the following

procedure. First, wash the chelate resin following the pro-

cedure given in the section on removal of metals from the

humic substance solution. Wash the ammonium saturated

chelate resin with 1 M $Cu(NO_3)_2$ to saturate the resin with

$Cu^{2+}$. Thoroughly wash the resin with distilled-deionized

water to remove excess $Cu(NO_3)_2$ and equilibrate with 2 M

$NH_4OH$ in a 1000 ml beaker. Transfer the resin to the col-

umn and place <u>at the exit end of the column</u> a layer of the

ammonium chelate resin ≈ 1 cm thick to remove $Cu^{2+}$ that

might bleed off the column. No bleeding was observed in

the 9 months of continuous chromatography. Prior to use

and between samples the column is eluted with $NH_4OH$ solu-

tions used in the chromatography to ensure the resin equili-

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brated with the eluting solution when samples were applied
to the column.

## SAMPLE PREPARATION FOR AND MEASUREMENT OF NUMBER-AVERAGE MOLECULAR WEIGHTS USING VAPOR PRESSURE OSMOMETRY.

The humic substances must be in the $H^+$-ion form for
the measurements of molecular weights since the apparent
weights by VPO will be corrected for dissociation of the
weak acid groups later using the method by Hansen and
Schnitzer (69).

Place a **strong** acid cation exchange resin that has been
acid, base, and organic washed, and which is in the $H^+$-ion
form, in a 30 ml buchner funnel. Pass the humic substances
through the resin to acidify them and elute thoroughly with
distilled-deionized water to wash the resin free of the
acidified humis substances. Concentrate the now dilute sample
with rotary evaporation.

Prepare three solutions of each humic substance fraction;
these being 1-fold, 2-fold, and 3-fold dilutions of a stock
solution of the sample having a concentration within the
working range of the VPO. Prepare a standard curve using
0.01 M, 0.05 M, and 0.1 M NaCl, treating the NaCl as complet-
ly dissociated to give solutions 0.02 M, 0.1 M, and 0.2M in
ions.

Balance the Wheatstone bridge of a model 301-A Mechrolab
VPO using distilled-deionized water droplets on both thermis-
tors. Treat samples and standards in the same manner. Place

a droplet of solution on the sample thermistor and read
the resistance imbalance after 3 minutes.  Rinse the sample
thermistor with solution and repeat the measurement.  Take
at least three measurements for each solution being analyzed.
The apparent molar concentration of the humic substance
solution is determined from the standard NaCl curve.

## CORRECTING THE APPARENT MOLAR CONCENTRATION AND MOLECULAR WEIGHT FOR DISSOCIATION OF WEAK ACID GROUPS.

The method used was that described by Hansen and Schnitzer
(69).  After determining the weight concentration of the
humic substance solutions used in measuring the apparent
molar concentration, the samples were diluted 150-fold to
200-fold for the pH measurements to conserve sample.  The
data that was collected came from the pH measurements of
three solutions with differing weight concentrations.

## LIGAND-EXCHANGE CHROMATOGRAPHY.

Originally, the plan for this study of metal-humic substance complexation involved comparing the results of gel filtration of (1) humic substances that had been purified by the removal of the co-extracted metals and(2) solutions of humic substances (previously purified by removal of co-extracted metals) to which certain metals had been added. The idea was that when complexes formed, the molar volume of the species passing through the gel filtration column would be greater since the complex would be larger than free humic substance molecules. Since this process would be done on all fractions separated by the gel filtration of the metal-free humic substances, it might be possible to assign some qualitative ranking to each fraction for its role in complexing transition metals. Those fraction having a greater affinity would form complexes of higher coordination and would exhibit a greater relative increase in molecular volume.

In preliminary studies I found that gel filtration of the humic substances extracted from the soils used in this study would be impossible. The humic substances consistently adsorbed on the column, irregardless of the type of matrix used. Adsorption occured on dextran, polyacrylamide, and cross-linked polystyrene. This aspect of the study was, as a result, abandoned. But not before certain

interesting phenomena were observed.

In the process of removing the co-extracted metal ions from the humic substances, I passed solutions of the humic substances through a column of chelate resin in the H-ion form and eluted with distilled-deionized water. When this was done, a substantial fraction of the humic substances passed readily through the column. An equally significant fraction of the humic substances did not elute through the column using distilled-deionized water and were adsorbed on the column. In the H-ion form the chelate resin is a light-straw color and noticeable darkening ocurred when the humic substances were adsorbed on the column.

When I eluted the column containing the adsorbed humic substances with 2M $NH_4OH$, the adsorbed substances were readily displaced. It seemed likely that when the humic substances freshly extracted from the soil were passed through the chelate resin, some of the co-extracted metals became adsorbed on the resin via coordination by imino-diacetate groups on the resin. These adsorbed metals did not have all of their potential coordination sites occupied by the iminodiacetate group and coordinated some of the humic substances that were also present in the system. The humic substances coordinating the metals retained by the chelate resin formed complexes of sufficiently high stability that they were not displaced by water molecules and were therefore not eluted by the distilled-deionized water.

There are several studies in the literature of analytic biochemistry that used ligand-exchange chromatography to separate solutions containing mixtures of amino acids, polypeptides, as well as nucleic acids and their derivatives (these were cited in the literature review). Thus it seemed that ligand-exchange chromatography could be used to resolve fractions having differing stabilities from a mixture of humic substances that had been extracted from the soil.

If such a resolution were possible, and if there were fractions present that did complex metals and other fractions that did not significantly form complexes, these fractions could be separated, in principle, prior to evaluation of the metal complexing stability of the humic substances. The presence of fractions forming weak complexes in a mixture would in effect dilute the system. The results would not give a complete picture of the nature of the metal complexing behavior and properties of the humic substance mixture.

Furthermore, if a range of compounds with differing metal complexing stabilities existed or if distinct fractions existed, it would be useful to know the range of the stabilities and the distribution of the humic substances with differing complexing stabilities.

When a 1% (w/v) solution of a metal-free humic substances was applied to a $Cu^{2+}$-saturated column that had been equilibrated with 0.5 M $NH_4OH$ and eluted with 0.5 M $NH_4OH$, the humic substances eluted as a single unresolved

peak with a tail. The absorbance of the eluted fractions were measured using a spectrophotometer at 215 nm. Typical elution curves are presented in **Figures** (1) and (2).

Since the resolution involves distribution of the humic substance between stationary and mobile phases depending on the competition between the humic substance molecule and the ammonium ion for coordination sites on the stationary metal, it follows that a more dilute ammonium solution would result in the humic substances being more strongly adsorbed by the metal saturated column. If this is done, the humic substances would pass through the column more slowly and prehaps the large peak may be resolved into several peaks if distinct fractions exist in the mixture.

I decided to pass the combined solutions that were eluted as the single large peak; i.e., the "bulk" fraction; and not pass the "tail" through a column equilibrated and eluted with a more dilute ammonium solution. This was done since, given the fact that the humic substances would take longer to pass through the ligand-exchange column when eluted with the more dilute solution, the compounds eluted as the "tail" with 0.5 N $NH_4OH$ would also be eluted as the "tail" with a more dilute solution the only difference being that they would take longer. It was considered unlikely that there would be any further fractionation of this fraction.

Once the unfractionated humic substances had been separated into "bulk" and "tail" fractions on the 0.5 M
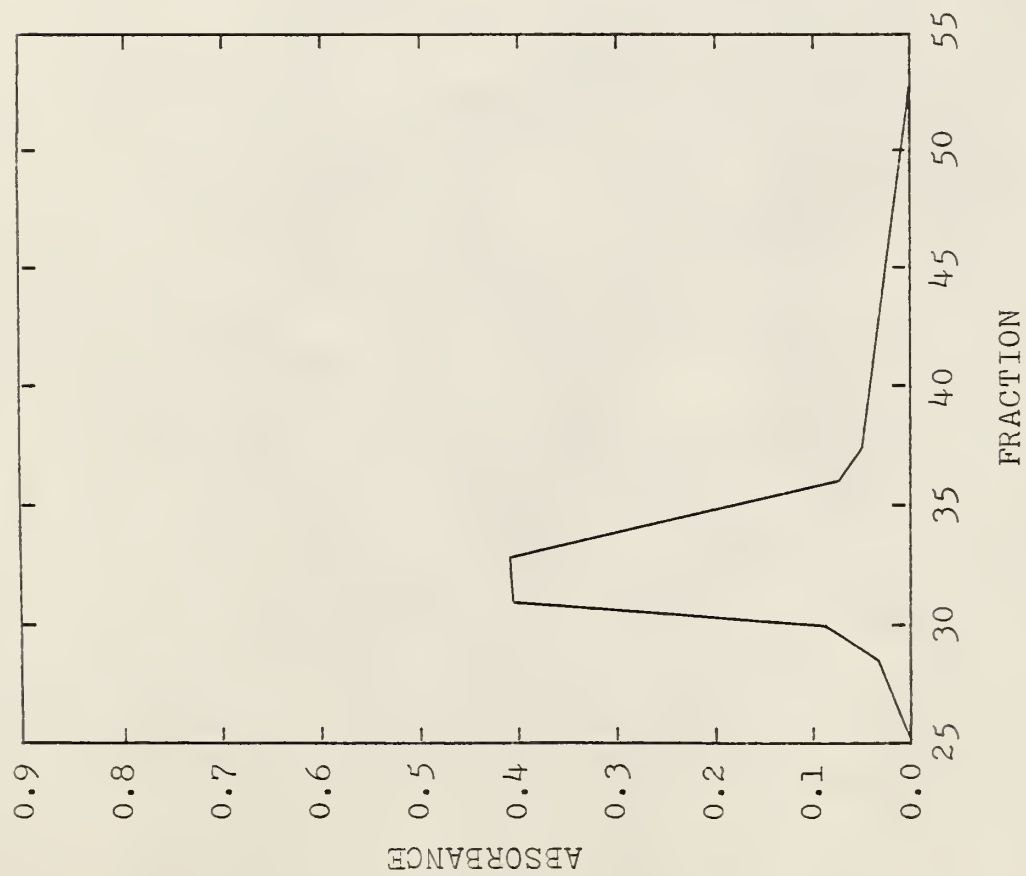
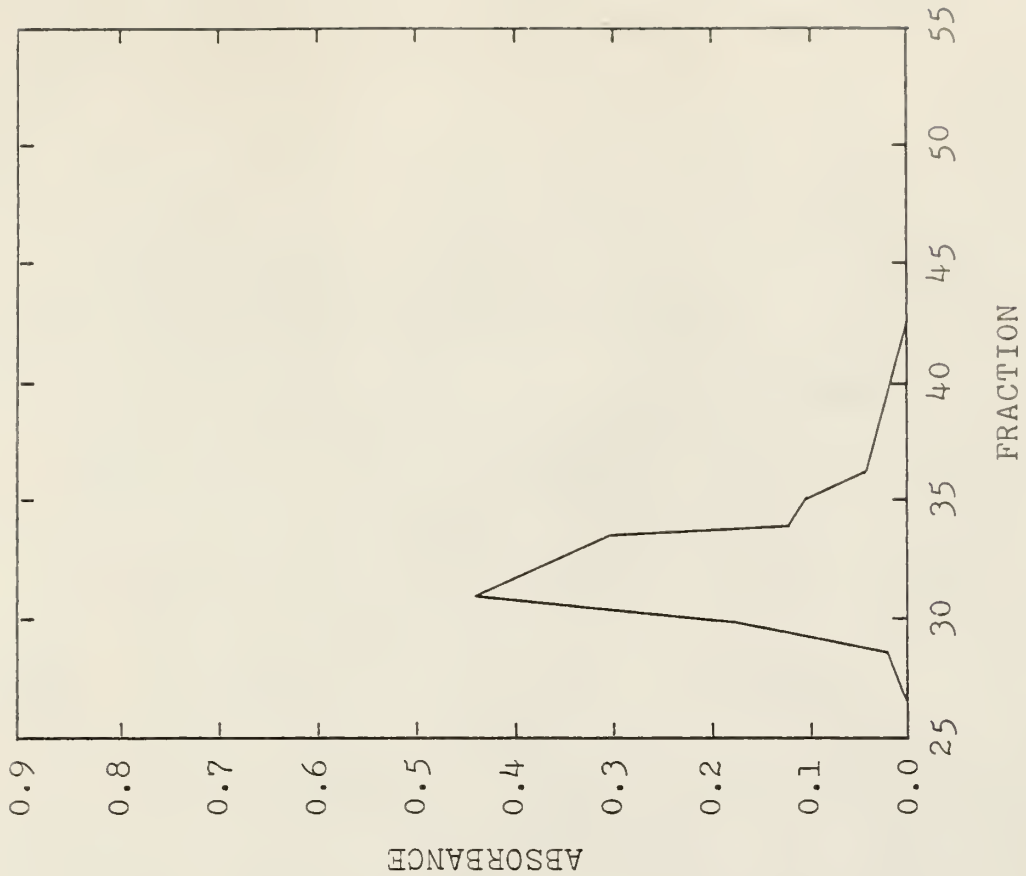Figure 1.  Elution profile for humic substances from plot #1, eluted with 0.5 M $NH_4OH$ ($\lambda = 215$ nm).

Figure 2.  Elution profile for humic substances from plot #2, eluted with 0.5 M $NH_4OH$ ($\lambda = 215$ nm).

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equilibrated column, the column was re-equilibrated with
0.005 M $NH_4OH$ and the "bulk" fraction eluted.  The "tail"
from the initial **fractionation became fraction #3.**  **The**
results for the four plots when the "bulk" fraction was eluted
with the more dilute ammonium solution are seen in **Figures**
(3) through (6).

In all cases a second peak was resolved from the **main**
peak when the humic substances were eluted with the 0.005 M
$NH_4OH$ solution.  There is some qualitative similarity between
the humic substances extracted from the soils comming from
plots #1 and #3, and between the substances from plots #2
and #4.  This qualitative similarity is suggested since
the humic substances from plots #1 and #3, although having
a second peak, it was not completely resolved and could
be detected only by monitoring absorbance at 215 nm.  The
second peak was more completely resolved from the main
peak for the humic substance samples comming from plots #2
and #4.  The second peak was apparent by visual inspection
of the eluted fractions as well as absorbance at 215 nm for
the humic substances comming from plots #2 and #4.

This result was unexpected and cannot be explained
given the limited nature of this study.  Interestingly,
there is no correlation between the results from the ligand-
exchange chromatography and the sludge treatments on the
sampling plots.  If this were the case then we would expect
greater similarity between #1 and #2 and between #3 and #4,
given the nature of the treatments.

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Figure 3. Elution profile for humic substances from plot #1, eluted with 0.005 M $NH_4OH$ ($\lambda$ = 215 nm).
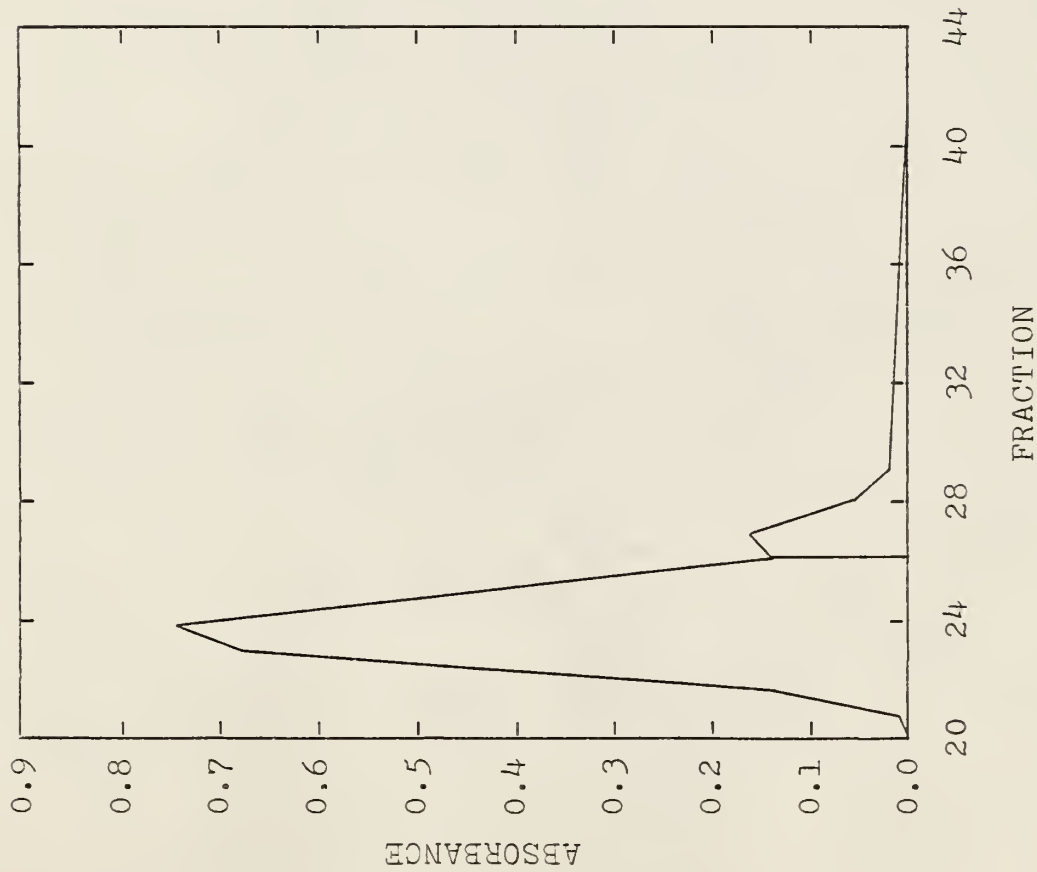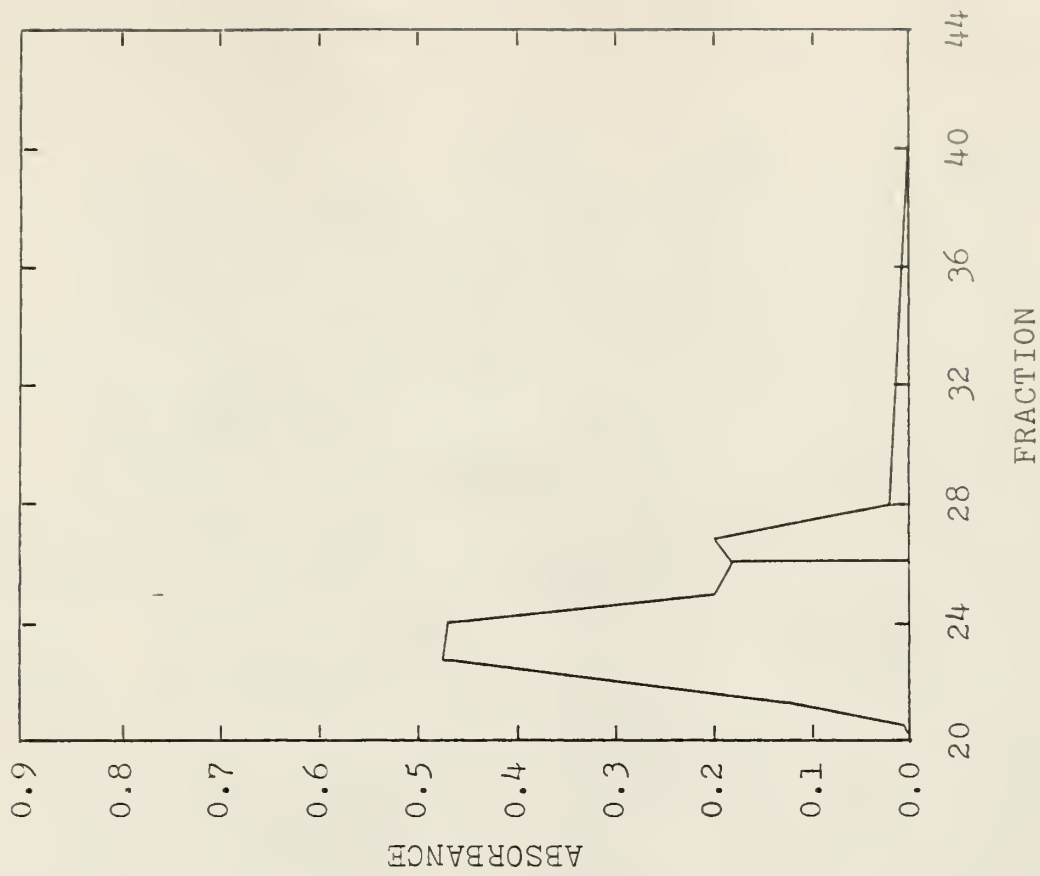
Figure 4. Elution profile for humic substances from plot #3, eluted with 0.005 M $NH_4OH$ ($\lambda$ = 215 nm).

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Figure 5.   Elution profile for humic substances from plot #2, eluted with 0.005 M NH$_4$OH ($\lambda$ = 215 nm).

Figure 6.   Elution profile for humic substances from plot #4, eluted with 0.005 M NH$_4$OH ($\lambda$ = 215 nm).

The soils in plots #2 and #4 had **noticeably finer** texture than the soils from plots #1 and #3, the latter were located nearer the Kansas river on the floodplain and included inclusions of the Carr series, a coarser-textured soil series than the Haynie series found in plots #2 and #4. The similarity in the properties of the humic substances _may_ be related to the texture of the soils, but this is only speculation.

## NUMBER-AVERAGE MOLECULAR WEIGHTS OF THE HUMIC SUBSTANCES.

The results for the measurement of the humic substances number-average molecular weights are given in **Table 2.**

TABLE 2

## DISSOCIATION CORRECTED NUMBER-AVERAGE MOLECULAR WEIGHTS.

| Plot | Fraction | Molecular Weight |
|------|----------|------------------|
| 1 | 1 | 169 |
| 1 | 2 | 62 |
| 1 | 3 | 70 |
| 2 | 1 | 276 |
| 2 | 2 | 112 |
| 2 | 3 | 84 |
| 3 | 1 | 340 |
| 3 | 2 | 87 |
| 3 | 3 | 92 |
| 4 | 1 | 431 |
| 4 | 2 | 73 |
| 4 | 3 | 65 |

For each plot there were three fractions: (1) fraction #1 was the first large peak eluted by 0.005 M $NH_4OH$ from the ligand-exchange column equilibrated with 0.005 M $NH_4OH$, (2) fraction #2 was the second peak eluted from the column equilibrated and eluted with 0.005 M $NH_4OH$, and (3) fraction #3 was the "tail" separated after the initial fractionation on the column equilibrated and eluted with 0.5 M $NH_4OH$.

Concerning the molecular weight results, we can make three comments. First, and most obviously, the number-average molecular weight of fraction #1 from all plots was higher than that for the other two fractions. Second, the number-average molecular weights of fractions #2 and #3 are almost the same for all plots. Third, the amount of humic substances in each sample available for analysis was very limited. The column used for the chromatography did not have the capacity for handling large quantities of humic substances. Considerable time ($\approx$ 2 months) was spent in obtaining 15 to 20 ml. of solution for each fraction with an average concentration of $\approx$ 0.01 M.

Each sample represented the combined fractions from 8 to 10 chromatographic runs. Significant amounts were required for weight concentration measurements. This required heating and drying at $110^{\circ}C$ in a **porcelain dish.** The humic substances would not completely re-dissolve after this treatment. As a result, that portion of sample used for determining weight concentration could not be recovered for further use.

The number-average molecular weights listed in Table 2 were not measured with the highest precision possible. Had there been more sample available for the analyses we could have greater confidence in the results since more replicate measurements could have been made. Nonetheless, given the fact that the soils are similar and the humic substances from then are also similar, one criteria for the reliability of the measurements would be agreement among the molecular weights of different samples.

The number-average molecular weights for fractions #1 from the four plots range from 169 to 431. The apparent molecular weights, before correction for weak acid dissociation, were: 156 (plot #1), 128 (plot #2), 210 (plot #3), and 292 (plot #4). In all cases, except for the sample from plot #1, there was a considerable change in molecular weight when dissociation was corrected for. The corrected molecular weight from plot #1 may not be as good as the values for the weights of humic substances comming from plots #2, #3, and #4. There is better agreement among the molecular weights of samples comming from different plots for fractions #2 and #3.

In conclusion, first, it seems that the humic substances with the least affinity for the $Cu^{2+}$ held on the chelate exchange column (fractions #1) from all soils have higher number-average molecular weights than those fractions interacting more strongly with $Cu^{2+}$ and eluted as fractions #2 and #3. Second, there seems to be little difference in

the number-average molecular weights of fractions #2 and
#3 from the different plots. This seems logical since
the criteria for the separation of fractions #2 and #3
was arbitrary. The observation that the fractions having
a lower affinity for $Cu^{2+}$ also have a higher molecular weight
is consistent with the commonly held view that the so-called
fulvic acids form complexes of higher stability and have
generally lower molecular weights than the humic acids.
In the results presented here, the two major fractions were
not separated on the basis of acid solubility as is tradi-
tionally done, although I observed that the substances
in fractions #1 from the various plots were less soluble ·
in the protonated form, but on the basis of their affinity
for a transition metal and the tendency to form complexes
of higher stability.

## MEASURING "STABILITY CONSTANTS" FOR HUMIC SUBSTANCE-METAL COMPLEXES USING FRONAEUS' METHOD.

As was stated in the introduction, the next phase
in this study was the measurement of "stability constants"
for transition complexes of those humic substance fractions
separated using ligand-exchange chromatography. The re-
sults from this phase of the study were not acceptable
for the reasons outlined below.

First, the method I originally chose for measuring
the "stability constants" of the metal-humic substance com-
plexes was the method described by Fronaeus (51). In the

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literature review section on the topic of measuring "stability constants" using ion exchange methods it was pointed out that this method fails to measure stability constants in a system of mixed ligands and the reasons the method fails were discussed. It was not until after the results from the experiments using this method were collected that I became aware of the inappropriateness of the method.

Second, the basic parameter in Fronaeus' method, $C_{mr}/C_m$ as a function of the dissociated ligand concentration $(A^-)$, could **conceivably** be used to indicate the stability of the complexes formed and give information about the range in complex stabilities with the fractionated humic substances. For this information to be useful, however, these measurements must be **supplemented with other** information about the system. The pH of the metal-humic substance solutions were not measured. The humic substance solutions were not titrated to determine the acidic behavior of the samples and determine the extent of dissociation as a function of the pH.

Third, as pointed out earlier, throughout this study I was working with limited amounts of sample. These amounts were insufficient to properly conduct the analyses that were performed and obtain results that were reliable. At each stage in the process there are errors, and since the final measurements depend on earlier analyses, the errors **accumulate throughout** the process. Weight concentration measurements and measurements to correct for weak acid

dissociation were not done with sufficient replications, and the latter without a sufficiently wide range in concentrations, to be as reliable as required given the fact that the errors accumulate.

Fourth, the method requires that the total ligand concentration be much greater than the metal concentration. At least two orders of magnitude difference is necessary for an error of less than 1% in approximating the free ligand concentration by the initial ligand concentration. The metal concentrations required to satisfy this condition were quite close to the lower limits for determination of Cd and Zn (the metals studied) using atomic absorption spectroscopy. I did not have sufficient humic substances to repeat determinations and was not satisfied with the precision of the measurements.

Finally, in order to obtain the relation: $C_{mr}/C_m$ as a function of the free ligand concentration (dissociated), the results were curve fitted using a least squares method. The humic substance solutions were: a stock solution (the concentration of the stock solutions were all approximately $10^{-5}$ M), 2-fold, 3-fold, 4-fold, and 5-fold dilutions of the stock solution. This particular choice of solution concentrations gave the maximum number of test solutions for the amount of humic substance stock solution available. However, the least squares method works best when the concentrations of the humic substance solutions analyzed are evenly spaced throughout the concentration range used rather than clustered

at the lower end of the concentration range as was the case
for the solutions I had prepared.

This creates a dilemma since, in order to have solu-
tions with concentrations evenly spaced throughout the
range there would have been only enough sample for three
different concentrations, when in fact more than the five
that were used would be considered necessary for reliable
results. Thus, once again, lack of sufficient sample meant
that the number of measurements made were less than neces-
sary for reliable results.

## CONCLUSIONS

These following conclusions can be made concerning
the discussion presented in the literature review and the
experimental results.

1) The humic substance molecules are capable of co-
association by a variety of mechanisms, among these can be
included the "bridging" of molecules through coordination
of a transition metal ion. Experiments concerned with most
properties of humic substances should probably include
removal of co-extracted metals as a preliminary step in
preparing the samples. The presence of these metals will
strongly influence the solubility, the apparent molecular
weights, and will interfere with measurements of complexing
stability if not carefully removed.

2) Fractionation of humic substances prior to eval-
uation of their properties is best done using well-defined

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chemical and/or physical properties. Separation of humic substances using ligand-exchange chromatography will fractionate solutes on the basis of their metal complexing stabilities. This method can be extremely useful not only in characterizing soil humic substances, but also as a preparitive treatment for subsequent analyses of the substances. Use of columns with greater diameters; i.e., higher capacity; is advisable since large quantities of sample are required for most analyses. Furthermore, use of longer columns and more dilute ammonium solutions should lead to better separations.

3) The basic objective in studying metal-humic substance interactions is to evaluate its affect on the fate of metals in the soil. This requires some knowledge of the capacity of the humic substances to complex metals and the stability of those complexes. Because the humic substance solutions are complex mixtures of similar but non-identical compounds, a number; i.e., a constant; that characterizes the stability of the complex cannot be measured. Rather, the extent of formation of a metal-humic substance complex will be a function of pH, the ionic strength, the acidic properties of the humic substance solution (i.e., the dissociation behavior of the compounds in the mixture over the pH range), and the nature of the coordination sites available in the system. We should consider all results we obtain; the degree of formation of the system, extent of dissociation, etc.; as conditional and lacking

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well-defined thermodynamic values.

4) Characterization of metal complexing behavior
of a given humic substance solution should include certain
information about the system. The acid dissociation behavior
of the mixture should be characterized with titration curves.
The degree of formation of the system; i.e., the average
number of ligands around the metal center as a function
of the dissociated ligand concentration; would give informa-
tion about progressive formation of complexes of higher
coordination. Molecular and equivalent (i.e., titratable-
proton equivalence) weights of the mixture should be deter-
mined to adequately specify the concentration of the humic
substances, keeping in mind that these are "number-average"
values. Finally, measurement of the extent of complexation
by one of the methods discussed in literature review should,
combined with these other results, give a great deal of
information about the system.

5) Finally, given the complexity of the system, we
cannot be certain that simple model compound(s) will ade-
quately model the behavior of the system. As of yet, there
have been few attempts, MacCarthy (77) being an exception,
to consider the effect mixtures have on measured values.
I believe that the validity of modeling complex systems with
simple compounds is open to serious question. As was pointed
out earlier, a mixture of two or three simple acids may
model the titration curve of a humic substance solution,
but it would not distinguish low and high stability complex

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structures in a mixture. Reproduction of macroscopic behavior is not sufficient evidence to characterize the microscopic details of the system.

# APPENDIX I

## A THEORETICAL BASIS FOR MEASURING MOLAR CONCENTRATION BY VAPOR PRESSURE OSMOMETRY.

Relations developed using classical thermodynamics allow us to use measurement of colligative properties as a means of determining the number-average molecular weights of humic substance fractions. What follows is a complete development of the thermodynamic relations which permit use of vapor pressure osmometry to determine molecular weights.

For a system of variable composition; eg., two phases such as liquid and vapor; the differential form of the Gibb's function can be writter:

$$dG = VdP - SdT + \mu_1 dn_1 + \mu_2 dn_2 \qquad I.1$$

where $\mu_i$ is the chemical potential of the component in the $i^{th}$ phase and $n_i$ denotes the number of moles of the component in the $i^{th}$ phase. In our case there are only two phases. At constant temperature and pressure for a system at equilibrium:

$$dG = \mu_1 dn_1 + \mu_2 dn_2 = 0 \qquad I.2$$

Since the system is at equilibrium, any transfer of an infinitesimal mass from one phase to another is such that:

$$dn_1 = -dn_2$$

This gives us:

$$\mu_1 = \mu_2 \qquad\qquad\qquad I.3$$

Since the chemical potential at constant T and P is also the partial molar Gibbs function we can write I.3 using the chemical potential expressed with partial molar volumes and entropies in its differential form:

$$\overline{V}_1 dP - \overline{S}_1 dT = \overline{V}_2 dP - \overline{S}_2 dT$$

$$\frac{dP}{dT} = \frac{(\overline{S}_2 - \overline{S}_1)}{(\overline{V}_2 - \overline{V}_1)} = \frac{\Delta\overline{S}}{\Delta\overline{V}} \qquad\qquad I.4$$

For a reversible process we can write: $\Delta\overline{S} = \Delta\overline{H}/T$. We can substitute this into I.4 because our system consists of two phases at equilibrium and equilibrium phase changes are reversible processes. Thus:

$$\frac{dP}{dT} = \Delta\overline{H} \;/\; T\Delta\overline{V} \qquad\qquad I.5$$

This equation can be modified further if we make two assumptions. First, if the transition involves a gaseous and a condensed phase then $\Delta\overline{V} \approx \overline{V}_{vapor}$ since the molar volume of a vapor is much greater than the molar volume of a condensed phase. Second, we will assume the vapor behaves like an ideal gas, a valid approximation at 1 atmosphere and 298 K. This allows us to use: $\overline{V}_{vapor} = RT/P_{vapor}$, where $P_{vapor}$ is the vapor pressure of the condensed phase at the temperature T. Whenever the condensed phase is

present the pressure of the gas is the vapor pressure of the condensed phase. The result upon substitution is the Clausius-Clapeyron equation:

$$\frac{dP_{vapor}}{dT} = \frac{P_{vapor}\Delta\overline{H}_v}{RT^2} \qquad\qquad I.6$$

Raoult's Law of Ideal Mixtures, which all solutions follow in the limit of infinite dilution, states that:

$$P_1 = P_1^{\Theta}x_1 \quad \text{and} \quad P_2 = P_2^{\Theta}x_2$$

where the subscripts indicate solvent and solute respectively, $P_1^{\Theta}$ is the vapor pressure of the pure solvent, and $x_1$ is the mole fraction of the solvent in the mixture. If the solute is non-volatile, then $P_2^{\Theta} \approx 0$ and we can write the total pressure as:

$$P_T = P_1 + P_2 = P_1^{\Theta}x_1$$

$$P_T = P_{vapor} = P_{vapor}^{\Theta}(1-x_2) \qquad\qquad I.7$$

$$x_2 = 1 - (P_{vapor}/P_{vapor}^{\Theta})$$

Differating $P_{vapor}$ with respect to $x_2$ gives:

$$\frac{dP_{vapor}}{dx_2} = -P_{vapor}^{\Theta} \qquad\qquad I.8$$

We can substitute I.7 into the Clausius-Clapeyron equation I.6 and invert the expression so that it gives:

$$\frac{dT}{dP_{vapor}} = \frac{RT^2}{\Delta H_v}[P_{vapor}^{\Theta}(1-x_2)]^{-1} \qquad\qquad I.9$$

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We can now use the chain-rule to combine I.8 and I.9:

$$\frac{dT}{dx_2} = \frac{dT}{dP} \frac{dP}{dx_2}$$

$$\frac{dT}{dx_2} = \left[\frac{RT^2}{\Delta H_v P^{\ominus}_{vapor}(1-x_2)}\right]\left[-P^{\ominus}_{vapor}\right]$$

$$\frac{dT}{dx_2} = \left[\frac{-RT^2}{\Delta H_v(1-x_2)}\right] \qquad\qquad I.10$$

From I.10 we have an expression showing how the temp-
erature of the solution, and hence the thermistor itself,
on the thermistor in the vapor pressure osmometer varies as a
function of the mole fraction of the solute in the solu-
tion. The next step will be to show that is possible to
derive a relation using an expression for the temperature
dependence of the resistance of a conductor ( in this case,
the thermistor ). The final result is a linear dependence
of the resistance imbalance on the mole fraction (and hence
the concentration) of the solute in a solution. This is
consistent with the findings of many workers using VPO
(eg., Muller and Stolten, 53 and Wachter and Simon, 69)

Muller and Stolten (53), who developed one of the
early vapor pressure osmometers, give the temperature de-
pendence of a resistor over a range of several hundred degrees
as:

$$r = r_0 \exp\left[B\left(\frac{1}{T} - \frac{1}{T_0}\right)\right] \qquad\qquad I.11$$

where $r_0$ is the resistance at $T_0$ measured with a current

low enough to ensure there is no self-heating of the conductor, B is an approximate constant depending upon the nature of the conductor, and temperatures are in degrees Kelvin. It is more convenient if I.11 is rewritten as:

$$\ln (r/r_o) = B(\frac{1}{T} - \frac{1}{T_o})$$  I.12

Returning to the expression I.10, this can be rearranged to give:

$$(-1/T^2)dT = \frac{R}{\Delta H_v} (\frac{dx_2}{1-x_2})$$  I.13

Since: $d(1/T) = (-1/T^2)dT$ and $d(1-x_2) = -dx_2$, we can write I.13 as:

$$d(1/T) = - \frac{R}{\Delta H_v} \frac{d(1-x_2)}{(1-x_2)}$$  I.14

Integration of I.14 yields:

$$(1/T) = - \frac{R}{\Delta H_v} \ln (1-x_2) + I$$  I.15

The integration constant "I" can be evaluated in this manner, the dissolving of the solute in the solvent will lower the vapor pressure of the solvent. If the vapor pressure in the system is held constant, as it is in the chamber containing the thermistors in the VPO used, then condensation of vapor will take place at the solution droplet on the sample thermistor due to the lower vapor pressure of the solution droplet vis-a-vis the solvent droplet at the reference thermistor and the solvent reservior used to saturate the atmosphere of the chamber. This conden-

sation will cause the temperature of the solution droplet to increase. Therefore, if we let $T_O$ be the temperature of the solution when $x_2 = 0$; i.e., the solvent droplet; then: $I = (1/T_O)$. We can now write I.15 as:

$$\left(\frac{1}{T} - \frac{1}{T_O}\right) = - \frac{R}{\Delta H_v} \ell n \ (1-x_2) \qquad\qquad I.16$$

We see, however, that I.12 can be written as:

$$\left(\frac{1}{T} - \frac{1}{T_O}\right) = (1/B) \ \ell n \ (r/r_O)$$

and setting these two expressions equal to one another:

$$\ell n \ (r/r_O) = - \frac{B \cdot R}{\Delta H_v} \ell n \ (1-x_2) \qquad\qquad I.17$$

$$\frac{r}{r_O} = (1-x_2) \ exp[-B \cdot R/\Delta H_v] \qquad\qquad I.18$$

where $r_O$ is the resistance at $T_O$; i.e., $r_O$ is the resistance of the reference thermistor which is at the temperature $T_O$ of the thermostated chamber; and $r$ is the resistance of the thermistor holding the sample solution. In the VFC the resistance across a Wheatstone bridge is measured and not the respective absolute resistances so that we are interested in:

$$\Delta r = r_O - r \qquad\qquad I.19$$

$$\frac{\Delta r}{r_O} = 1 - \frac{r}{r_O}$$

$$\frac{r}{r_O} = \frac{r_O - \Delta r}{r_O} \qquad\qquad I.20$$

Thus, we can write I.18 as:

$$\frac{r_o - \Delta r}{r_o} = (1-x_2) \exp[-B \cdot R/\Delta H_v] \qquad \text{I.21}$$

$$\Delta r = r_o - r_o(1-x_2) \exp -B \cdot R/\Delta H_v$$

$$\Delta r = r_o x_2 \exp[-B \cdot R/\Delta H_v] + (r_o - r_o \exp[-B \cdot R/\Delta H_v])$$

Let:

$$\alpha = r_o \exp[-B \cdot R/\Delta H_v]$$

$$\beta = r_o - \alpha$$

Then:

$$\Delta r = \alpha x_2 + \beta \qquad \text{I.22}$$

This expression tells us that the resistance imbalance
across the Wheatstone bridge for the sample and reference
thermistors in a VPO is a linear function of the mole frac-
tion of the non-volatile solute. Of course, this means
that the resistance imbalance is a linear function of the
concentration of the solute in what ever form it is expressed.

## CORRECTING THE APPARENT MOLECULAR WEIGHT FOR DISSOCIATION OF WEAK ACID GROUPS.

Hansen and Schnitzer (69) reported a method for correcting the apparent molecular weight of compounds with weak acid groups for measurements based on colligative properties of the solvent. Consider the generalized polyprotic acid:

$$H_iA \rightleftarrows jH^+ + H_{i-j}A^{j-}$$
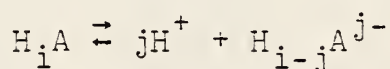
where: $0 \leq j \leq i$. If the initial concentration of the acid is taken as "c" moles per liter, then at equilibrium:

$$(H_iA) = (1-\alpha)c \qquad \qquad \text{II.1}$$

$$(H^+) = j\alpha c \qquad \qquad \text{II.2}$$

$$(H_{i-j}A^{j-}) = \alpha c \qquad \qquad \text{II.3}$$

where "$\alpha$" is the extent of dissociation; i.e., the fraction of $H_iA$ dissociated. We can then write:

$$j = M/\epsilon \qquad \qquad \text{II.4}$$

where "M" is the molecular weight and "$\epsilon$" is the "equivalent weight" at <u>equilibrium</u>. That is, "$\epsilon$" is the molecular weight divided by the number of protons per molecule dissociated at the equilibrium pH. Hansen and Schnitzer call "j" the total number of protons per molecule (i=j) and call "$\epsilon$" the true equivalent weight. This will not be true in general for a

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polyprotic acid sonce the protons will not all have the same dissociation constant and the acid at equilibrium will have sone of its groups dissociated and some undissociated; i.e., $0 \leq j \leq i$; where i is the total dissociable protons per molecule.

Furtnermore, the molar concentration of the acid can be written as:

$$c = W/M \qquad \qquad II.5$$

where "W" is the weight concentration of the polyprotic acid initially and "M" is the molecular weight of the acid. Using II.3, II.4, and II.5 the proton concentration can be written:

$$(H^+) = 10^{-pH} = (M/\epsilon)(W/M)\alpha \qquad II.6$$

$$\log (H^+) = \log W + \log (\alpha/\epsilon) \qquad II.7$$

If $(H^+)$ is measured at a series of different weight concentrations "W", plot the data, and extrapolate to: $W = 1$; i.e., $\log W = 0$; then at that point: $\log (H^+) = \log (\alpha/\epsilon)$ and $(H^+) = (\alpha/\epsilon)$. The total number of species in solution at equilibrium, expressed as moles per liter, is given as:

$$(H_iA) + (H_{i-j}A^{j-}) + (H^+) = (1-\alpha)c + \alpha c + j\alpha c$$

$$= c + j\alpha c$$

$$= (1 + j\alpha)c \qquad II.8$$

$$= [1 + (\tfrac{M}{\epsilon})\alpha][\tfrac{W}{M}]$$

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Finally, $(1 + j\alpha)c$ in II.8 can be written:

$$(1 + j\alpha)c = W[\frac{1}{M} + \frac{\alpha}{\epsilon}]$$

But this equals: $W/M_{app}$, where "$M_{app}$" is the apparent molecular weight measured by some colligative property technique in which the weak acid groups are dissociated to some extent. Thus,

$$W/M_{app} = W [\frac{1}{M} + \frac{\alpha}{\epsilon}] \qquad\qquad II.9$$

$$M_{app}^{-1} = M^{-1} + \frac{\alpha}{\epsilon}$$

Finally,

$$M = \frac{M_{app}}{[1 - M_{app}(\alpha/\epsilon)]} \qquad\qquad II.10$$

The method described by Wilson and Weber (77) for correcting the apparent molecular weight for weak acid dissociation is essentially the same as the one given by Hansen and Schnitzer (69). This is not readily apparent since they use different symbols. Wilson and Weber use a parameter which, although given a mathematical definition, is never assigned its true physical meaning. This parameter, $\theta$, is defined mathematically as a power series of the weight concentration "W":

$$\theta = a W + b W^2 + . . . \qquad\qquad II.11$$

Furthermore:

$$\overline{MN} = K/a \qquad\qquad II.12$$

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where "$\overline{MN}$" is the number-average molecular weight, "$K$" is an instrument constant, and "$a$" is the coefficient on the first term in the power series of "$\theta$". In very dilute solutions Wilson and Weber neglect squared and higher powers of the weight concentration. Thus, it follows that: $a = \theta/W$ and $\overline{MN} = K \cdot W/\theta$. The apparent molecular weight measured using some colligative property in which the weak acid groups are partially dissociated is:

$$\overline{MN}_{app} = K \cdot W/\theta_{app} \qquad\qquad II.13$$

The relation between the two parameters: $\theta$ and $\theta_{app}$ is given by Wilson and Weber as:

$$\theta = \theta_{app}/[1 + (H^+)/C_a] \qquad\qquad II.14$$

where $(H^+)$ is the proton concentration and "$C_a$" is the total acid concentration (equivalent to Hansen and Schnitzer's "$c$"). Wilson and Weber maintain that it is necessary to account for the stepwise dissociation of the different types of acidic groups, using the two types proposed by Gamble (70, 72), and replace "$C_a$" with $(C_I + C_{II})$. Here, $C_I$ and $C_{II}$ denote the concentration of type I and II acids respectively.

Although they explicitly separate "$C_a$" into two parts, it is never carried beyond that and the result remains the same as if the terms were not denoted explicitly since no distinction is made between the two groups.

If we examine the relations given by Wilson and Weber

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closely we can see that "$\theta_{app}$" represents the <u>total moles of particles</u> (including moles of $H_iA$, $H_{i-j}A^{j-}$, and $H^+$ in Hansen and Schnitzer's notation). The parameter "$\theta$" represents the moles of acid $H_iA$; i.e., the moles of particles if the acid did not dissociate. The ratio:

$$\theta_{app}/\theta = 1/\ 1+ (H^+)/C_a \qquad\qquad II.15$$

which is II.14 rearranged, is just:

$$1/\ 1 + (H^+)/C_a\ = 1/(1 + j\alpha) \qquad\qquad II.16$$

using Hansen and Schnitzer's notation. Thus, we can write:

$$\overline{MN}_{app} = K \cdot W/\theta_{app} = W/(1 + j\alpha)c \qquad\qquad II.17$$

where the last expression is written using Hansen and Schnitzer's notation. If we rearrange this:

$$W/\ \overline{MN}_{app} = (1 + j\alpha)c \qquad\qquad II.18$$

This is identical to the expression given by Hansen and Schnitzer (cf., the expression II.8 and II.9).

## USE OF THE SALICYLATE MODEL FOR HUMIC SUBSTANCE-METAL COORDINATION SITES.

This is a short discussion of the question as to whether the o-hydroxybenzoate (also refered popularly as the salicylate) model for the coordination sites on humic substance molecules is a valid choice.

The use of the o-hydroxybenzoate model can be traced to the work done by Schnitzer and Skinner (65b). In this work, these workers report experiments in which carboxylic groups were blocked by esterification, phenols by saponification, and carboxyls and phenols simultaneously by methylation. Metal retention before and after treatment were compared. These workers found that the reduction in metal complexing capacity was about the same whether either carboxyls or phenols were blocked separately. They found that the simultaneous blocking of both types of acidic groups did not significantly further reduce the complexing capacity of the humic substances. On this basis they concluded the carboxylic and phenolic groups were not acting independently when metal complexes formed.

Because the chemical modification blocking these groups rendered the humic substances insoluble in aqueous solution, measurements of metal retention for both the untreated and treated substances were done in aqueous acetone ( acetone: water = 9:1). The metals were introduced via ion exchange

resins saturated with the metals of interest.

Himes and Barber (57) report blocking of phenolic groups by methylation of <u>soil</u> using dimethyl sulfate (DMS) to assess the importance of phenols compared to the contribution from carboxylic groups in metal retention by the soil. They used diazomethane to block both types of groups, again using soil and not extracted humic substances, for comparison. They found that metal retention by the soil was reduced just as affectively by DMS methylation as by treatment by diazomethane. Himes and Barber concluded that carboxylic and phenolic groups do <u>not</u> act independently when coordinating metals.

Lewis and Broadbent (61) compare treatments by diazomethane and DMS and found that, while diazomethane reduced $Cu^{2+}$ retention to nearly zero, methylation by DMS reduced $Cu^{2+}$ retention by only 25%. Davies et al. (69) report blocking carboxylic and phenolic groups independently and simultaneously by a variety of methods. They found that when carboxyls and phenols were blocked separately or simultaneously metal retention was reduced about the same amounts. Metal retention was reduced 50% to 60%. The blocking reactions were not complete. Davies et al. (69) conclude that carboxyls and phenols in humic substances do <u>not</u> act independently when complexing metals.

Tan et al. (71b) report infra red data that show the frequency they assign to "-OH" stretching shifts from 3500 $cm^{-1}$ to 3200 $cm^{-1}$ upon the addition of $Zn^{2+}$. They interpret

this perturbation as evidence that phenols coordinate $Zn^{2+}$ in a o-hydroxybenzoate bidentate chelate.

Many other workers refer to the work done by Schnitzer and Skinner (65b) and Himes and Barber (57) as evidence for the existence of these types of chelate sites (eg., Cheam, 73; Gamble, 70; Gamble et al., 70; and Van Dijk, 71). Gamble (70) presents the argument that the weaker acidity of o-phthalate sites is the reason this type of site does not react to form complexes as readily as the o-hydroxybenzoate sites.

In a separate paper, Gamble et al. (70) further argues in support of the model proposed by Schnitzer and Skinner, suggesting since Schnitzer and Skinner did the blocking reactions in aqueous acetone (not the most suitable solvent for such reactions) the reactions were incomplete. This would explain, he says, the residual metal retention capacity remaining after the treatments. Yet, in the same paper Gamble et al. (70) list acid dissociation constants and stability constants for $Cu^{2+}$ complexes with both o-hydroxybenzoic and o-phthalic acids compiled by Ringbom (63).

The acid dissociation constants listed were: for o-phthalic acid, $pK_1 = 2.8$ and $pK_2 = 5.1$; and for o-hydroxybenzoic acid, $pK_1 = 2.9$ and $pK_2 = 13.1$. The stability constants were: $K_s = 0.01$ and $K_s = 0.0032$, for o-phthalic acid and o-hydroxybenzoic acid respectively. Not only is o-phthalic acid just as acidic, if not slightly more acidic, if we consider only the dissociation of the first proton; but it forms

complexes of slightly higher stability. All reported values
for the acidities of these two compounds show that o-phthalic
acid is more acidic.

Randhawa and Broadbent (65) used DMS to methylate
phenolic groups and found that it had little effect on
metal retention. They conclude that either the methylation
reaction did not block the phenols or these groups were not
involved in metal retention to any significant degree.

Manning and Ramamoorthy (73) disagree with the o-hydroxy-
benzoic acid model for the chelation sites in humic substances.
They argue that displacement of the phenolic proton would
require a formation constant for a $Cu^{2+}$ complex of $10^{10.4}$,
certainly the value compiled by Ringbom (63) does not approach
this value. This would mean that the free $Cu^{2+}$ in their
experiments in the presence of a chelate having such a forma-
tion constant would be no higher than $10^{-7}$ M. They measured
free $Cu^{2+}$ using ISE ranging from $2.5(10^{-4})$ to $6.0(10^{-4})$ M.

The arguement given by Manning and Ramamoorthy is just-
ifiable. We see that a phenol ortho to a carboxyl on an
aromatic ring is a much weaker acid than a phenol on an
unsubstituted ring. The phenol on a o-hydroxybenzoic type
structure is weaker than the weakest carboxyls of any type
by at least eight orders of magnitude. It seems highly
unlikely that displacement of the phenolic proton would occur
before coordination of the metal by another carboxyl in
the system unless the stability of the complex so formed
was very high; and this, we have seen, is not the case.

DISCUSSION OF THE DERIVATION OF "$\bar{n}$", THE DEGREE OF FORM-
ATION OF THE SYSTEM, GIVEN BY FRONAEUS (51).

Fronaeus (51) describes a method for measuring the
"complexity constants" for the formation of metal complexes.
The method used cation exchange resin to introduce a com-
peting equilibria, which allows determination of the free
metal concentration in the presence of the ligand. Fronaeus
derives an expression designed to allow determination of
Bjerrum's "$\bar{n}$", "the degree of formation of the system."

To begin with, Fronaeus defines a function:

$$\bar{Q} = \frac{C_{mr}}{C_m} \qquad\qquad IV.1$$

where $C_{mr}$ is the total concentration of metal in all forms
exchanged on the cation exchange resin, expressed as moles
per kilogram of resin, and $C_m$ is the total metal concentra-
tion in solution in all forms. This expression is then
expressed as:

$$\bar{Q} = C_{mr} / \sum_{i=0}^{n} \{MA_i^{(i-2)-}\} \qquad\qquad IV.2$$

where $\{MA_i^{(i-2)-}\}$ is the concentration of the $i^{th}$ complexed
specie in solution.

The formation reaction for the $i^{th}$ complex specie is:

$$M^{2+} + i\,A^- \;\rightleftarrows\; MA_i^{(i-2)-}$$
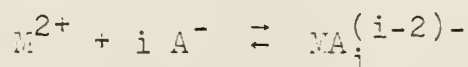
The "complexity constant" is defined by Fronaeus as:

$$\beta_i = \frac{\{MA_i\}}{\{M\}\{A\}^i} \qquad\qquad IV.3$$

omitting the charge signs on the ions for simplicity. The remainder of the derivation is not from Fronaeus (51).

Using IV.3, IV.2 can be written in the following form:

$$\phi = C_{mr} \Big/ \sum_{i=0}^{n} [\beta_i \{M\}\{A\}^i] \qquad\qquad IV.4$$

$$\phi = \frac{C_{mr}}{\{M\}} \Big/ \sum_{i=0}^{n} [\beta_i \{A\}^i]$$

This can be rearranged to give:

$$\phi \cdot \frac{\{M\}}{C_{mr}} = 1 \Big/ \sum_{i=0}^{n} [\beta_i \{A\}^i] \qquad\qquad IV.5$$

It is clear that the L.H.S. of IV.5 is a function of $\{A\}$ only; i.e., the dissociated ligand concentration. The derivative of: $\phi\{M\}/C_{mr}$ with respect to $\{A\}$ is:

$$\frac{d}{d\{A\}}\left[\frac{\{M\}\cdot\phi}{C_{mr}}\right] = - \frac{\sum\limits_{i=0}^{n}[i\beta_i\{A\}^{i-1}]}{(\sum\limits_{i=0}^{n}[\beta_i\{A\}^i])^2} \qquad\qquad IV.6$$

Rearranging IV.6:

$$- (\sum_{i=0}^{n}[\beta_i\{A\}^i]) \cdot \frac{d}{d\{A\}}\left[\frac{\{M\}\cdot\phi}{C_{mr}}\right] = \frac{\sum\limits_{i=0}^{n}[i\beta_i\{A\}^{i-1}]}{\sum\limits_{i=0}^{n}[\beta_i\{A\}^i]}$$

Multiplying this expression through by: $\{A\}$ and $\{M\}/\{M\}$,

$$- \{A\} \; \frac{\sum\limits_{i=0}^{n} [\beta_i \{M\}\{A\}^i]}{\{M\}} \cdot \frac{d}{d\{A\}} \left[ \frac{\{M\}\cdot\phi}{C_{mr}} \right] = \frac{\sum\limits_{i=0}^{n} [i\beta_i \{M\}\{A\}^i]}{\sum\limits_{i=0}^{n} [\beta_i \{M\}\{A\}^i]} \qquad \text{IV.7}$$

By definition, the degree of formation of the system is:

$$\bar{n} = \frac{\sum\limits_{i=0}^{n} [i\{MA_i\}]}{\sum\limits_{i=0}^{n} [\{MA_i\}]} = \frac{\sum\limits_{i=0}^{n} [i\beta_i \{M\}\{A\}^i]}{\sum\limits_{i=0}^{n} [\beta_i \{M\}\{A\}^i]} \qquad \text{IV.8}$$

Thus, we see that:

$$\bar{n} = - \{A\} \; \frac{C_m}{\{M\}} \cdot \frac{d}{d\{A\}} \left[ \frac{\{M\}\cdot\phi}{C_{mr}} \right] \qquad \text{IV.9}$$

This expression is not the same as the expression given by Fronaeus (51). His expression for "$\bar{n}$" was:

$$\bar{n} = - \frac{C_a}{\phi} \cdot \left[ \frac{\partial \phi}{\partial C_a} \right]_{C_{mr}} \qquad \text{IV.10}$$

$$= - (C_a) \frac{C_m}{C_{mr}} \left[ \frac{\partial \phi}{\partial C_a} \right]_{C_{mr}}$$

where $C_a$ is the total ligand concentration and Fronaeus makes the approximation: $C_a \approx \{A\}$ when $C_a \gg C_m$.

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METAL COMPLEXATION BY
SOIL HUMIC SUBSTANCES


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This study is concerned with evaluating the metal complex-ing behavior of soil humic substances. Perhaps the most impor-tant feature of soil humic substances, and humic substances in general from sewage sludge and surface waters, is the heterogene-ity of these substances. Humic substances are most likely poly-mers derived from plant lignin alteration products, poly- and monosaccharides, polypeptides, amino acids, and other bio-organ-ic compounds. The compounds in a solution of humic substances are characterized by a wide range in molecular weights, functional group acidities, and metal complexing stabilities.

Soils from a municipal sewage sludge disposal site were extracted with a $NH_4(HCO_3)$ solution. The metals co-extracted with the humic substances were removed by passing the extracts through an ammonium-saturated iminodiacetate ion exchange (the so-called chelate exchange) resin column. The metal-free extracts were fractionated on a $Cu^{2+}$-saturated chelate exchange resin column equilibrated and eluted with aqueous ammonium solutions. A separation into two fractions occured, presumably on the basis of metal complexing stability.

An attempt to measure "stability constants" for the humic substance fractions was unsuccessful. The major reason for this, other than insufficient amounts of sample, was that it is not possible to measure such a constant for a solution consisting of a mixture of ligands and complexes of different types.

Metal complexing behavior of humic substances can be described by measuring the distribution of metal between free and complexed forms as a function of pH, ligand concentration, and ionic stren-

gth.  Further information that supplements information on the metal complexing behavior of humic substances would include number-average molecular weights, acid dissociation behavior as a function of pH, and the "degree of formation of the system"; i.e., the mean number of ligands around the metal center.