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BARD

FINAL REPORT

PROJECT No. I-500-82

Anion Adsorption by Soil Organic Matter and Peat

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1987

Standard BARD Cover Page for Scientific Reports

Date 1.6.1987

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. I-500-82

Title of Research Project:

Anion adsorption by soil organic matter and peat

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Project's starting date: 1.9.1983

Type of Report: 1st Annual 2nd Annual Final X

Signatures:

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Institution's Authorizing Official

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A. ABSTRACT

Emphasis was given to interactions of chloride, nitrate, borate, EDDHA, and FeEDDHA with humic substances and soil organic matter. Reactions of trace elements and aluminum were reviewed and Donnan and double-layer theories were used to model anion exclusion in a system containing humic acid. The research included: (1) the development of rapid analytical procedures for the routine determination of inorganic anions (chloride, nitrate, sulfate) and FeEDDHA by anion exchange chromatography, (2) interference of humic substances on the nitrate and chloride ion-selective electrodes, (3) adsorption of boron by composted organic matter in relation to plant growth and boron uptake, and (4) theoretical and experimental studies on cation-anion interactions with humic acids, including application of two geometric models for determining charge spacings and the configuration of humic acids in solution.

Boron sorption by organic matter was 3 to 5 times higher as compared to smectite clays. Additions of organic matter to soil resulted in decreases in the concentration of boron in the soil solution and inhibited its uptake by plants. Adsorption of FeEDDHA to humic substances was appreciable at low pH (4-5) but negligible in slightly acidic to neutral soils (pH 6-7). In accordance with theory, exclusion occurred at high pH values (> 7). Measured particle radii of a soil humic acid, as obtained from nitrate exclusion data, ranged from 0.9-1.5 nm at low degrees of ionization (i.e., low pH) to 0.3-0.6 nm at near complete ionization of carboxylic functional groups (neutral pH). Charge spacings on humic acids, as obtained from chloride exclusion data (planer model), were of the order of 3 to 28 Å for three soil humic acids; for a lignite humic acid, the range was 10 to 100 Å. A byproduct of the research with the chloride ion-selective electrode was the quantification and determination of stability constants for the binding of Ag^+ by humic substances.

B. OBJECTIVES OF THE RESEARCH

(a) To characterize the adsorption-desorption behavior of anions with components of the soil organic matter (e.g., humic and fulvic acids). The main concern will be nitrate, borate, chloride and FeEDDHA.

(b) To ascertain the effect of pH, anion concentration, ionic strength, and competing anion on the adsorption process.

(c) To determine the interactions of anions with composted organic matter and with humic substances originating from soils in the USA and Israel.

(d) To determine the relative affinity of soil organic matter components to the various anions.

(e) To develop models for the prediction of adsorption, desorption and exclusion of anions by the organic fractions of mineral soils.

(f) To elaborate particle size and shape of humic substances from acid-base titrations and anion exclusion.

C. DESCRIPTION OF COOPERATION

A close and fruitful cooperation existed between investigators of the two countries during the course of the study. Drs. Chen and Stevenson had previously engaged in cooperative research while Dr. Chen was at the University of Illinois on sabbatical leave from the Hebrew University of Jerusalem (1981-82 school year) and the research initiated at that time was expanded and carried out to a successful conclusion. Graduate students from both Israel (P. Barak and U. Yermiah) and the USA (F. J. Sikora, C. Stubbs, and G. F. Vance) were closely involved in the research and thereby benefited from the joint effort.

The research consisted largely of laboratory studies on factors affecting adsorption-desorption of anions (and cations) by peat, mineral soils, and isolated humic and fulvic acids. Two major lines of work (separation of anions by ion chromatography and use of the nitrate ion-selective electrode in binding studies) were initiated at the University of Illinois but completed at the University of Jerusalem in Rehovot. Through mutual agreement and planning, Dr. Chen and his students emphasized research with borate, nitrate, and anionic chelating agents (EDDHA); Dr. Stevenson and his students emphasized chloride exclusion and binding, the latter being of importance under acidic conditions and can lead to errors in the estimation of acidic functional groups by methods based on ion-exchange. Models were developed jointly by investigators at the two institutions to determine charge spacing and the shapes of humic substances in solution from data on anion exclusion, and included applications of a cylindrical model (Dr. Chen and co-workers) and a planar model (Dr. Stevenson and co-workers).

Site visits were made and a series of seminars were presented by both principle investigators, as follows: Dr. Chen at the University of Illinois (1. Effect of Humic Substances on Plant Growth; 2. Effect of Soil Sterilization on the Chemical and Biological Properties of Soils; and 3. The Physical Chemistry of Humic Substances); Dr. Stevenson at the Hebrew University of Jerusalem and at the Technion, Haifa (1. Metal Ion Binding by Humic Substances; and 2. The Dynamics of Soil Nitrogen Transformations).

D. RESEARCH ACHIEVEMENTS AND BENEFITS TO AGRICULTURE

Soil organic matter contains a large number of oxygen and nitrogen containing functional groups. Charge density on these groups varies with pH and ion strength. Mechanisms involved in anionic interactions with soil organic matter include ion exchange, ion exclusion, specific complex formation, hydrogen bonding, and hydrophobic adsorption. Although organic matter can function as a sink for nitrite, borate, and phosphate, research on anionic interactions with reactive components of soil organic matter (i.e., humic substances and polysaccharidides) are limited. Information obtained in this study will be of interest to scientists in several scientific disciplines and will contribute to our understanding of the role of organic matter (particularly humic substances) in practical agriculture.

The concentration of ions in the soil solution is critical for human and animal health. Some anions exhibit a narrow margin between sufficiency and toxicity. For example, 0.8-1.3 mg L⁻¹ of fluoride is considered optimal for drinking water while 0.3-2.0 mg L⁻¹ of boron in irrigation water is optimum for sensitive and semi-tolerant plants. The concentration of nitrate in drinking water is critical for the health of infants; values of 45-90 mg L⁻¹ are considered as the upper safe limit in most western countries.

In the course of this work, new methods were developed for the rapid and accurate analysis of nitrate, chloride, and sulfate in soil solutions and irrigation waters by high pressure liquid chromatography (HPLC). This procedure allows soil scientists to avoid tedious and less accurate procedures for the analysis of nitrate and sulfate. An HPLC procedure was also developed for the quantitative determination of EDDHA, FeEDDHA (Sequestrene 138) and its isomers, and of similar compounds in chelate mixtures or soil solutions. The procedure provides a tool for further investigations on the fate of iron-containing chelate fertilizers in soil, thereby leading to improved management

practices for prevention of lime-induced iron chlorosis on sensitive crops.

Soluble humic substances were found to interfere with measurements of nitrate and chloride using the corresponding ion-selective electrodes (ISE). The results clearly showed that ISE techniques cannot be used for the determination of these ions in solutions containing significant concentrations of humic substances. Further research is being done with the nitrate ISE to establish the significance of interference at low concentrations. The practical importance of this work arises from the fact that the nitrate ISE is sometimes used to monitor and control nitrate concentrations in nutrient solutions in commercial greenhouse operations.

As already mentioned, FeEDDHA serves as the most important remedy for lime-induced chlorosis in field crops, horticulture crops, and ornamentals. Rapid adsorption of applied FeEDDHA by soils has been noted in the literature and poses a serious problem in the agricultural use of this chelate as a fertilizer. Experiments with EDDHA and FeEDDHA in sodic and calcic systems with humic acid show that, at low pH values, both EDDHA and FeEDDHA are repelled by sodium humate; calcium humate shows little interaction with FeEDDHA at neutral pH. These data would seem to fit a hydrogen bonding mechanism for adsorption coupled with anion exclusion for the sodium system at neutral pH. It would appear that humates do not provide an explanation for FeEDDHA fixation in soils. A preliminary experiment with montmorillonite suggests that reactions with soil minerals are of importance and bear further investigation.

The borate anion is of special concern in arid and semiarid zone soils. Toxicities are often found in such areas, sometimes in association with saline conditions. Since borate is known to bind with polysaccharides, which contain functional groups similar to those of humic substances, adsorption and desorption of borate by composted organic matter (OM) was studied. Strong

adsorption was observed. Adsorption isotherms were modeled, thereby providing a predictive tool for soil scientists. Application of organic matter significantly reduced soluble boron levels due to irreversible adsorption, thereby reducing boron uptake by plants.

Exclusion of NO_3^- and Cl^- by humic substances was found to be a dominating effect, as compared to adsorption. Exclusion was modeled by both the Donnan equilibrium theory and the double layer theory. The latter was used along with acid-base titrations to calculate particle size of HS which is of importance to soil scientists and environmentalists who study transport phenomena in relation to soil OM. Evidence provided in this study shows that charged cylinder or fiber shape particle seem to be the most likely for HS. Calculated radii of the particles were about 0.9-1.3 nm at low pH and they gradually decline to 0.3-0.6 nm at neutral or basic pH levels. The models developed can be applied to other anions that do not show specific adsorption to HS in systems that do not contain metal ions (which could provide metal bridging between anions and OM).

Binding of cations such as Al, Fe, Mn, Zn and Cu to HS has been reviewed in two extensive review articles that will assist soil scientists interested in further pursuing the metal bridging concept as well as transport of metal complexes of humic substances.

E. Articles in Journals, Chapters in Books and Dissertations Originating From This Project.

1. Barak, P. 1987. Interactions of Humic Substances with Anions. Ph.D. thesis. Submitted to the Hebrew University of Jerusalem.
2. Barak, P., and Y. Chen. 1987. Three-minute analysis of chloride, nitrate and sulfate by single column anion chromatography. Soil Sci. Soc. Am. J. 51:257-258.
3. Barak, P., and Y. Chen. 1987. Determination of FeEDDHA in soils and fertilizers by anion exchange chromatography. Soil Sci. Soc. Am. J. (in press).
4. Barak, P., and Y. Chen. 1988. Reactions of EDDHA and FeEDDHA with humic substances. (in preparation).
5. Barak, P., and Y. Chen. 1988. Apparent radius of humic molecules from acid-base titration and nitrate exclusion. (in preparation).
6. Chen, Y., and F. J. Stevenson. 1986. Soil organic matter interactions with trace elements. p. 73-116. In: Y. Chen, and Y. Avnimelech (Eds.). The Role of Organic Matter in Modern Agriculture. Martinus Nijhof, The Netherlands.
7. Chen, Y., P. Barak and F. J. Stevenson. 1988. Nitrate exclusion by humic substances. (in preparation).
8. Chen, Y., V. Yermiahov and R. Keren. 1988. Boron adsorption on soil organic matter in relation to pepper plants growth and to boron uptake. (in preparation).
9. Sikora, F. J. 1986. Cation-Anion Interaction with Humic Substances. Ph.D. thesis, Department of Agronomy, University of Illinois.
10. Sikora, F. J., and F. J. Stevenson. 1987. Interference of humic substances on chloride analysis using chloride ion-selective electrode. Soil Sci. Soc. Am. J. (in press).
11. Sikora, F. J., and F. J. Stevenson. 1987. Silver complexation by humic substances: Conditional stability constants and nature of reactive sites. Geoderma (submitted).
12. Sikora, F. J., and F. J. Stevenson. 1988. Application of Donnan and counterion condensation theories to model anion interactions with humic substances (in preparation).
13. Sikora, F. J., and F. J. Stevenson. 1988. Application of Donnan counterion condensation theories to K^+ and Cl^- interactions with humic acids. Soil Sci. Soc. Am. J. (in preparation).
14. Stevenson, F. J., and G. F. Vance. 1987. naturally occurring aluminum-organic complexes. In: G. Sposito (ed.). The Environmental Chemistry of Aluminum. CRC Press, Boca Raca, Florida, USA (in press).

15. Yermiah, V. 1987. Boron Sorption on Soil Organic Matter and its Effect on Plant Growth and Boron Uptake. MSc. thesis. Submitted to the Hebrew University of Jerusalem (Hebrew).
16. Yermiah, V., R. Keren, and Y. Chen. 1987. Boron adsorption on composted organic matter. Soil Sci. Soc. Am.J. (submitted).

4 . SUMMARY AND CONCLUSIONS

1. SUMMARY AND CONCLUSIONS

Organic matter can affect anion concentration in the soil solution in the following ways: (1) anion exclusion can occur due to negative net charges on soil organic colloids; (2) specific adsorption of chelate complex formation of hydroxy ions can occur at di-carboxy or carboxy-hydroxy sites on organic matter; (3) positively charged NH_3^+ (and possibly OH_2^+) groups can be formed by protonation, thereby providing sites for anion exchange; (4) organometallic complexes can be formed with humic substances, (5) oxyanions or organic anions can be bound through H-bonding, and (6) large organic anions can be bound through hydrophobic adsorption. Some of these mechanisms and their significance in interactions of anions with humic substances and soil organic matter were studied.

Development of Methods for Measuring Anion Interaction with Humic Substances

In the course of this work, requirements of analytical speed and accuracy necessitated development of methods of analysis of nitrate and FeEDDHA by ion chromatography. The techniques developed were expanded to more general applications in soil and fertilizer analysis and were published in Soil Science Society of America Journal as two articles.

Nitrate -- Analysis of nitrate by ion chromatography is an accepted analytical technique which completely separates the analyte from the rest of the sample and places the analytical peak in a standard chemical matrix before the analyte reaches the detector. Standard chromatographic techniques using 5 mM potassium hydrogen phthalate at pH 4.5 as an eluent for a 25 cm column routinely required 10-15 minutes for separation of chloride, nitrate, and sulfate and regeneration of the column for the next injection. This was considered to be impractical for a large number of analyses together with the necessary blanks and standards. Changes were made in the standard equipment configuration and eluents in order to reduce analysis time to under three minutes without sacrificing accuracy; these changes brought concomitant reduction in the pressure required from the pump and significant reduction of column costs. The improvement in technique was tested for its general applicability to the analysis of natural waters including groundwater and soil solution.

Accepted techniques of single column ion chromatography of inorganic anions were extended to separation of chloride, nitrate, and sulfate using 15 mM phthalic acid as an eluent, permitting reduction of column length to 30 mm and thereby reducing analysis time to three minutes. Using a 50 μL sample loop, detection limits were 0.05 mmol L^{-1} and coefficient of variation values ranged from 0.8 to 1.7% using peak height measurements and 0.8 to 8.1% using peak area measurements. This configuration is appropriate for routine analysis of soil water extracts and ground water. This procedure was published by Barak and Chen (1987) (section 5).

FeEDDHA -- Published analytical techniques included spectroscopic measurement of the colored chelate (Hill-Cottingham, 1962-section 5) solvent extraction followed by spectroscopic measurement and gel chromatographic separation followed by spectroscopic measurement. No published results were found, other than those of the original author, in which these methods were

used; Fe by atomic adsorption spectroscopy was usually employed instead (see chapter on FeEDDHA). These methods were deemed either insufficiently specific or especially tedious, and it was thought desirable to develop a chromatographic procedure based on the ionic properties of FeEDDHA. The results of the development of an improved method for measurement of FeEDDHA was accepted for publication as "Determination of FeEDDHA in soils and fertilizers by anion exchange chromatography" by Phillip Barak and Yona Chen in Soil Science Society of America Journal 51:000-000, and summarized as follows:

The specific conditions for the quantitative analysis of FeEDDHA (ethylenediamine di-o-hydroxyphenylacetic acid) by high pressure liquid chromatography (HPLC) were studied and defined. Using a 3 cm anion exchange column and 5 mM H_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$ as an eluent, FeEDDHA was separated into the di-racemic and meso isomers. With the same column and 3 mM H_2SO_4 + 50 mM Na_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$, FeEDDHA eluted as a single peak. Standard calibration curves for FeEDDHA were obtained with regression coefficients of 0.9999 at concentrations up to 0.25 mM. The coefficient of variation is 1.5%, or $1.8 \mu\text{mol L}^{-1}$ using a 50 μL sample; the detection limit was $1.2 \mu\text{mol L}^{-1}$. FeEDDHA was separated easily on anion exchange HPLC columns from other chelates such as CDTA, EDTA, and DTPA. The proposed method was used to determine FeEDDHA concentrations in water extracts of soils that were incubated with the compound. With variation of the eluent, separation of ferrated and unferrated EDDHA is possible.

Measurement and Modeling of Boron Adsorption on Composted Organic Matter in Relation to Plant Growth and Boron Uptake

Boron (B) adsorption and desorption on composted organic matter at various pH levels has been determined. The compost consisted of 158 mg g^{-1} and 56 mg g^{-1} humic acid and fulvic acid fractions, respectively, totaling 21.4% of the organic matter. At pH 8, the amount of adsorbed B increased by about 57% with an ionic strength increase up to 0.15 M. For solutions of CaCl_2 at concentrations lower than $5 \times 10^{-3} \text{ M}$, the effect of CaCl_2 on B adsorption was negligible. Adsorption isotherms of B on organic matter were determined at pH levels of 7.0, 7.9, and 8.9. Adsorption increases with increasing B concentration in solution and pH.

A comparison of literature data for clays to those measured for the organic matter shows that adsorption on the latter at similar pH levels and total B concentration is at least times higher. A phenomenological equation based on a modified Langmuir equation was successfully used to fit the measured data. Based on the isotherms, adsorption coefficients were derived and used to calculate the predicted dependence on pH of B adsorption on organic matter at various total B levels. Desorption studies of B from organic matter at pH 8 and increasing levels of adsorbed B exhibited strong hysteresis effects and the adsorbed B was removed to a partial extent only. The formation of di-hydroxy and/or hydroxy-carboxy bonds of borate to humic substances and/or carbohydrates is proposed as an adsorption mechanism that agrees with the irreversibility of the adsorption process.

Effects of increasing application rates of boron (B) to a soil mixed with increasing levels (0-10%) of composted organic matter on B concentrations in pepper leaves and plant growth response was studied. Boron application levels

were 0, 1, 5 and 10 mmol kg⁻¹ soil mixture, and organic matter (OM) levels were 0, 1, 3, 6 and 10%. Adsorption isotherms were determined on soil:OM mixtures, and the data were modeled and calculated according to a modified Langmuir equation. Adsorption increased with pH and OM levels. Calculated data for the adsorption curves agreed well with the measured ones. Calculated values for the soil solution in the plant growth experiment correlated well with the measured ones but were somewhat higher. Boron application levels effected plant yield at 0% OM only where high B levels resulted in a yield decrease. At higher OM levels plant yield responded positively to OM and the yields were similar for increasing B applications. However, B concentrations in the leaves were strongly dependent upon OM levels: at low OM and high B application rates B in leaves approached toxic levels and at 0% OM exceeded the toxicity limit (330 mg kg⁻¹). Increasing OM levels to 1% or more prevented this excessive uptake of B by adsorption thereby reducing B concentration in the soil solution. Measured and calculated B concentrations in pepper leaves correlated well with B concentration in the soil solution suggesting an optimal concentration of 40-60 mg kg⁻¹ in the leaves when soil solution concentrations are about 1 mg L⁻¹, and a linear increase of about 30 mg B kg⁻¹ dry weight of leaves for further increase of 1 mg B L⁻¹ soil solution. Application of the findings to other plants requires further testing.

Adsorption and Exclusion of Nitrate and FeEDDHA (Sequestrene).

FeEDDHA -- Removal of FeEDDHA from solution by soils immediately upon contact has been noted in the literature and poses a serious problem in the agricultural use of this chelate as a fertilizer. Experiments with FeEDDHA and EDDHA in sodic and calcic systems with humic acid show that at low pH both FeEDDHA and EDDHA are adsorbed. At neutral pH, both FeEDDHA and EDDHA are repelled by sodium humate while calcium humate shows little interaction with FeEDDHA at neutral pH. These data would seem to fit a hydrogen bonding mechanism for the adsorption phenomenon coupled with anion exclusion for the sodic systems at neutral pH. It would appear that humates do not provide an explanation for FeEDDHA fixation in soils but a preliminary experiment with montmorillonite suggests that reactions with soil minerals bear further investigation.

Nitrate -- Nitrate exclusion as determined by material balance equations using nitrate determination in HA-free equilibrium solutions separated by dialysis membrane from humic substances show significant nitrate exclusion which agrees with the anionic nature of humic substances. Determination of nitrate activity either inside the dialysis cell with HA or in titration vessels shows strong interference (in opposite directions) for both the Radiometer and the Orion nitrate specific electrodes, leading to interpretations supporting either anomalous exclusion effects or strong adsorption; the use of nitrate specific electrodes directly in humic acid solutions should be discouraged. On the other hand, ion chromatography and ISE both provide reliable information on the external, HA-free equilibrium solution.

In this study, data gathered by dialysis and ion chromatography were combined with additional information on the nature of the acidic groups and a quantitative model of the humic macromolecule and its double electric layer was defined and formulated.

Although nitrate exclusion was the prominent phenomenon observed at all pH levels other evidence from IR data and nitrate retention in soils suggest that adsorption may also take place. It seems however, that exclusion is significantly higher than adsorption. Therefore, a net exclusion effect was recorded.

Interference of Humic Substances in the Determination of the Chloride Ion by the Chloride Ion-Selective Electrode.

Humic substances were found to interfere with Cl^- determination using the Cl^- ion-selective electrode by complexing Ag^+ , thereby reducing the activity of the potential determining ion. Proof of Ag^+ binding was obtained through a separate technique that involved equilibrium dialysis and Ag^+ determination by atomic absorption spectroscopy. Competition between Ag^+ and H^+ for binding sites was evident from an increase in mV response of the Cl^- ion-selective electrode with addition of HNO_3 and release of protons upon Ag^+ binding in the dialysis experiment. Selectivity coefficients ($k_{\text{Cl}^+/\text{HA}}$) for 11 humic and fulvic acids ranged from 0.59×10^{-4} to $1.3 \times 10^{-4} \text{ ml mg}^{-1}$ at pH 6.5. Interference would be negligible in solutions containing less than 0.1 mg mL^{-1} humic substances but would be serious for samples containing low concentrations of Cl^- ($<10^{-4.5} \text{ M}$) and high amounts of organic matter ($>0.1 \text{ mg mL}^{-1}$).

Complexation of Ag^+ by a wide range of humic and fulvic acids was determined using a Cl^- ion-selective electrode sensitive to the concentration of Ag^+ in solution. Maximum binding capacities (MBC) ranged from 0.75 to 1.95 mmol Ag g^{-1} at pH 6.5 (ionic strength, I, of 0.1) and were more closely related to the N contents of the humic substances (0.5 to 2.8 mmole N g^{-1}) than to acidic functional group content (3.5 to 7.0 $\text{mmoles COOH groups g}^{-1}$). Scatchard plots of data obtained by titrations with Ag^+ (pH 6.5, I of 0.1) were concave upward, indicating a continuum of binding sites. Apparent stability constants for binding at low saturation of binding sites ($\log K^*$) ranged from 3.57 to 4.80; constants for binding at high saturation ($\log K^{\frac{n}{2}}$) ranged from 2.64 to 3.17. Higher values (6.34 to 8.82) were obtained using an approach that permitted measurements to be made at low Ag^+ concentrations. The evidence indicated that complexation occurred predominantly on N (and possibly S) functional groups, as opposed to COOH and other acidic functional groups.

Chloride Exclusion From Humic Acid

Donnan and counterion condensation theories were used to model the distribution of ions (specifically K^+ and Cl^-) in a Donnan system containing humic acid. A general equation was derived to determine Donnan distribution of ions with variable valence at any pH and ionic strength. For soil humic acids, Donnan ratios for K^+ (r_D^K) were greater than for Cl^- (r_D^{Cl}), indicating that a greater amount of K^+ was present in the interior solution than would be expected for an ideal Donnan system. The data were analyzed on the basis that the non-ideal behavior was due to: i) lower activity coefficients for the ions in the interior, as compared to the exterior solutions, and ii) counterion condensation of K^+ on the humic polymer. Whereas the latter could not be dismissed entirely, the major cause of the unequal Donnan ratios appeared to be due to a difference in the activity coefficients.

Inferences on charge spacings and the configuration of humic acids in solution were made from experimental data on Cl^- exclusion. For three soil humic acids, charge spacings ranged from 3 to 28 Å; for a lignite humic acid, the range was 10 to 200 Å. A rather constant charge spacing over the pH range from 3.5 to 6.8 supported the hypothesis that humic acid is a flexible molecule in solution.

Theories and Modeling of Anion Interactions with Humic Substances and of Particle Size and Dimensions of Humic Substances

Information on the interaction of anions with humic substances is generally lacking. Although there is some evidence for adsorption of anions by Fe- and Al-humates, and of borate by cis-hydroxyl groups, most anions would be expected to be repelled from the negatively charged humic colloid.

Exclusion of anions from humic substances can best be visualized in a Donnan-type system, where the negatively charged macromolecule is restrained in the internal phase and anions are repelled due to electrostatic interactions. Whereas Donnan theory has been used successfully to examine ion interactions with silicate minerals very little information was available on application of the theory to model anion interactions with humic substances. Since humic and fulvic acids in solution act as weak-acid polyelectrolytes, counterion condensation would also be expected to play a role in ionic interactions.

As part of the research carried out during the course of this project, Donnan and counterion condensation theories were used to model the distribution of ions in a system containing humic acids, the objectives being to quantify the interactions (i.e., exclusion of Cl^- and retention of K^+) and to determine charge spacing and the configuration of humic acids in solution. Donnan theory and models for counterion condensation were discussed. Applications of the theory to ionic interactions with humic acids are given in section 6, using a universal equation developed in section 3 to account for charge characteristics of the humic acid colloid and carbonate species in solution.

Acid-base titration data for a humic acid extracted from peat soil is not consistent with a model of a simple organic acid behaving in accordance with simple activity considerations. When the humic acid was considered to be a linear polyelectrolyte with cylindrical geometry and charge evenly distributed on the cylinder surface, solutions of the relevant Boltzmann-Poisson equations showed that cylinders of radius from 0.85 to 0.35 nm would show the same pH/ionic strength response as measured. The apparent radius measured by this method agrees closely with the apparent viscometric radius reported for other humic substances. The carboxylic groups, when cleared on behavior related to macromolecular nature, may be well-described as the result of two or more types of carboxylic groups.

Nitrate exclusion data for the same humic acid shows that the radius of a charged cylinder which would cause nitrate exclusion identical to that actually measured is strikingly similar to that measured for cation condensation. Radii measured are 0.9 - 1.3 nm at low degrees of ionization (low pH), gradually declining to 0.3 - 0.6 nm at near total ionization of the carboxylic functional group (neutral pH). The degree of convergence of

results of two complementary phenomena may be regarded as enhancing the credibility of both the model and the data for the humic acid employed herein. The thinning of the humic molecule with increasing ionization is consistent with prevailing views of humic substances as randomly coiled polyelectrolytes which extend due to ionic repulsion.

Based on viscometric behavior of humic substances analyzed with the Simha equation, Chen and Schnitzer (1976a) (Section 6) found that the measured particle radius of a humic acid and a fulvic acid was between 0.30 and 0.34 nm over the range of pH 3 to 10, while the particle length over the same range was 3.06 - 4.43 nm for the fulvic acid and 7.86 - 9.91 nm for the humic acid. The particle radii measured viscometrically were virtually identical for humic and fulvic acids and agree well with the apparent radius determined here potentiometrically (cation condensation) and chromatographically (anion exclusion); furthermore, the viscometric measurements show the particles to be sufficiently long that the assumption of infinitely long rods, and therefore negligible end effects, to be a reasonable approximation.

The dimensions of the humic macromolecule fall in the range of those of a number of other fibrous biomacromolecules, such as the DNA double helix (1.0 nm radius), tropocollagen triple helix (0.75 nm radius) and the α -helix (0.23 nm radius). It should be born in mind that the biomolecules of proteins and DNA differ essentially from those of humic substances because the proteins and DNA have a well-defined, repeating structure whereas the humic substances are heterogeneous.

The dimensions of the peat soil-derived humic acid examined in this study further suits several of the structural models which have been developed over the years by veteran researchers of humic substances. Given that the width of a benzene ring is about 0.25 nm, the experimental dimensions are consistent with structural models of Dragunov, Flaig, Buffle and Stevenson, especially when permitting strands to condense lengthwise at low pH. The small apparent radius of humic substances have thwarted but numerous attempts to prepare single-particle preparates for electron microscopy and to resolve such particles once prepared. Additionally, the small radius must contribute significantly to the great flexibility of humic electrolytes and their ability to undergo conformational changes in the presence of high salt concentrations and even low concentration of di- and trivalent metals.

Interaction of Soil Organic Matter with Aluminum and Micronutrients

The binding of aluminum (Al) by naturally occurring organic substances is a subject of considerable importance in several scientific disciplines including crop science, forestry, geochemistry, limnology, soil science, and environmental chemistry. Organic substances are involved in the weathering and neogenesis of Al-bearing minerals, and they serve as agents for the transport of Al in leached terrestrial soils, notably those classified as Spodosols, or Podzols. Aluminum can act as a bridging cation for the binding of humic substances to clay minerals, thereby affecting the physical properties of soils.

The interaction of Al with organic substances is of considerable importance in controlling soil solution levels of the highly toxic Al^{3+} ion in acid soils and natural waters. Research on acid deposition has implicated

organic substances in the solubilization and transport of Al from terrestrial environments to natural waters. A knowledge of the nature of organic ligands that form complexes with Al, and of the properties of the complexes thus formed, will lead to a better understanding of the behavior of Al in terrestrial and aquatic systems.

The significance of Al-organic matter interactions in soils has been reviewed (Stevenson and Vance, 1987) (Appendix) with an emphasis on the distribution of naturally occurring organic ligands that bind Al and the nature and stability of Al-organic matter complexes. Although primary consideration was given to terrestrial soils, many of the concepts also apply to aquatic systems.

Naturally occurring organic substances of various types play a prominent role in the binding of Al in soils and sediments. Both soluble and insoluble complexes are formed. Biochemical compounds produced by microorganisms (e.g., aliphatic organic acids, phenolic constituents, hydroxamate siderophores) are particularly important in environments where microbial activity is intense, such as in the rhizosphere and the organic rich layers of forest soils, notably Alfisols and Spodosols. Humic substances are of greater importance in modifying the reactions of Al on clay and oxide surfaces, in reducing toxicities of Al in acid soils, in regulating the sorption-desorption of phosphate in allophonic soils, and in transporting Al in natural waters.

Organic matter plays a key role in the behavior of micronutrients in soil. Both soluble and insoluble complexes are formed. Substantial evidence has accumulated to indicate that complexing agents (e.g., defined biochemical compounds and humic substances) play a prominent role in the dissolution of micronutrients and their transport to plant roots. This topic has been reviewed by Chen and Stevenson (1986) (Appendix).

Whereas natural organic substances show promise as carriers of trace elements in micronutrient deficient soils, their use has not been widely adopted. Further research and technological advancements in fertilizer manufacture may lead to more extensive use in the future.

Stability constants are metal complexes with humic and fulvic acids are highly variable and differ by as much as three orders of magnitude for the same experimental conditions (pH, ionic strength, etc.). In many instances, comparisons cannot be made because of differences in approaches for modeling the experimental data. A variety of modeling methods have been applied, but, thus far, there is no sound basis for selecting one approach at the exclusion of all others. Consideration needs to be given to standardization of methods so that results from various laboratories can be compared. In the future, attention should be given to competitive binding (i) between metal ions for reactive sites on humic and fulvic acids, (ii) between naturally occurring ligands (e.g., humic acids, fulvic acids, biochemical compounds) for the metal ion, and (iii) between soluble and insoluble organic matter for the metal ion.

2 . INTRODUCTION

2. INTRODUCTION

One generalization that can be made about soils is that the particles of which they are composed have a net negative charge. Clay, organic matter, and free oxides are the main soil colloids contributing to the negative charge, and thereby the ability to retain cations in nonleachable forms. It is nevertheless a fact that anions are also bound to varying extents by the soil complex (see reviews of Parfitt, 1978; Hingston, 1981; and Mott, 1981). In the past, major emphasis has been given to anion sorption by inorganic constituents (e.g., Fe- and Al-oxides) but there is evidence to indicate that the soil organic fraction is also involved.

It has been suggested that organic matter can affect the anion concentration in the soil solution in the following ways: (i) positively charged NH_3^+ (and possibly OH_2^+) groups can be formed by protonation, thereby providing sites for anion exchange; (ii) a chelate complex can be formed with the anion (e.g., borate), thereby removing it from solution (Davis, 1978), (iii) organometallic complexes can be formed with humic substances, specifically phosphato-Fe(III) and phosphato-Al(III), and (iv) anionic species (e.g., phosphate, nitrate) can be incorporated into organic forms through biological action (Parfitt, 1978; Mott, 1981).

The significant role of organic matter in phosphate adsorption (mechanism III) has been widely recognized (Parfitt, 1978). Attention has also focused on boron fixation, and it is well accepted that much of the boron in soils is associated with organic matter (Ellis and Knezek, 1972). However, interactions of other anions with soil organic matter have not been studied in detail, including those of major importance such as nitrate, sulfate, chloride, and fluoride. Since the charge on organic matter is strongly affected by pH, it is important to understand the role of pH in adsorption-desorption. Soil liming and acid rain are examples of situations where pH substances may be affected.

Nitrate pollution problems exist in numerous locations in the world where conditions are known to be suitable for anion adsorption. Excesses of boron are known in arid zone soils of both Israel and the USA and fluoride is of increasing concern due to fertilization with phosphates containing fluoride. Thus, it is of great importance to clarify the role of organic matter in adsorption-desorption of inorganic anions in soil.

Retention of anions has implications of humus chemistry. Research by one of the principal investigators (FJS) has shown that humic acids, as normally prepared, contain HCl (possibly in bound form), and that the residual acid leads to erroneous values for acidic functional groups by methods commonly used for their determination.

Whereas humic substances may retain anions under certain circumstances, as noted above, they can also be expected to exclude (repel) anions due to negative charges on the humic colloids. Donnan theory has been used successfully to examine ion interactions with silicate minerals but little application has been made of the theory to model cation-anion interactions with humic substances.

1.1 Evidence for Anion Sorption by Soil Organic Matter.

The possibility that the organic fraction of soil contributes to retention of anions by soils has been indicated in studies where sorption measurements have been carried out before and after destruction of organic matter by hydrogen peroxide oxidation or dry combustion. In some cases, sorption has been found to be positively correlated with organic matter content of the soil (Brown and Jenkinson, 1971). However, it should be noted that sorption of anions by soils containing high amounts of Fe- and Al-oxides can be reduced in the presence of organic matter, due undoubtedly to mutual blocking of reactive sites.

Espinoza et al. (1978) concluded that NO_3^- retention by the organic fraction of some Andept soils of Chile was the same, on a weight basis, as for the mineral fraction. Sorption was attributed to pH-dependent positive charges on the soil organic matter. Black and Waring (1979) concluded that NO_3^- sorption on an Australian Krasnozems soil was due partly to organic matter. Fluhler et al. (1982) found that a Rendoll soil containing 25.5% organic carbon adsorbed $1.2 \text{ meq F}^- \text{ g}^{-1}$ and that leaching of F^- was slow and late as compared to mineral soils. In other studies, Jee and De (1967) added varying amounts of humic acid to a series of alluvial, lateritic, and black cotton soils. Isotherms for I^- adsorption in the absence of humic acid generally showed negative adsorption. However, as increasing amounts of humic acid were added, increasing amount of I^- were adsorbed and varied from 1 to $32 \text{ } \mu\text{eq g}^{-1}$ of humic acid, depending on the amount of humic acid added to the soil.

1.2 Adsorption Mechanisms

Potential mechanisms for sorption of anions by humic substances include protonation of weakly basic functional groups, ion-dipole or dipole-dipole interactions (mainly H-bonding), ligand exchange, and retention within the fiber-like lattice of humic and fulvic acids. Protonation of weakly basic oxygen-containing and NHx groups of humic and fulvic would lead to associations such as $\text{R-COH}_2^+\text{Cl}^-$, $\text{R-C=OH}^+\text{Cl}^-$, and R-NH_3^+ .

Arnett (1963) has given an account of the acid-base properties of weak organic bases. In general, protonation of oxygen-containing functional groups requires extremely acidic environments. On this basis, the pH of soil, peat, and natural waters containing humic substances (including the soil solution) would not be sufficiently low for protonation of oxygen-containing functional groups; NHx groups would be slightly more susceptible. However, it is possible that the environment of functional groups in complex macromolecules, such as humic and fulvic acids, are conducive for protonation. It should also be noted that the effective H^+ concentration near colloidal surfaces may be several times greater than in the bulk solution and thereby protonation of oxygen-containing functional groups would occur, with alcoholic-OH being a likely candidate. An alternate explanation for Cl^- retention by humic acids has been given by Stevenson (1978), who concluded that sorption may result from the formation of carbonium ions, such as may result from acid catalyzed keto-enol transformations ($-\text{C}=\text{C}-\text{CHO} + \text{HCl} = (-\text{C}-\text{C}-\text{COH})^+\text{Cl}^-$).

Indirect evidence for retention of anions by humic substances has come from studies on the sorption of mineral acids by coals and activated

carbons. Edgcombe (1956) found that the total Cl^- content of 29 coals varied from 0.2 to 1.0%, a significant portion of which was held as the Cl^- ion, with the coal acting as an anion exchanger. Other studies indicating the presence of organically bound Cl^- in coals include those of Daybell and Pringle (1958) and Gluskoter and Ruck (1971). Potential sites for anion sorption have been postulated to include COOH , C=O , ether, and phenolic OH (Haggin, 1982). A similar array of functional groups is believed to be involved in anion sorption by activated carbons (Snoeyink and Weber, 1967). Wachowska et al. (1983) attributed retention of HCl and HF by coals to basic nitrogen present in pyridine-like structures.

The considerations mentioned above indicate that anions can be adsorbed by coals, activated charcoals, and possibly humic substances. Sorption is pH dependent and increases with decreasing pH. Sorption of HCl has implications for the extraction and purification of humic and fulvic acids, and for the determination of acidic functional groups by methods based on ion exchange, such as the calcium acetate method for COOH groups.

1.3 Anion Exclusion by Humic Substances

The acidic nature of humic substances has been known since the time of Berzelius, and therefore also the anionic character of humic substances when ionized, which is easily verified by the migration of humic substances toward the anode under the influence of an applied electric field (Flaig et al., 1975). Central to the theory of the double electric layer is the accumulation of counter ions and exclusion of coions from the vicinity of the charged surface. In the case of humic acids which develop a negative charge due to ionization of weakly acidic and very weakly acidic functional groups, the accumulation of protons at the surface of the humic macromolecule will affect the acid-base behavior in response to changes in ionic strength. By the same token, anions are expected to be repelled from the negatively charged humic surface. Unlike the acid-base behavior which is a function of the electric potential at the humic surface, and is therefore unmeasurably directly in the bulk solution, anion exclusion is the total anion deficit from the humic surface to an infinite distance, and is measurable in the bulk solution.

Anion exclusion phenomena have been extensively investigated for clays and soil clays with planar configuration, notably montmorillonite and illite (Edwards and Quirk, 1962; Bolt and de Haan, 1979). Anion exclusion has also been noted for the organic macromolecules; for example, chloride exclusion in humic acid solutions has been measured by Tschapek and Torres-Sanchez (1978) and by Sikora (1986): the data of Tschapek and Torres-Sanchez for a cola-derived humic acid were analyzed using calculations for a planar charged surface while the data of Sikora for three soil-derived humic acids were analyzed using a Donnan equilibrium model which did not reflect specifically the geometry or dimensions of humic substances. Calculations by Bentz (1982) show that the planar case may approximate the cylinder only with restrictions regarding cylinder radius, ionic strength, and surface potential. More specifically, the approximation is suitable when $\kappa a \geq 10$ or ≥ 1 and the dimensionless surface potential $\phi_s < 2$, where $\kappa = 3.29 \cdot 10^7 \sqrt{I}$; a is the cylinder radius, and I is ionic strength; for molecules of unknown radius and surface potential, it cannot be assumed a priori that the above conditions are met (i.e., $\kappa a \geq 1$ or 10).

The anion exclusion phenomenon is typically measured by adding a known amount of tracer ion to a known volume of dissolved or suspended sample. Measurement of the activity of the tracer ion in an equilibrium solution, usually separated from the colloidal sample by a dialysis membrane, permits calculation of the volume accessible to the tracer ion. The latter volume is subtracted from the total volume to yield the excluded volume.

Special attention to analytical techniques is required since the nature of the exclusion phenomenon is such that the data require measuring either very small differences at moderate concentrations or moderate differences at very low concentrations; Bolt and de Haan (1979) recommend precision of 0.1% for an extended concentration range. Exclusion of chloride is conveniently measured by either radioactive isotopes (Bolt and de Haan, 1979) or chloride specific electrodes (Edwards and Quirk, 1962; Tschapek and Torres-Sanchez, 1978; and Sikora, 1986). However, radioisotope techniques are not available for nitrate since the two natural isotopes of nitrogen, N^{14} and N^{15} , are both stable, other isotopes have half lives of 9.97 min or less. Rechnitz (1969) has shown that ion specific electrodes for a number of ions are capable of producing high quality data consistent with the best measurements of such diverse quantities as the solubility products of perchlorate salts, formation constants of fluoride complexes with H^+ and Fe^{3+} , and formation constants of calcium complexes of EDTA, citrate, malate, lactate, etc. The use of electrodes specific for metal ions immersed in solutions of humic substances during the course of titration is an accepted practice in studies of metal binding capacities and stability constants of metal-humic complexes (Stevenson, 1982). Ion specific electrodes incorporating an ion-exchange membrane specific for nitrate are commercially available and may be suitable for measurement of nitrate activity in solutions of humic substances, permitting the measurement of exclusion phenomena by titration. Techniques of ion chromatography, a variation of high pressure liquid chromatography employing an ion exchanger as the stationary phase, have been developed since 1975 (Small, 1983; Fritz, 1987). Ion chromatography may be found suitable for the task of measuring nitrate exclusion data in batch experiments with dialysis membranes where samples of equilibrium solution without substantial levels of humic substances can be obtained. Ion chromatography and ion specific electrodes will both be employed in this research aiming to measure interactions of anions with humic substances.

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3 . MEASUREMENTS OF ANION ADSORPTION AND EXCLUSION BY HUMIC SUBSTANCES

3.1 Three-Minute Analysis of Chloride, Nitrate, and Sulfate by Column Anion Chromatography

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ABSTRACT

Accepted techniques of single column ion chromatography of inorganic anions were extended to separation of chloride, nitrate, and sulfate using 15 mM phthalic acid as an eluent, permitting reduction of column length to 30 mm and thereby reducing analysis time to three minutes. Using a 50 μ L sample loop, detection limits were 0.05 mmol L^{-1} and coefficient of variation values ranged from 0.8 to 1.7% using peak height measurements and 0.8 to 8.1% using peak area measurements. This configuration is appropriate for routine analysis of soil water extracts and ground water.

INTRODUCTION

Ion chromatography is an analytical technique which essentially was developed in the mid-1970s and new configurations suitable for specific applications are still evolving rapidly. Recently, Nieto and Frankenberger (1985 a, b) have shown single column ion chromatography to be suitable for analysis of inorganic anions and cations in aqueous soil extracts, following earlier work by Tabatabai and Dick (1983) showing

the use of chemically suppressed ion chromatography for anion analysis of natural waters. Other branches of analytical chemistry have applied ion chromatography to more exotic solutions such as pickling brines, electroplating baths, and paper pulping liquor (Small, 1983). The great versatility of ion chromatography stems from the variety of equipment configurations made possible by varying the column length and composition; eluent composition, pH, concentration, and flow rate; and method of detection, which may be based either on conductivity, ultraviolet spectroscopy, refractive index, or amperometry. Separation time for inorganic anions on standard 150 or 250 mm columns is commonly on the order of 10 minutes; attempts by Nieto and Frankenberger (1985a) to speed analysis by using a 30 mm column with a standard eluent did not produce quantitative results due to poor separation of NO_3^- and SO_4^{2-} . While testing a configuration similar to that reported by Jupille (1985) for rapid single-ion analysis of SO_4^{2-} , we found an equipment configuration suitable for 3-minute analysis of Cl^- , NO_3^- , and SO_4^{2-} in soil extracts and other natural waters.

MATERIALS AND METHODS

The equipment configuration consisted of a Perkin-Elmer Series 10 Liquid Chromatograph pump, a 50 μL sample injection loop, a Wescan Ion-Guard anion cartridge (269-003, 30 x 4.6 mm internal dimensions, 40 x 6.3 mm external), a Wescan Ion-Guard holder (269-002), a Jasco Uvidec-100-V UV Spectrophotometer with a flow-through cell (10 mm

optical path), and a LDC/Milton Roy CI-10 Computing Integrator. The eluent used was 15 mM phthalic acid (uncorrected pH, 2.5) at a flow rate of 5 mL min⁻¹, producing a back pressure of 7 x 10⁵ kg m⁻² or less. Detection was based on UV absorbance at 300 nm.

Soil extracts were prepared from saturated pastes which were initially filtered with Whatman no. 40 paper on a Buchner funnel and refiltered immediately before injection with a Millipore membrane filter (0.45 µm). Standards were prepared from reagent grade sodium salts of Cl⁻, NO₃⁻, and SO₄²⁻; concentrations of the anions in the standards were adjusted to reflect typical concentrations and ratios commonly found in natural waters so as to minimize need for dilutions.

RESULTS AND DISCUSSION

The configuration described above uses indirect UV detection based on the difference between UV absorbance of the phthalate eluent and the inorganic anions. Compared to 4.5-mM phthalate at pH 4.0 used by Nieto and Frankenberger (1985a), the use of a phthalate eluent at pH 2.5 increases the retention of the inorganic anions and additionally forces the system peak to elute before Cl⁻ instead of after SO₄²⁻ (Jupille, 1985), while at the same time the higher concentration of eluent together with the shorter column length cause elution at shorter retention times. Within 3 min, Cl⁻, NO₃⁻, and SO₄²⁻ have been separated and eluted, and the column is fully conditioned for the next injection.

Typical chromatograms are shown in Fig. 1. Calibration plots for Cl^- , NO_3^- , and SO_4^{2-} are linear in the range tested (Fig. 2) and permit easy interpolation for calculating sample concentration based on either peak height or peak area. Regression coefficients for the calibration curves for Cl^- , NO_3^- , and SO_4^{2-} were 0.9991, 0.9992, and 0.9958, respectively, when peak heights were employed and 0.9997, 0.9939, 0.9867, respectively when peak areas were used ($n = 12$ throughout). Precision for the three anions, as determined by repetitive injection of a standard of intermediate concentration is between 0.02 to 0.04 $\text{mmol}_c \text{L}^{-1}$ when based on peak height, and 0.03 to 0.16 $\text{mmol}_c \text{L}^{-1}$ based on peak area (Table 1); the coefficients of variation range from 0.8 to 1.7% based on peak height and 0.7 to 8.1% based on peak area, which does not exceed the variation reported by Nieto and Frankenberger (1985a) for a full length column. These results suggest that either peak height or peak area can be successfully employed for anion concentration determinations by the proposed method. Detection limits were established by injection of dilute standards; for all three anions, a 50 μL sample containing 0.05 $\text{mmol}_c \text{L}^{-1}$ caused peak detection and initiated peak height and area measurement.

Concentration ranges are to a large extent a function of sample injection loop size, which thereby determines the amount of analyte injected; a 50 μL loop was used here, but we have employed loop sizes ranging from 6 μL for an extremely saline soil extract to 200 μL for rain water analysis. Routine measurement of electrical conductivity in order to estimate total anion concentration and thereby assure that the sample falls within the standards range is recommended. We have

Fig. 1: Chromatograms of: a) combined standard containing 8, 4, and 4 mmol_C L⁻¹ Cl⁻, NO₃⁻, and SO₄²⁻, respectively, b) a saturated extract of a clay loam soil (Mitzpeh Massuah, xerorthent, 4.41, 0.78, and 2.82 mmol_C L⁻¹ Cl⁻, NO₃⁻, and SO₄²⁻, respectively), and c) groundwater from the coastal aquifer of Israel (Rehovot, 1.99, 0.81, and 0.37 mmol_C L⁻¹ Cl⁻, NO₃⁻, and SO₄²⁻, respectively).

Fig. 2: Calibration plots for Cl⁻, NO₃⁻, and SO₄²⁻ as measured by indirect UV detection. Each point is the average of three injections; regression equations and correlation coefficients were calculated with all 12 injections.

Table 1: Precision of analysis of Cl^- , NO_3^- , and SO_4^{2-} based on 12 injections of 50 μL of combined standard.

Anion	Concentration Injected	Peak Height		Peak Area	
		Standard Deviation	Coefficient of Variation	Standard Deviation	Coefficient of Variation
	$\text{mmol}_c \text{ L}^{-1}$		%	$\text{mmol}_c \text{ L}^{-1}$	%
Cl^-	4.0	0.047	1.18	0.029	0.74
NO_3^-	2.0	0.016	0.81	0.162	8.08
SO_4^{2-}	2.0	0.034	1.70	0.116	5.82

performed hundreds of analyses over the course of up to three months on a single column. We attribute the relative longevity of our columns to the fact that at pH 2.5, the organic contaminants, including humic substances, are almost uncharged whereas at more conventional eluent pH values, i.e., pH 4.5, the organic components are near their pK values and are anionic and polyanionic, causing extensive, nearly irreversible adsorption. The use of a guard column as an ultra-high-speed separation column in the manner described above both reduces initial column cost and increases the output of the equipment and personnel.

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3.2 Determination of FeEDDHA in Soils and Fertilizer by Anion Exchange Chromatography

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ABSTRACT

The specific conditions for the quantitative analysis of FeEDDHA (ethylenediamine di-o-hydroxyphenylacetic acid) by high pressure liquid chromatography (HPLC) were studied and defined. Using a 3 cm anion exchange column and 5 mM H_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$ as an eluent, FeEDDHA was separated into the dl-racemic and meso isomers. With the same column and 3 mM H_2SO_4 + 50 mM Na_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$, FeEDDHA eluted as a single peak. Standard calibration curves for FeEDDHA were obtained with regression coefficients of 0.9999 at concentrations up to 0.25 mM. The coefficient of variation is 1.5%, or $1.8 \mu\text{mol L}^{-1}$ using a 50 μL sample; the detection limit was $1.2 \mu\text{mol L}^{-1}$. FeEDDHA was separated easily on anion exchange HPLC columns from other chelates such as CDTA, EDTA, and DTPA. The proposed method was used to determine FeEDDHA concentrations in water extracts of soils that were incubated with the compound. With variation of the eluent, separation of ferrated and unferrated EDDHA is possible.

INTRODUCTION

Abnormalities of Fe nutrition are common for many plants grown either in calcareous soils or in neutral or alkaline nutrient solution. Chelated Fe is often used as an Fe fertilizer. In most soil applications, ferric EDTA (ethylenediamine tetraacetic acid) is relatively ineffective, but phenolic analogs of EDTA have proven successful (Knoll, 1957). For example, Fe chelated as NaFe(ethylenediamine di- α -hydroxyphenylacetic acid) [NaFeEDDHA], also known as NaFe(N,N'-ethylene bis (α -hydroxyphenylglycine) is widely used as an effective Fe fertilizer for plants grown in calcareous soils (Hagstrom, 1984); this chelate contains 6.5% Fe. The most effective of the Fe chelates are marketed at prices 200 to 500 times those of N-P-K fertilizers (by weight) and when required, iron chelate may be second only to N in fertilizer input cost. Effective identification and quantification of the active ingredients is therefore desirable for both following reactions of the chelate with soil components and for evaluating fertilizer composition.

The quantitative analysis of FeEDDHA in fertilizers and soils by previously reported methods is difficult because neither of its two outstanding characteristics — Fe chelation and deep red color — are sufficiently unique for unambiguous interpretation. Because soil extracts often contain Fe complexed with soluble soil organic matter, total Fe concentration in soil solution cannot be used as an indicator for FeEDDHA without a degree of uncertainty. For example, for the saturation extracts of 68 California soils, the water-soluble Fe

contents were equivalent to between 0.2 and 12.8 mg FeEDDHA L⁻¹, without any FeEDDHA actually being present in the extracts (Bradford et al., 1971). The deep red color of the FeEDDHA due to Fe-phenol interaction, which might be used as the basis for spectrophotometric determination of FeEDDHA, overlaps the broad absorption spectra of soluble soil organic matter in soil extracts and poses an analytical problem. Both of these analytical difficulties were addressed by Johnson and Young (1973) in their investigations of EDDHA as an extracting agent to assess Fe status of soils. Batra and Maier (1964) separated soluble soil organic matter from FeEDDHA with saturated ammonium sulfate and isoamyl alcohol, followed by spectrophotometric determination of FeEDDHA; in practice, this method is tedious and noxious.

EDDHA is found in two isomeric forms. Using paper chromatography with a 4:1:5 butanol:acetic acid:water eluent, Hill-Cottingham (1962) separated NaFeEDDHA into two bands, red ($R_f = 0.44$) and violet ($R_f = 0.65$), which were identified as the meso and dl-racemic isomers of NaFeEDDHA, although assignation of isomer to band was unsuccessful (Ryskiewich and Boka, 1962). Crystallographic analysis of a number of salts of FeEDDHA has shown that the meso isomer has the cis-configuration for both the phenolic and carboxylic groups whereas the dl-racemic isomer has trans carboxylic groups and phenolic O roughly coplanar with the N atoms (Bailey et al., 1981). Bailey et al. (1981) found that most salts of FeEDDHA form regular crystal structures containing one-half meso and one-half dl-racemic isomers (Fig.1), whereas crystals of the Mg salt may be crystallized so as to contain only the dl-racemic form. Plant uptake of radioiron chelated with

each of the isomers would seem to indicate no significant difference in plant response to the two isomers (Ryskiewich and Boka, 1962).

Some chromatographic separations have been reported for other chelating agents. Floor (1984) has reported separation on a 10-cm anion exchange column of unferrated tetraacetic acids, including EDTA, in 10 min. Preliminary experiments with FeEDDHA showed that this complex was strongly retained and did not elute within 120 min under the conditions reported.

This research was undertaken to optimize conditions for the specific quantitative determination of FeEDDHA by high pressure liquid chromatography (HPLC). The procedure developed was further applied to quantitative determinations of the isomers of FeEDDHA, of FeEDDHA in chelate mixtures, and to recovery tests on soils that were incubated with FeEDDHA.

MATERIALS AND METHODS

Primary standards of FeEDDHA were prepared by dissolution of EDDHA acid (Sigma Chem. Co., 90% declared purity) in an equivalent amount of NaOH, followed by addition of excess ferric chloride to form the FeEDDHA complex at concentrations up to 0.25 mmol L^{-1} .

Solutions were injected into an anion exchange chromatography system consisting of a 50- μL sample loop, a Wescan Ion-Guard anion cartridge (no. 269-003, 30- by 4.6-mm internal dimensions, 40- by 6.3-mm external) used as a high-speed separation column, a Jasco Uvidec-100-V UV spectrophotometer, and an LDC/Milton Roy CI-10B integrator.

Commercial-grade NaFeEDDHA from Ciba-Geigy Corp. was separated into meso and dl-racemic isomers using paper chromatography with a 4:1:5 butanol:acetic acid:water eluent (Hill-Cottingham, 1962). The two compounds were dried and redissolved in distilled water; spectra of the solutions of the purified isomers were measured in the UV and visible range with a Hewlett-Packard 8415A diode array spectrophotometer. A pure preparation of the dl-racemic isomer was prepared by crystallization of $\text{Mg}(\text{FeEDDHA})_2 \cdot 9\text{H}_2\text{O}$ prepared from H_4EDDHA (Sigma Chem. Co.) following the procedure of Bailey et al. (1981). The crystals were dissolved in distilled water before injection.

Four soils of Israel representing a variety of soil types (Table 1) were incubated at field capacity under aerobic conditions with 2-mg Fe as FeEDDHA g^{-1} dry soil for up to 7 d. Immediately upon contact and then at intervals for 7 d, residual FeEDDHA was extracted with water (1:1) by shaking for 30 min. Extracts were filtered through 0.45- μm Millipore filters and injected for chromatographic analysis of FeEDDHA using 3 mM H_2SO_4 + 50 mM Na_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$ as the eluent.

Iron complexes of EDTA, DTPA (diethylenetriaminepentaacetic acid), CDTA (trans-1,2-diaminocyclohexane-N,N',N'-tetraacetic acid) and a number of commercial Fe chelates were also injected for comparison with FeEDDHA.

Table 1. Selected properties of soils used in this study.

Location	Soil type	Texture	CaCO ₃	Organic matter	CEC	pH
			-- g kg ⁻¹ --	--	cmol _c kg ⁻¹	
Kfar Rupin	Calciorthid	Heavy clay	582	31	16.7	7.8
Har Raihan	Rhodoxeralf	Heavy clay	7	25	33.4	7.3
Kisalon	Haploxeroll	Heavy clay	28	107	50.5	8.1
Mitzpeh Massua	Xerorthent	Light clay	630	17	18.0	8.0

RESULTS AND DISCUSSION

The two FeEDDHA isomers were well separated by paper chromatography into a red band and a violet band, as reported by Hill-Cottingham (1962). The FeEDDHA isomers had very similar absorbance spectra in the UV and visible range (Fig. 2), both exhibiting peaks at 206 and 280 nm, the first due to the benzene ring and the second due to ortho-substitution of the benzene ring (Jaffe and Orchin, 1962). Both isomers showed a broad band of absorption in the visible range typical of Fe-phenol complexes, peaking at about 480 nm; the difference in color between the two isomers is due to a difference of about 10 nm in the placement of the peak. For spectrophotometric detection, any of the three peaks (206, 280, and 480 nm) would be acceptable, but the 206-nm wavelength was used for detection because it has the strongest absorbance.

Under conditions typical for single column anion chromatography of inorganic anions, such as 4 mM potassium hydrogen phthalate at pH 4.5 and conventional 25-cm long column, the FeEDDHA anion does not elute within 120 min. Reduction of retention time may be achieved by either increasing the eluent concentration, use of a more strongly displacing eluent anion such as sulfate, reduction of the anionic nature of FeEDDHA by decreasing the eluent pH, or a combination of a concentrated, strongly displacing anion and low pH, as proposed in this method. In contrast, reduction of pH reduces the anionic nature of the weak acid eluent such as phthalate and increases elution time of the inorganic anions. A small concentration of Fe(III) may be desirable in the eluent

Fig. 1. Schematic diagrams of FeEDDHA: A, meso isomer, $R_f = 0.65$ (paper chromatography); and B, dl-racemic isomer, $R_f = 0.44$.

Fig. 2. Ultraviolet and visible spectra of red and violet isomers of FeEDDHA at a concentration of 0.022 mM.

to prevent deferration of FeEDDHA while adsorbed on the column. The number of plates of the 30-mm column used here was estimated at 115 based on the retention time and peak width of FeEDDHA relative to that of the solvent (Saunders, 1977).

The two FeEDDHA isomers were well separated using 5 mM H_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$, eluting at 4 and 6 min at a flow rate of 3 cm min^{-1} . The dl-racemic isomer prepared from $\text{Mg}(\text{FeEDDHA})_2 \cdot 9\text{H}_2\text{O}$ was found upon injection to be almost completely free of contamination with the meso isomer and had a retention time identical to that of the red band, thus making possible the identification of the dl-racemic isomer as the red ($R_f = 0.44$) band and therefore the meso isomer as the violet ($R_f = 0.65$) band (Fig. 3).

For chromatography of FeEDDHA without isomeric separation, 3 mM H_2SO_4 + 50 mM Na_2SO_4 + 0.01 mM $\text{Fe}_2(\text{SO}_4)_3$ was found to be a suitable eluent, with a retention time of 1.6 min for a flow rate of 3 cm min^{-1} . With a 50- μL sample loop, calibration curves for FeEDDHA were linear for concentrations up to 0.25 mM, or 12.5 nmol/sample ($r^2 = 0.9999$ for peak height and 0.9990 for peak area), and facilitated easy quantification of FeEDDHA in samples of unknown content (Fig. 4). The detection limit for a 50- μL sample, as determined by automatic peak detection by the integrator, was 0.0012 mmol FeEDDHA L^{-1} , or 0.06 nmol/sample; the sample loop size can easily be increased to 500 μL to further increase sensitivity. Based on 10 injections of a 0.12-mmol L^{-1} standard, the coefficient of variation is 1.5%, equivalent to 0.018 mmol L^{-1} .

With regard to other defined iron chelates, the conditions for chromatographic separation presented here provides clear resolution

against EDDHA since retention times for NaFeCDTA, NaFeEDTA, and NaHFeDTPA were 17, 22, and 74% of that of FeEDDHA, respectively, indicating good separation of FeEDDHA from polycarboxylic ligands. A number of commercial iron chelates of the phenolic type but of unknown composition produced well-separated peaks with retention times greater than that of FeEDDHA, but no further investigation of these phenolic iron chelates was undertaken at this time.

FeEDDHA recovered after incubation in soils by water extraction, and determined by the chromatographic techniques reported here, declined rapidly in two of the soils immediately upon contact, followed by a further gradual decline (Fig. 5). Two other soils showed only a gradual decline in extractable FeEDDHA over the course of 7 d. This pattern was similar to that found by Fillett and Lindsay (1971) using Fe determination in a DTPA extract and by Hill-Cottingham and Lloyd-Jones (1958) using colorimetric titration of a water extract.

Another example of a possible use of this chromatographic technique is the analysis of soil extracts using H_4EDDHA as a measure of soil iron availability. The filtered extracts were separated using 3 mM H_2SO_4 + 50 mM Na_2SO_4 [without addition of $Fe_2(SO_4)_3$]. In the absence of Fe to ferrate EDDHA, the protonated and uncharged ligand passes down the length of the column with the solvent, permitting the distinction between FeEDDHA and EDDHA, and therefore the use of EDDHA as an extractant for the evaluation of soil iron status (Johnson and Young, 1973).

All separations reported above were performed on an anion exchange column 3 cm in length in order to optimize speed of analysis; this

Fig. 3. Chromatograms of (A) original NaFeEDDHA solution, (B) violet and (C) red isomers separated from A by paper chromatography, and (D) solution of $\text{Mg}[\text{Fe}(\text{rac-EDDHA})]_2$.

Fig. 4. Calibration curves for FeEDDHA isomers based on peak height of absorption at 206 nm. (ABU = absorbance unit).

Fig. 5. Recovery of FeEDDHA applied to soil as measured by chromatography.

length was satisfactory because of the strong binding of FeEDDHA with the exchanger, which necessitated an extremely strong eluent. However, if separation is not acceptable, the column length may be easily extended to 6 cm by adding an identical 3-cm column to the flow path or, in extreme cases, using the 10- or 25-cm columns of identical composition available from the same manufacturer. Longer column length necessarily increases analysis time and is not indicated unless specific separation difficulties arise.

CONCLUSIONS

A fast and quantitative method for the determination of FeEDDHA in fertilizers and soils was developed. This method can also be used to differentiate ferrated and unferrated EDDHA, thereby facilitating research on the usefulness of EDDHA as an alternative to DTPA as an indicator of iron availability to plants.

ACKNOWLEDGEMENT

The assistance of Dr. Leon Margolis in assignation of spectral data to chemical structure is gratefully acknowledged.

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3.3 INTERFERENCE OF HUMIC SUBSTANCES ON CHLORIDE ANALYSIS USING THE CHLORIDE ION-SELECTIVE ELECTRODE¹

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ABSTRACT

Humic substances were found to interfere with Cl^- determination using the Cl^- ion-selective electrode by complexing Ag^+ , thereby reducing the activity of the potential determining ion. Proof of Ag^+ binding was obtained through a separate technique that involved equilibrium dialysis and Ag^+ determination by atomic absorption spectroscopy. Competition between Ag^+ and H^+ for binding sites was evident from an increase in mV response of the Cl^- ion-selective electrode with addition of HNO_3 and release of protons upon Ag^+ binding in the dialysis experiment. Selectivity coefficients ($k_{\text{Cl},\text{HA}}$) for 11 humic and fulvic acids ranged from 0.59×10^{-4} to 1.3×10^{-4} ml mg^{-1} at pH 6.5. Interference would be negligible in solutions containing less than 0.1 mg ml^{-1} humic substances but would be serious for samples containing low concentrations of Cl^- ($<10^{-4.5}$ M) and high amounts of organic matter (>0.1 mg ml^{-1}).

¹Contribution from the Dep. of Agronomy, Univ. of Illinois, Urbana, IL. 61801. Supported in part by a grant from the United States-Israel Agricultural Research and Development Fund (BARD 1-500-82).

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INTRODUCTION

The chloride content of soil extracts, drainage waters (including those from peat and forest soils), and effluents from feedlots, landfills, and compost heaps is attracting considerable attention. Many models for predicting the speciation of metal ions in the soil solution require accurate values for anionic composition, including Cl^- . In this paper, consideration is given to the possible interference of humic substances in the determination of Cl^- using the Cl^- ion-selective electrode (ISE).

The Cl^- ISE offers a simple technique for the determination of Cl^- in environmental samples (Adriano and Doner, 1982; Lamm et al., 1972; Van Loon, 1968). Determination of Cl^- in soil extracts and water samples using the Cl^- ISE produced results that agreed well with a more time consuming colorimetric method using $\text{Hg}(\text{SCN})_2$ and Fe^{+3} (Selmer-Olsen and Oien, 1973).

Accurate Cl^- analysis with an ISE can be difficult in the presence of inorganic ions (OH^- , Br^- , I^- , S^{-2} , CN^- , NH_4^+ , and $\text{S}_2\text{O}_3^{-2}$) that form complexes with the potential determining Ag^+ ion (Hulanicki and Trojanowicz, 1979; Orion, 1983; Van Loon, 1968). Little information is available on interference by organic molecules that bind Ag^+ . Higher values have been recorded for Cl^- in plant tissues and soil extracts using the Cl^- ISE method, as compared to a potentiometric titration method (Hipp and Langdale, 1971; La Croix et al., 1970). These higher values may have been due, at least in part, to complexing Ag^+ by organic molecules.

Objectives of the present study were: i) to determine if humic substances have the capacity to interfere with Cl^- determination through Ag^+ complexation, and ii) to quantify the interference.

THEORETICAL CONSIDERATIONS

The Cl^- ISE contains a solid state AgCl membrane in which Ag^+ is the potential determining ion (Koryta and Stulik, 1983). The appropriate Nernstian equation for Ag^+ analysis is

$$E = E''_{\text{Ag}} + \frac{RT}{F} \ln a_{\text{Ag}} \quad [1]$$

where a_{Ag} is the Ag^+ activity and E''_{Ag} is the intercept for the linear portion of the Ag^+ standard curve. In terms of the activity of Cl^- (a_{Cl}), Eq. [1] can be written as

$$E = E''_{\text{Cl}} - RT/F \ln a_{\text{Cl}}. \quad [2]$$

The intercept for the linear portion of the Cl^- standard curve (E''_{Cl}) is equivalent to ($E''_{\text{Ag}} + RT/F \ln K_{\text{sp}}$), where K_{sp} is the solubility product of AgCl .

Equations [1] and [2] only apply when the activity of Ag^+ (or Cl^-) in the experimental solution is much greater than the activity of the ions produced from dissolution of the AgCl membrane. Functions for electrode mV response for all activity levels of Ag^+ and Cl^- can be shown to be (Kortya and Stulik, 1983; Morf et al., 1974):

$$E = E''_{\text{Ag}} + RT/F \ln (a_{\text{Ag}} + (a_{\text{Ag}}^2 + 4K_{\text{sp}}^{1/2})^{1/2})/2 \quad [3]$$

and

$$E = E''_{\text{Cl}} - RT/F \ln (a_{\text{Cl}} + (a_{\text{Cl}}^2 + 4K_{\text{sp}})^{1/2})/2. \quad [4]$$

At high Ag^+ and Cl^- activities, Eqs. [3] and [4] become the linear forms shown by Eqs. [1] and [2]. At low Ag^+ and Cl^- activities, dissolution of AgCl controls the electrode response and the lower limits of detection become $K_{\text{sp}}^{1/2} = 10^{-4.88}$ M (Morf et al., 1980). Equations [3] and [4] indicate that standard curves of Ag^+ and Cl^- should be symmetrical.

The general Nernstian equation for Cl^- in the presence of an interfering ion is (Kortya and Stulik, 1983)

$$E = E''_{\text{Cl}} - RT/F \ln a_{\text{Cl}} + k_{\text{Cl},\text{B}} (a_{\text{B}})^{-1/z_{\text{B}}} \quad [5]$$

where the subscripts Cl and B refer to the analyte and interferent, respectively, z_{B} is the valence of the interferent, and $k_{\text{Cl},\text{B}}$ is the selectivity coefficient for interferent B. As with Eqs. [1] and [2],

Eq. [5] is only valid at moderate to high activities of Cl^- . A more general equation for the effect of an interferent at all activity levels of Cl^- is

$$E = E''_{\text{Cl}} - RT/F \ln \zeta + k_{\text{Cl},\text{B}}(a_{\text{B}})^{-1/z_{\text{B}}} \quad [6]$$

where ζ is equivalent to the logarithmic term in parenthesis in Eq. [4]. Equation [6] indicates that $k_{\text{Cl},\text{B}}$ establishes the lower limit of Cl^- detection in the presence of an interfering ion, whereas $K_{\text{sp}}^{1/2}$ sets the limit of Cl^- detection in the absence of an interfering ion. In the presence of an interferent ion with a low activity, both $k_{\text{Cl},\text{B}}$ and $K_{\text{sp}}^{1/2}$ can establish the lower limit of detection.

The IUPAC Commission for Analytical Nomenclature has suggested two methods for determining $k_{\text{Cl},\text{B}}$ (Guilbault, 1979), which are the separate solution method and the fixed interference method. The latter approach was used in the present study. In this method, interferent activity is kept constant while analyte concentration is increased. The selectivity coefficient is calculated from

$$k_{\text{Cl},\text{B}} = a_{\text{Cl}} / (a_{\text{B}})^{-1/z_{\text{B}}} \quad [7]$$

where a_{Cl} is obtained from the intersection of the linear function of the Cl^- standard curve with the asymptote at the lower limit of detection in the presence of the interferent.

Equation [7] can only be used when the selectivity coefficient or activity of the interferent is sufficiently high to offset the effect of $K_{\text{sp}}^{1/2}$ in determining the limiting mV response, that is, when $k_{\text{Cl},\text{B}}(a_{\text{B}})^{-1/z_{\text{B}}} \gg K_{\text{sp}}^{1/2}$. Equation [6] can be modified to represent the asymptote for the limit of analyte detection in the presence of interferent for the case when $a_{\text{Cl}} \ll K_{\text{sp}}^{1/2}$:

$$E_1 = E''_{\text{Cl}} - RT/F \ln K_{\text{sp}}^{1/2} + k_{\text{Cl},\text{B}}(a_{\text{B}})^{-1/z_{\text{B}}} \quad [8]$$

The linear function of the standard curve for the activity of Cl^- in the absence of an interferent is given by Eq. [2]. At the intersection of

the limiting asymptote and the linear function, E from Eq. [2] equals E_1 yielding

$$\ln K_{sp}^{1/2} + k_{Cl,B}(a_B)^{-1/z_B} = \ln a_{Cl} \quad [9]$$

or

$$k_{Cl,B} = (a_{Cl} - K_{sp}^{1/2}) / (a_B)^{-1/z_B}. \quad [10]$$

Equation [10] is a general equation that can be used when $k_{Cl,B}(a_B)^{-1/z_B}$ approximates $K_{sp}^{1/2}$.

A selectivity coefficient cannot be determined from Eq. [10] for substances that contain detectable background levels of the analyte ion (Cl^-) since a limiting mV response cannot be easily obtained from the experimental data. For samples containing residual Cl^- , an iterative procedure can be used in which estimated values for $k_{Cl,B}$ are incorporated into Eq. [6] until the calculated response of E vs a_{Cl} fits the experimental data. The value of $k_{Cl,B}$ used to obtain the plot of best fit can be taken as the selectivity coefficient for the interferent, excluding the effect of background Cl^- .

EXPERIMENTAL METHODS

The Cl^- ISE and double junction reference electrode used were Orion models 94-17B and 90-02, respectively. The inner chamber of the reference electrode was filled with Orion inner chamber solution (No. 90-0002) and the outer chamber was filled with 1 M KNO_3 . A standard glass electrode was used with the double junction reference electrode for simultaneous measurements of pH and Ag^+ or Cl^- . A Fisher Accumet model 825 MP pH meter was used in conjunction with a Fisher electrode switch model 753 for all ion and pH measurements. The mV response for the Cl^- ISE was offset -70 mV from the absolute value. A strip chart recorder was used to insure that equilibrium was established before measurements were recorded. Equilibration time was generally between 2 and 5 minutes. The temperature of the analyte solution was $25.0 \pm 1.0^\circ C$.

The humic and fulvic acids were taken from a collection in the soil chemistry laboratory at the University of Illinois. Their source and preparation are summarized in Table 1. Humic and fulvic acid solutions

Table 1. Source, extractant used, and preparation of humic substances.

Sample	Source	Extractant	Preparation
Humic Acids			
HA1	A horizon Illinois Sable silt loam (Typic Haplaquoll)	0.15 M $\text{Na}_4\text{P}_2\text{O}_7$	Shaken repeatedly with 0.3 M HF: 0.1 M HCl, dialyzed, and freeze-dried
HA2	Same as HA1	Same as HA1	Same as HA1 plus hydrolyzed with 3M HCl for 12 hours.
HA3	Surface Layer Ohio Rifle Peat (Typic Borochemist)	Same as HA1	Same as HA1
HA4	Same as HA3	Same as HA1	Same as HA1 plus hydrolyzed with 0.1 M HCl for 8 hours.
HA5	A horizon of Michigan Blue Lake Sand (Alfic Haplorthod)	Same as HA1	Same as HA1
HA6	B horizon of Michigan Blue Lake Sand (Alfic Haplorthod)	Same as HA1	Same as HA1
HA7	North Dakota Lignite (Leonardite)	0.5 M NaOH	Same as HA1
HA8	A horizon Harpster Silt Loam (Calcic Argaquoll)	0.15 M $\text{Na}_4\text{P}_2\text{O}_7$ and subsequent extraction with 0.5 M NaOH	Same as HA1
HA9	A horizon Illinois Drummer Silt Loam (Typic Haploquoll)	0.15 M NaOH	Same as HA1
Fulvic Acids			
FA1	Same as HA3	Same as HA1	Recovered by sorption/desorption from activated charcoal (Stevenson and Goh, 1971).
FA2	Humic Spodosol on the west coast of Newfoundland (marketed by CONTECH E.T.C., Ltd., Ottawa, Canada)	Unknown	Unknown
FA3	A horizon Illinois Drummer Silt Loam (Typic Haploquoll)	Protolytic solvent (95:4:1 acetone: H_2O : conc. HCl)	Extract dialyzed and soluble fraction freeze-dried
FA4	Same as FA3	Same as FA3	Insoluble precipitate of FA3 recovered by centrifugation

were prepared by dissolving the freeze-dried material in 0.1 M KOH and adjusting the pH to approximately 10. The humic substance solution was stirred under N_2 gas for one hour. After complete dissolution, the pH was adjusted to 6.0 with HNO_3 and appropriate dilution was made to obtain the concentration of interest. All experimental solutions were maintained at a constant ionic strength (I) of 0.1 M with respect of KNO_3 . The pH of each experimental solution was adjusted by dropwise additions of 0.1 M KOH or 0.1 M HNO_3 . Activities of Ag^+ and Cl^- were determined from concentration values using an activity coefficient of 0.782 from the Davies' equation (Lindsay, 1980).

Standard curves for Ag^+ and Cl^- were constructed by titrating 10^{-4} M, 10^{-3} M, and 10^{-2} M $AgNO_3$ or KCl into a 50 ml solution of 0.1 M KNO_3 with subsequent mV measurements taken with the Cl^- ISE. Ionic strength (I) was maintained at 0.1 M with respect to KNO_3 by making up the titrant solutions of Ag^+ and Cl^- in 0.1 M KNO_3 .

Preliminary studies indicated that concentrations of humic acid between 1 and 5 $mg\ ml^{-1}$ were required to obtain a Nernstian response with a plot of mV vs. log humic acid concentration. Because of the high concentrations, the effect of humic acid on mV response was determined by diluting 20 ml solutions containing 5 $mg\ ml^{-1}$ HA1, HA3, or HA7 with 0.1 M KNO_3 . Titrations were performed at pH 3.5 and 6.5.

For the analysis of Ag^+ binding through equilibrium dialysis, Spectrapor dialysis tubing with a 3500 molecular weight cutoff (MWCO) was soaked in 4 M HNO_3 overnight at room temperature and rinsed with copious amounts of deionized water the next morning. Because Ag^+ is readily complexed by sulfide (Lindsay, 1980), and since sulfide is a normal constituent of the dialysis tubing (Spectrapor, 1984), the acid pretreatment was necessary to oxidize and remove sulfide. A second wash with 0.1 M KOH was performed to neutralize residual acidity in the tubing. The humic acid was placed in the interior of the analysis tube at a concentration of 0.5 $mg\ ml^{-1}$, pH of 6.95, and volume of 20 ml. The sample was then placed in a 2 x 20 cm screw-top glass tube containing 20 ml of a solution consisting of $AgNO_3$ at concentrations ranging from 6×10^{-4} M to 4×10^{-3} M. The dialysis cell was rotated on a platelet rotary shaker (Fisher Model No. 438) at 3 rotations min^{-1} . After a pre-

determined equilibrium time of five hours, Ag^+ and H^+ were determined in the interior and exterior solutions by atomic absorption spectroscopy and the pH glass electrode, respectively. Bound Ag^+ was determined as the difference between Ag^+ in the interior and exterior solution. Background Cl^- was negligible since levels in the HAs used (HA1, HA3, and HA7) were less than 0.02 mmol g^{-1} (see below).

Background levels of Cl^- in the humic substances were also determined by equilibrium dialysis, using the procedure outlined for Ag^+ binding. The interior solution consisted of 15 ml of humic substance at a concentration of 1 mg ml^{-1} and pH of 7. Fifteen ml of 0.1 M KNO_3 was placed in the exterior solution. The I of the interior and exterior solution was maintained at 0.1 to eliminate unequal distributions of Cl^- due to Donnan effects. After rotation on the platelet rotary shaker for 2 hours the exterior solution was removed and analyzed for Cl^- using the Cl^- ISE. The pH was lowered to 4.0 by addition of one drop of 1 M HNO_3 to subdue any interference that may have occurred due to leakage of humic substances into the exterior solution. Each sample was analyzed in triplicate.

Selectivity coefficients were determined using principles of the fixed interferent method. Chloride was titrated into 50 ml of 0.5 mg ml^{-1} humic substance solution with 10^{-4} M , 10^{-3} M , 10^{-2} M , $10^{-1.7} \text{ M}$, and 10^{-1} M KCl . The titrant volume was kept low (total volume $< 6 \text{ ml}$) to minimize dilution of the sample. The pH was maintained at 6.5.

RESULTS AND DISCUSSION

Standard curves for Ag^+ and Cl^- from the Cl^- ISE are presented in Fig. 1. The lower linear limits were determined by a procedure outlined by Liteanu et al. (1976), in which a function is obtained for the curvilinear section of the standard curve by linearizing the data. The intersection of the curvilinear and linear functions is the limit of linearity. The curvilinear and linear equations for Ag^+ and Cl^- , represented by the broken lines in Fig. 1, are shown below:

$$\begin{aligned} \text{Ag}^+: \quad & \text{mV} = 192 + 20100 \exp(1.49 \log a_{\text{Ag}}), \quad a_{\text{Ag}} < 10^{-4.66} \\ & \text{mV} = 60 \log a_{\text{Ag}} + 491, \quad a_{\text{Ag}} > 10^{-4.66} \\ \text{Cl}^-: \quad & \text{mV} = 195 - 13800 \exp(1.41 \log a_{\text{Cl}}), \quad a_{\text{Cl}} < 10^{-4.34} \\ & \text{mV} = -57.0 \log a_{\text{Cl}} - 82.7, \quad a_{\text{Cl}} > 10^{-4.34} \end{aligned}$$

Theoretical responses in Fig. 1 (solid line) were calculated from Eqs. [3] and [4], using values of 490 and -87.1 mV for E''_{Ag} and E''_{Cl} , respectively.

Results obtained with our Cl^- ISE were very similar to those obtained by Morf et al. (1974). The limiting potentials for the experimental response curves tend to fall below the theoretical values at low Ag^+ and Cl^- activity, indicating that the equilibrium activity of Ag^+ was less than that predicted from the solubility product of $AgCl$. The lower linear limit for Ag^+ , as compared to Cl^- , was accounted for by the discrepancy between theory and experiment. If the experimental values were exactly symmetrical, the linear limit would be the same for both Ag^+ and Cl^- .

Typical response curves for Ag^+ and Cl^- in the presence and absence of humic acid are shown in Fig. 2 for HA7. The curve for Cl^- in the presence of humic acid is typical of an interferent effect (Freiser, 1978; Kortya and Stulik, 1983); that for Ag^+ is indicative of Ag^+ binding. Fitch et al. (1986) observed a similar electrode response upon the binding of Cu^{+2} using the Cu^{+2} ISE.

Electrode potentials as a function of humic acid concentration are shown in Fig. 3 for HA1, HA3, and HA7. An increase in humic acid concentration resulted in a decrease in mV response. This result is to be expected if Ag^+ was being complexed by the humic acid, thereby reducing the free activity of the potential determining Ag^+ ion. Further proof of Ag^+ binding was indicated by the effect of pH on the mV response. At pH 3.5, H^+ effectively competed with Ag^+ for binding sites, resulting in free Ag^+ activities and mV responses greater than that at pH 6.5.

Equilibrium dialysis confirmed Ag^+ binding by humic acid (Table 2). For the three humic acids, competition between Ag^+ and H^+ for binding sites was evident from a decrease in pH with an increase in Ag^+ concentration of the exterior equilibrium solution. Because the pH was not controlled, no attempt was made to model the binding data.

Results presented thus far indicate that the decrease in mV response of the Cl^- ISE in the presence of humic acid was most likely due to Ag^+

Figure 1. Calculated (solid line) and experimental (broken line) standard curves for Ag^+ and Cl^- using the Cl^- ISE at $I=0.1$. The solid line was calculated from Eqs. [3] and [4] using E''_{Ag} and E''_{Cl} values of 490 and -87.1 mV, respectively. Experimental data represent an average of 7 replications.

Figure 2. Electrode response curves for Ag^+ and Cl^- in the absence and presence of 0.5 mg ml^{-1} HA7 at pH 6.5.

Figure 3. Response of the Cl^- ISE at pH 3.5 and 6.5 in the presence of increasing concentration of HA1, HA3, and HA7.

Figure 4. Response of the Cl^- ISE in the presence of humic and fulvic acids (0.5 mg ml^{-1}). The solid line represents the Cl^- standard curve in the absence of interferent. The broken line is the calculated response using the determined value of $k_{\text{Cl},\text{HA}}$ from Table 4 in Eq. [11].

Table 2. Binding of Ag^+ by humic substances as determined by equilibrium dialysis at an initial pH of 6.95 and ionic strength of 0.1 M with respect to KNO_3 .

Humic acid	Equilibrium conc. of Ag^+ in exterior solution	Adsorbed Ag^+	Interior pH	Exterior pH
	-----M x 10^{-4} -----	-----mmol g $^{-1}$ -----		
HA1	1.29	0.56	6.27	6.55
	1.94	0.71	6.21	6.44
	2.51	0.84	6.09	6.41
	5.98	1.38	5.91	6.24
	10.20	1.66	5.70	6.07
	14.40	1.78	5.57	5.81
HA3	1.47	0.49	6.26	6.38
	2.28	0.55	6.22	6.32
	2.82	0.65	6.08	6.30
	6.90	1.02	5.96	6.14
	11.70	1.08	5.90	6.18
	16.00	1.20	5.87	6.05
HA7	1.76	0.36	6.43	6.51
	2.69	0.44	6.39	6.50
	3.29	0.61	6.35	6.44
	7.23	0.95	6.21	6.34
	12.00	1.02	6.08	6.20
	16.20	1.06	6.07	6.22

complexation. Any background Cl^- in the humic preparations would also be expected to decrease the mV response. Therefore, selectivity coefficients for the interference of humic substances on Cl^- analysis cannot be obtained without a knowledge of background Cl^- levels.

Background Cl^- concentrations, as determined by equilibrium dialysis, are shown in Table 3. Chloride concentrations in HA1, HA3, HA4, HA6, and HA7 were below the detection limit of 0.02 mmol g^{-1} . High amounts of Cl^- were found in HA9 and FA4 (0.293 and $0.627 \text{ mmol g}^{-1}$). The Cl^- contents of the fulvic acids were generally greater than for the humic acids.

Selectivity coefficients for the interference of humic substances on Cl^- analysis are presented in Table 4. Since the molecular weight of humic substances is unknown, Eq. [6] was modified to yield

$$E = E'_{\text{Cl}} - \frac{RT}{F} \ln (a_{\text{Cl}} + (a_{\text{Cl}}^2 + 4K_{\text{sp}})^{1/2}/2) + k_{\text{Cl,HA}} g_{\text{HA}} \quad [11]$$

where g_{HA} is the concentration of humic substance in units of mg ml^{-1} . The selectivity coefficient ($k_{\text{Cl,HA}}$) is therefore given in units of ml mg^{-1} . Since some humic materials contained Cl^- , a_{Cl} in Eq. [11] refers to added Cl^- plus Cl^- derived from the humic preparations. Electrode responses (mV vs $\log a_{\text{Cl}}$) in the presence of 0.5 mg ml^{-1} HA1, HA3, HA5, and FA1 are shown in Fig. 4. The intercept and slope values for the calculated standard curves (solid line) were determined at high Cl^- additions where Eq. [11] becomes the linear form shown by Eq. [2]. Similar responses were found for the other humic substances listed in Table 4.

Equation [10] could not be used to determine selectivity coefficients for humic substances containing Cl^- since a limiting mV response was unattainable without extrapolation (e.g., HA5 and FA1 in Fig. 4). However, the selectivity coefficient could be determined by estimating values for $k_{\text{Cl,HA}}$ until the calculated value for E as a fraction of (a_{Cl}) in Eq. [11] fit the experimental data. A computer program was developed to determine a value for $k_{\text{Cl,HA}}$ that would produce the greatest coefficient of determination (r^2) for the experimental data

Table 3. Background levels of Cl^- in humic and fulvic acids as determined by equilibrium dialysis.

Humic acid	Cl^- content	Fulvic acid	Cl^- content
	----mmol g^{-1} ----		---mmol g^{-1} ---
HA1	nd [†]	FA1	0.069 ± 0.004
HA2	0.020 ± 0.008	FA2	0.049 ± 0.002
HA3	nd	FA3	0.028 ± 0.011
HA4	nd	FA4	0.628 ± 0.021
HA5	0.027 ± 0.009		
HA6	nd		
HA7	nd		
HA8	0.044 ± 0.004		
HA9	0.293 ± 0.006		

[†] Values were below the detection limit of $0.020 \text{ mmol g}^{-1}$.

of interest using the intercept and slope at high Cl^- addition. Selectivity coefficients determined from the modified fixed interferent method are presented in Table 4. There was no apparent interference from HA9 and FA4 since the mV response could be explained almost entirely by the amount of background plus added Cl^- (data not shown). For these samples, the decrease in mV response appeared to be dominated by background Cl^- .

Selectivity coefficients for humic substances in Table 4 can be used to give an indication of errors involved in overestimation of Cl^- in natural waters and soil extracts using the Cl^- ISE. For a water sample or soil extract (pH 6.5) containing $10^{-4.5}$ M Cl^- and 0.5 mg ml^{-1} HA1, a Cl^- ISE mV reading of the solution could mistakenly be taken to represent 10^{-4} M Cl^- (see Fig. 4). This level of Cl^- would be more than two times that of the actual amount. Since Ag^+ binding is a function of pH (see Fig. 3), errors in Cl^- analyses will be particularly large at low Cl^- concentrations and moderate to high pH.

In most surface and ground waters, errors will generally be less than presented above since the usual range of organic matter concentration in natural waters is 0.0001 to 0.01 mg ml^{-1} (Stumm and Morgan, 1981). Higher organic matter concentrations would be expected in leachate waters from peat, the organic layers of forest soils, compost heaps, and cattle feedlots. Moore's (1985) values for organic C in peat extracts (0.001 to 0.05 mg ml^{-1}) correspond to approximate weight concentrations of 0.002 to 0.1 mg ml^{-1} . These low levels indicate that interference of humic substance on Cl^- determination may seldom pose a problem, but care must be taken with research or environmental samples containing low concentrations of Cl^- and very high amounts of organic matter.

Table 4. Selectivity coefficients ($k_{Cl,HA}$) for interference of humic and fulvic acids on Cl^- analysis with the Cl^- ISE as calculated by a modification of the fixed interferent method.

Humic acid	$k_{Cl,HA}$	Fulvic acid	$k_{Cl,HA}$
	--ml $mg^{-1} \times 10^{-4}$ --		--ml $mg^{-1} \times 10^{-4}$ --
HA1	1.20	FA1	0.59
HA2	1.00	FA2	1.10
HA3	0.83	FA3	0.94
HA4	0.84		
HA5	1.30		
HA6	0.60		
HA7	0.78		
HA8	0.75		

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3.4 SILVER COMPLEXATION BY HUMIC SUBSTANCES: APPARENT STABILITY CONSTANTS AND NATURE OF REACTIVE SITES¹

F.J. Sikora and F.J. Stevenson ²

ABSTRACT

Complexation of Ag^+ by a wide range of humic and fulvic acids was determined using a Cl^- ion-selective electrode sensitive to the concentration of Ag^+ in solution. Maximum binding capacities (MBC) ranged from 0.75 to 1.95 mmol Ag g^{-1} at pH 6.5 (ionic strength, I, of 0.1) and were more closely related to the N contents of the humic substances (0.5 to 2.8 mmole N g^{-1}) than to acidic functional group content (3.5 to 7.0 mmoles COOH groups g^{-1}). Scatchard plots of data obtained by titrations with Ag^+ (pH 6.5, I of 0.1) were concave upward, indicating a continuum of binding sites. Apparent stability constants for binding at low saturation of binding sites ($\log K_1^*$) ranged from 3.57 to 4.80; constants for binding at high saturation ($\log K_n^*$) ranged from 2.64 to 3.17. Higher values (6.34 to 8.82) were obtained using an approach that permitted measurements to be made at low Ag^+ concentrations. The evidence indicated that complexation occurred predominantly on N (and possibly S) functional groups, as opposed to COOH and other acidic functional groups.

Additional Index Words: Silver complexation, Scatchard analysis, fulvic acid, humic acid.

¹Contribution from the Dep. of Agronomy, Univ. of Illinois, Urbana, IL. 61801. Supported in part by a grant from the United States-Israel Agricultural Research and Development Fund (BARD I-500-82).

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INTRODUCTION

Complexation of metal ions by humic substances has important implications in environmental soil chemistry. Most studies have dealt with di- and trivalent cations (e.g., Fe^{3+} , Al^{3+} , Cu^{2+} , Pb^{2+} , Cd^{2+} , Zn^{2+} , etc.), and apparent stability constants have been obtained for the complexes thus formed. Recent work indicates that humic substances also play an important role in the cycling of Ag^+ in soils (Jones et al., 1986).

Soils typically contain less than $0.5 \mu\text{g Ag g}^{-1}$ but substantially higher amounts are found in contaminated soils near Ag mining operations (Jones, 1986; Jones et al., 1986). Elevated levels are also found in areas where AgI has been used for cloud seeding and rain stimulation (Klein and Mulvey, 1978).

A knowledge of the chemistry of Ag^+ complexation by humic substances is required for an understanding of the behavior of Ag in contaminated soils. Soluble forms of inorganic Ag are highly toxic to microorganisms (Cornfield, 1977), and they have an adverse effect on soil biochemical processes (Al-Khafaji and Tabatabai, 1979). Soil adsorption and plant uptake studies have indicated a strong affinity between free Ag^+ and soil organic matter (Jones, et al. 1986; Jones and Peterson, 1986).

Objectives of the present study were: (i) to quantify Ag^+ complexation by humic substances through the determination of apparent stability constants, and (ii) to determine plausible Ag^+ binding sites on humic substances.

THEORETICAL ASPECTS

Recent work on metal ion binding by humic substances has been based on the assumption that the macromolecule is the central group to which the metal ion is bound (see review of Stevenson and Fitch, 1986). This approach was adopted in this study to model Ag^+ binding data. The extent of binding was expressed in the usual way as a formation function, $\bar{v}$, defined as:

$$\bar{v} = \text{sites bound/macromolecule conc.} = (\text{Ag}_b)/(\text{L}_t) \quad (1)$$

where (Ag_b) and (L_t) are the molar concentrations of the bound metal and

total ligand, respectively. The former was calculated as the difference between total and free Ag^+ in the experimental solution (i.e., $(\text{Ag}_b) = (\text{Ag}_t) - (\text{Ag}^+)$).

Stability constants for the Ag^+ complexes were determined by the Scatchard plot technique. The general form of the equation (one class of binding sites) is (Tanford, 1961):

$$\bar{v} = \frac{nK_o(\text{Ag}^+)}{1 + K_o(\text{Ag}^+)} \quad (2)$$

where n is the total number of binding site and K_o is a microscopic association constant for the reaction of Ag^+ with an unfilled reactive site.

For two or more classes of sites, Eq. (2) becomes:

$$\bar{v} = \sum \frac{n_i K_j(\text{Ag}^+)}{1 + K_j(\text{Ag}^+)} \quad (3)$$

where n_i is the number of sites of class j , and K_j is the intrinsic or microscopic association constant for the i th class of sites.

Rearrangement of Eq. (3) yields:

$$\frac{\bar{v}}{(\text{Ag}^+)} = nK_o - K_o \bar{v}, \text{ or } \frac{\theta}{(\text{Ag}^+)} = K_o - K_o \theta \quad (4)$$

where $\theta = \bar{v}/n$ = molar conc. of bound sites/molar conc. of reactive sites (nL_t).

In the absence of reliable information regarding the molecular weights of humic and fulvic acids, apparent stability constants ($\log K^*$) were obtained by the modified equation. As shown later, plots of $\theta/(\text{Ag}^+)$ vs. θ were concave upward, from which apparent stability constants were obtained for binding at low and high saturation of binding sites (i.e., $\log K_1^*$ and $\log K_n^*$, respectively).

The concentration of reactive sites (nL_t) required for estimating θ were obtained by analysis of the binding data using the Langmuir equation (Sposito, 1982, 1984).

$$q = \frac{bK'(\text{Ag}^+)}{1 + K'(\text{Ag}^+)} \quad (5)$$

where q is the amount of Ag^+ bound and b is the maximum binding capacity

on a gram basis (e.g., mmol g⁻¹), respectively. The symbol K' is an association constant often referred to as the binding strength. By defining a distribution coefficient as $K_d = q/(Ag^+)$, Eq. (5) can be transformed to the following (Sposito, 1984):

$$K_d = bK' - K'q \quad (6)$$

For more than one class of binding sites, a plot of K_d vs. q produces a curvilinear plot in which the subtending line to the X-axis (high saturation of binding sites) is the total binding capacity (Klotz and Hungston, 1971; Sposito, 1984).

$$x_{int} = \lim_{K_d \rightarrow 0} q = \sum_{i=1}^n b_i \quad (7)$$

The total binding capacity (MBC) obtained in this way was used to estimate the molar concentration of reactive sites (nL_t), and hence θ for use in Eq. (5).

Some of the humic substances used in this study contained background levels of Cl^- . A correction was thereby needed for the amount of Ag^+ that was precipitated as $AgCl$.

$$(AgCl) = (Cl^-) - K_{sp}/(Cl^-) \gamma_{\pm AgCl}^2 \quad (8)$$

where (Cl^-) is the molar concentration of Cl^- , λ_{AgCl} is the mean ionic activity coefficient of $AgCl$, and K_{sp} ($=10^{-9.75}$) is the solubility product of $AgCl$. The value for γ_{AgCl} (I of 0.1) was calculated from the Davies equation (Lindsay, 1979). The molar concentration of Ag^+ available for complexation was then obtained by subtraction: $(Ag^+) =$ molar conc. of added $Ag - (AgCl)$.

The basis for estimations of $\log K_1^*$ and K_n^* from curvilinear Scatchard plots has been outlined by Klotz and Hungston (1971). At low metal ion saturation, the slope of the Scatchard plot can be shown to be equivalent to:

$$\text{Slope (low } \theta) = - \frac{\sum_{i=1}^{\infty} f_i K_i^2}{\sum_{i=1}^{\infty} f_i K_i} = - \frac{\left[f_1 K_1^2 + \sum_{i=2}^{\infty} f_i K_i^2 \right]}{\left[f_1 K_1 + \sum_{i=1}^{\infty} f_i K_i \right]} \quad (9)$$

On the assumptions that

$$\sum_{i=2}^{\infty} f_i K_i^2 \ll f_1 K_1^2, \text{ and } \sum_{i=2}^{\infty} f_i K_i \ll f_1 K_1 \quad (10)$$

Eq. (9) reduces to: Slope (low θ) = $-K_1$.

At high metal ion saturation, the slope is equivalent to:

$$\text{Slope (high } \theta) = - \sum_{i=1}^{\infty} \frac{f_i}{K_i}^{-1} = - \left[\frac{\left(\prod_{i=1}^{n-1} K_i \right) K}{\sum_{i=1}^{n-1} \left(f_j \prod_{\substack{i=1 \\ i \neq j}}^n K_i \right) + f_n \prod_{i=1}^{n-1} K_i} \right] \quad (11)$$

On the assumptions that $f_n \ll 1$, and that

$$\sum_{j=1}^{n-1} \left(f_j \prod_{\substack{i=1 \\ i \neq j}}^n K_i \right) \ll f_n \prod_{i=1}^{n-1} K_i \quad (12)$$

Eq. (11) reduces to: Slope (high θ) = $-K_n$

Since the two log K values are obtained from the slopes, rather than the intercepts, errors in estimating nL_t , and hence θ are minimized.

Apparent stability constants for binding at low saturation of binding sites were also obtained by ligand titrations at $p(\text{Ag}^+) 9.8$ (activity of Ag^+ at the electrode surface). The basis for the measurements is given below.

The appropriate Nernstian equation for Ag^+ analysis is:

$$E = E_0 + 2.303 RT/F \log a_{\text{Ag}} = E_0 + 60.7 \log a_{\text{Ag}} \quad (13)$$

where a_{Ag} is the activity of the Ag^+ ion. The value for the slope of the response curve (60.7) was of the order of that generally found for the Cl^- ISE.

In the presence of a ligand, the response of the electrode is given by:

$$E = E_0 + 60.7 \log ((L\text{Ag})/K (L)) \quad (14)$$

where K is the apparent stability constant for the reaction:
 $\text{Ag} + \text{L} = \text{LAG}$. When a large excess of ligand is present, the quantities L and LAG can be considered equal to (L_t) and (Ag_t) , respectively. By appropriate substitutions into Eq.(14), the following is obtained:

$$E = E_o + 60.7 \log (\text{Ag}_t) - 60.7 \log (K^*) - 60.7 \log (L_t) \quad (15)$$

The quantity $(E_o + \text{Rt}/F \log (\text{Ag}_t))$ is constant and equal to the initial mV reading. Thus,

$$E = E'_o - 60.7 \log K^* - 60.7 \log (L_t) \quad (16)$$

A plot of E vs. $\log (L_t)$ yields a straight line, from which $\log K^*$ can be obtained from the intercept. The approach has been used to determine apparent stability constants for the binding of Cu^{2+} to humic acids (Fitch and Stevenson, 1984).

In concluding this section, it should be noted that the determination of binding parameters of complex macromolecules (such as humic substances) by plotting techniques is not as intuitively simple as one might suspect (see Klotz and Hunston, 1971). For example, curvilinear Scatchard plots can be obtained for reasons other than by complexation at more than one class of sites, such as through non-cooperative binding (Cantor and Schimmell, 1980). Also, difficulties can arise in estimating bound forms at the two extremes of the binding curve. In the present case, concave Scatchard plots were assumed to be due a continuum of binding sites, and values for $\log K_1^*$ were undoubtedly influenced by the availability (and reliability) of data obtained at low θ values, corresponding to the initial subtending portion of the Scatchard plot.

EXPERIMENTAL METHODS

Sources of the humic substances are shown in Table 1. Methods of extraction and preparation are described elsewhere (Sikora and Stevenson, 1987). The N contents of the humic substances and other pertinent data are presented in Table 2.

The humic and fulvic acids were prepared for analysis by dissolving

Table 1. Source of humic substances used for Ag^+ complexation.

Sample	Source
Humic Acids	
HA1	Extracted from the A horizon of Sable silt loam (Typic Haplaquoll) with 0.15 M $\text{Na}_4\text{P}_2\text{O}_7$ and shaken with 0.3 N HF:0.1 N HCl to remove mineral matter.
HA2	A subsample of HA1 hydrolyzed with 3 M HCl for 12 hours.
HA3	Surface layer Ohio Rifle peat (Typic Borohemist). Extracted and prepared as per HA1.
HA4	A subsample of HA3 that was hydrolyzed with 0.1 M HCl for 8 hours.
HA5	A horizon Michigan Blue Lake sand (Alfic Haplorthod). Extracted and prepared as per HA1.
HA6	B horizon Michigan Blue Lake sand (Alfic Haplorthod). Extracted and prepared as per HA1.
HA7	North Dakota Lignite (Leonardite). Extracted with 0.5 M NaOH and prepared as per HA1.
HA8	A horizon of Harpster silt loam. (Calcic Argaquoll). Extracted sequentially with 0.15 M $\text{Na}_4\text{P}_2\text{O}_7$ and 0.1 M NaOH and prepared as per HA1.
Fulvic Acids	
FA1	Same source as HA3. Recovered by sorption/desorption from activated charcoal (Stevenson and Goh, 1971).
FA2	Humic Spodosol on the west coast of Newfoundland (marketed by CONTECH E.T.C. Ltd., Ottawa, Canada)
FA3	A horizon of Drummer silt loam (Typic Haploquoll). Extracted with 95:4:1 (acetone: H_2O :conc.HCl) solvent, dialyzed, and freeze-dried.

Table 2. Apparent stability constants for Ag^+ complexes, maximum binding capacities (MBC), and N contents of humic and fulvic acids.

Sample	$\log K_1^*$	$\log K_n^*$	MBC	N Content
Humic Acids		----- mmol g ⁻¹ -----		
HA1	4.40	3.07	1.95	1.74
HA2	4.21	3.09	1.80	1.29
HA3	4.16	3.02	1.47	1.54
HA4	4.29	3.04	1.53	2.33
HA5	4.80	3.13	1.41	2.73
HA6	4.22	2.94	1.75	2.30
HA7	3.95	2.70	1.10	0.81
HA8	4.15	3.17	1.87	2.80
Fulvic Acids				
FA1	3.57	3.13	1.07	1.41
FA2	3.63	2.64	0.75	0.46
FA3	3.73	2.84	1.00	0.81

25 mg of freeze-dried material in 40 ml of 0.01 M KOH. The pH was immediately adjusted to 10 by addition of 0.1 M HNO_3 to minimize chemical changes at high pH's. The solution was then stirred under N_2 gas for one hour, the pH was adjusted to 6.5 with 0.1 M HNO_3 , and the sample was diluted to 50 ml to give a final concentration of humic material of 0.5 mg ml^{-1} . All solutions were kept at a constant I of 0.1 M KNO_3 .

Silver complexation was determined by incremental additions of 0.002 M AgNO_3 into 50 ml of a solution containing 25 mg of humic substance and measuring free Ag^+ with an Orion Cl^- ion-selective electrode (ISE). A complete description of the instrumental apparatus is described elsewhere (Sikora and Stevenson, 1987). Titrant volume was kept low (0 to 5 ml) to minimize dilution of humic substance. The pH was maintained at 6.5 through drop-wise addition of 0.1 M KOH or 0.1 M HNO_3 . The ligand titrations were carried out as described by Sikora and Stevenson, 1987).

Free Ag^+ concentration was determined from the linear portion of the Ag^+ standard curve extrapolated to $7 \times 10^{-6} \text{ M Ag}^+$. The concentration of Ag^+ , rather than activity, was directly obtainable from the standard curve since both experimental sample and standards were at a constant I of 0.1 M KNO_3 .

RESULTS AND DISCUSSION

Response curves of the Cl^- ISE (Ag^+ as the determinant ion) in the presence of HA1 and HA3 are shown in Fig. 1. Similar results were obtained for the other humic and fulvic acids. In each case, a decrease in mv response was observed, indicating complexation of Ag^+ . Measurements for binding at high concentration of Ag are subject to considerable error, both because the values represent the difference between two relatively large values and because of changes in the ionic composition of the experimental solution. Data used for the calculations noted below were restricted to mV readings between $p(\text{Ag}_t)$ 5 and 3.

The behavior of the Cl^- ISE in the presence of humic substances was similar to that observed for organic ligands known to complex Ag^+ (Sikora and Stevenson, 1987). Further evidence for Ag^+ complexation by the humic substances used herein was obtained by Sikora and Stevenson

(1987), using an approach that involved equilibrium dialysis and Ag^+ determination by atomic absorption spectroscopy. A strong affinity between Ag^+ and soil organic matter has also been postulated through studies of Ag^+ interactions with Welsh soils (Jones et al., 1986).

Typical plots obtained by application of Eq. (6) for calculating MBC are shown in Fig. 2. The values are approximations since X-intercepts of the limiting slope (Eq. (7)) are highly sensitive to the number of data points used for extrapolation (Klotz, 1982). In this study, ten data points were used.

Maximum binding capacities (MBC) for the humic and fulvic acids are given in Table 2. The values ranged from 0.75 to 1.95 mmole Ag g^{-1} (pH 6.5, I of 0.1) and were more closely related to the N contents of the humic substances (0.5 to 2.8 mmole N g^{-1}) than to acidic functional group content (3.5 to 7.0 mmoles COOH groups g^{-1}). Nitrogen-containing groups would therefore appear to play an important role in Ag^+ binding. Humic acids, in general, have higher N contents (but lower contents of O-containing functional groups) than fulvic acids, and, as noted in Table 2, they have a higher capacity for binding Ag^+ . The greater retention of Ag^+ by humic acids, as compared to fulvic acids, may explain the lower availability of Ag^+ to ryegrass in the presence of humic acid, as compared to fulvic acid (Jones and Peterson, 1986).

On a unit weight basis, the ability of humic acids to bind Ag^+ was not altered substantially by acid hydrolysis (compare HA2 vs. HA1 and HA4 vs. HA3 in Table 2). The hydrolysis step, which was included to remove proteinaceous substances and other easily hydrolyzable organic N compounds, was only partially effective in reducing the N contents of the humic acids (i.e., proportional amounts of C also removed). The findings suggests that the "structural N" of humic acids is also involved in Ag^+ binding.

Although the magnitude of the MBC values were of the general order of the N contents of the humic and fulvic acids, the correlation between the two was not highly significant (NS at $P=0.01$). This was not totally unexpected because of errors involved in estimating MBC (discussed earlier). Sulfur-containing functional groups may also have contributed to Ag^+ complexation. Due to the low S content of humic substances (see Stevenson, 1985), S would appear to play a subservient role to N in Ag^+ binding.

Fig. 1

Response of the Cl^- ISE to Ag^+ additions in the presence of humic acids HAL and HA3 (pH 6.5; $I = 0.1$)

Fig. 2.

Modified Langmuir plots for binding of Ag^+ to humic acids HAL and HA3, where K_d is the distribution coefficient and q is the amount of Ag^+ bound. The maximum binding capacity (MBC) was obtained from the X intercept.

The high affinity of Ag^+ for ligands containing N and S is well-known (see Stumm and Morgan, 1981; Cotton and Wilkinson, 1980). The Ag^+ ion is highly polarized and is classified as a B-type metal (Stumm and Morgan, 1981). Metal ions of this type form complexes with some degree of covalency due to their loosely held outer shell electrons. Sites of high electronegativity (i.e., O-containing functional groups) do not form stable complexes with Ag^+ since their capacity to donate electrons is minimal. On the other hand, sites of low electronegativity (i.e., N or S) form stable complexes, the most common structures being linear (Cotton and Wilkinson, 1980). Morf et al. (1974) provide a list of N- and S-containing ligands known to complex Ag^+ .

Synthetic polymers have been reported to bind Ag^+ through bridging of COOH groups (Strauss and Begala, 1980). Evidence that COOH was not involved in the binding of Ag^+ by humic and fulvic acids was obtained in a study of the influence of pH on Ag^+ binding. Results for HA1 and HA7 are shown in Fig. 3. If COOH groups were the primary binding site, a greater mV drop would have occurred at the lower pH's and would have followed the pattern expected for dissociation of COOH groups ($\text{COOH} = \text{COO}^- + \text{H}^+$). The pK_a 's of the humic acids were in the range 4.5 to 4.6 (I of 0.1), and dissociation was essentially complete at pH 7.5. The mV change in the pH range 9.0 to 11.5 corresponds to the pK_a range of aliphatic NH_2 groups (Morrison and Boyd, 1973) and suggests competition between Ag^+ and H^+ for NH_x reactive sites.

Typical Scatchard plots are shown in Fig. 4. The concave nature of the plots indicates binding at two or more independent classes of binding sites, non-cooperative binding, or a combination of the two (Cantor and Schimmel, 1980). In the discussion that follows, the assumption was made that an infinite number (continuum) of binding sites was involved (see Theoretical section). The constants thus obtained represent binding at low ($\log K_1^*$) and high ($\log K_n^*$) Ag^+ saturation. Results of Table 2 show that, for the humic and fulvic acids examined in this study, $\log K_1^*$ values (binding at stronger sites) ranged from 3.81 to 4.46; those for $\log K_n^*$ (binding at weaker sites) ranged from 2.67 to 3.14). Similar types of Scatchard plots have been recorded for Cu^{+2} complexation by humic substances (Fitch et al., 1986; Sposito et al., 1979; Bresnahan et al., 1978). $\log K_1^*$ and $\log K_n^*$ values of 6.75 and 5.09, respectively, were obtained by Fitch and Stevenson (1984) for the

Fig. 3. Effect of pH on response of the Cl^- ISE in the presence of humic acid ($I = 0.1$ with respect to KNO_3). The concentration of humic acids was 0.5 mg ml^{-1} .

Fig. 4. Modified Scatchard plots for complexation of Ag^+ by humic acids HA1 and HA3.

Cu^{+2} complexes of HAl (pH 4, I of 0.1.). The extent to which transition metal ions compete with Ag^+ for binding sites is unknown.

An unexpected finding was that there was a positive correlation between $\log K^*$ and the N contents of the humic and fulvic acids (Fig. 5). The regression equations were:

$$\log K_1^* = 3.55 + 0.332 N \quad r^2 = 0.54^*$$

$$\log K_n^* = 2.70 + 0.169 N \quad r^2 = 0.57^{**}$$

If all the N in the humic substances was in the same form (e.g., as an NH_2 group), and had binding occurred only at N-containing groups, the microscopic association constants would have been independent of total N content. The increases observed may reflect differences in the nature of the N groups in humic substances and/or to the occurrence of other binding sites. Information on the nature of the N and S in humic substances would provide a better understanding of Ag^+ binding.

The $\log K_1^*$ values reported above are tentative in that a limited number of data points were available at low θ values (see Fig. 2). Accordingly, an attempt was made to determine $\log K^*$ for binding at very low saturation of binding sites, using Eq. (16). Data reported in the previous study (Sikora and Stevenson, 1987) for the effects of HAl, HA3, and HA7 on response of the Cl^- ISE (pH 6.5, I of 0.1 M KNO_3) were used for this purpose. Results obtained for HAl and HA3 are shown in Fig. 6. Stability constants ($\log K^*$), as calculated from the linear portion of the curves, were: HAl, 6.34; HA3, 7.52; HA7, 8.82. These values are several orders of magnitude higher than the $\log K_1^*$ values given in Table 2, and can be attributed to failure to detect binding at low degrees of Ag saturation using the Ag^+ titration approach. The findings are consistent with the concept that a continuum of binding sites are present in humic substances, and they serve to emphasize that results obtained in different laboratories, and for different humic preparations, can only be compared over the same range of Ag saturation.

In summary, on the basis of the experimental results obtained herein, and from the known chemistry of Ag^+ in solution, an appropriate hypothesis for the complexation of Ag^+ by humic substances is that N-containing, and to a lesser extent S-containing, groups play an

Fig. 5. Relationship between $\log K_1^*$ and $\log K_n^*$ vs. N content of the humic substances. The solid lines represent significant linear regression models for $\log K_1^*$ ($p=0.05$) and $\log K_n^*$ ($p = 0.01$).

Fig. 6. Response of the Cl^- ISE by titration with two humic acids (HA1 and HA3) at pH 6.5 (I of 0.1 with respect to KNO_3).

important role; O-containing groups are relatively unimportant. As indicated by Stevenson (1985), the majority of environmentally important trace metals can be expected to bind to humic substances to a maximum value corresponding to their content of acidic functional groups. The Ag^+ ion is an exception to this rule in that the binding capacity was more closely related to the N contents of the humic substances. Further characterization of Ag^+ binding by humic substances would be beneficial in predicting the behavior of Ag in contaminated soils.

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5. THEORIES AND MODELING OF ANION INTERACTIONS WITH HUMIC SUBSTANCES

5.1 Donnan theory (F. J. Sikora and F. J. Stevenson)

Most soil chemists have formulated Donnan theory on the basis of equivalent chemical and electrochemical potentials of salt across the membrane (Babcock, 1960; Davis, 1942; Wiklander, 1964). The familiar equation relating the activities of cations and anions between the two phases is as follows:

$$r_D' = \left(\frac{a_M^\alpha}{a_M^\beta} \right)^{1/x} = \left(\frac{a_N^\beta}{a_N^\alpha} \right)^{1/y} \quad (1)$$

where r_D' is the true Donnan ratio, a is the activity of cation M (charge x) or anion N (charge y), and α and β refer to the inner and outer phases, respectively. In terms of concentration:

$$\left(\frac{m_M^\alpha}{m_M^\beta} \right)^{1/x} \frac{(\gamma_M^\alpha)^{1/x} (\gamma_N^\alpha)^{1/y}}{(\gamma_M^\beta)^{1/x} (\gamma_N^\beta)^{1/y}} = \left(\frac{m_N^\beta}{m_N^\alpha} \right)^{1/y} \quad (2)$$

where λ is the activity coefficient. To simplify this expression, the ratio of the activity coefficients is denoted as ϕ , yielding:

$$\left(\frac{m_M^\alpha}{m_M^\beta} \right)^{1/x} \phi = \left(\frac{m_N^\beta}{m_N^\alpha} \right)^{1/y} \quad (3)$$

The assumption is made that the activity coefficients for all cations are identical. Thus, for a uni-univalent salt (e.g. KCl), ϕ becomes:

$$\phi = (\gamma_{\pm KCl}^\alpha)^2 / (\gamma_{\pm KCl}^\beta)^2 \quad (4)$$

If the ionic activity coefficients are equal in the interior and exterior solution, $\phi=1$ and Eq. (3) becomes:

$$r_D = \left(\frac{m_M^\alpha}{m_M^\beta} \right)^{1/x} = \left(\frac{m_N^\beta}{m_N^\alpha} \right)^{1/y} \quad (5)$$

The Donnan ratio, r_D , in Eq. (5) refers to the molar concentration ratio of cation and anions. The true Donnan ratio, as shown by r_D' in Eq. (1), refers to the activity ratios of cations and anions. For a system containing one cation and one anion, ϕ must equal one for r_D to equal r_D' .

Equation (5) can be applied to a system containing KCl as a uni-univalent salt. The Donnan ratio for Cl^- is:

$$r_D^{\text{Cl}} = \frac{m_K^\alpha}{m_K^\beta} \quad \phi = \frac{m_{\text{Cl}}^\beta}{m_{\text{Cl}}^\alpha} \quad (6)$$

The constraint of electroneutrality in the inner phase dictates the following relationships:

$$m_{\text{Cl}}^\alpha + m_A^\alpha - m_K^\alpha = 0 \quad (7)$$

$$m_{\text{Cl}}^\beta - m_K^\alpha = 0 \quad (8)$$

The symbol m_A^α refers to the molar concentration of equivalent negative charge on the restrained colloid. The assumptions are made that H^+ , OH^- , and carbonate species concentrations are insignificant.

Since the concentrations of ions in the bulk solution phase are easier to measure in dialysis experiments, Eq. (7) can best be expressed in terms of ions in the bulk solution phase. By combining Eqs. (6) and (7), the following is obtained:

$$\frac{m_{\text{Cl}}^\beta}{r_D^{\text{Cl}}} + m_A^\alpha - r_D^{\text{Cl}} \frac{m_K^\beta}{\phi} = 0 \quad (9)$$

Since $m_{Cl}^{\beta} = m_K^{\beta}$, the above equation can be written as:

$$\frac{m_{Cl}^{\beta}}{r_D} + m_A^{\alpha} - r_D^{Cl} \frac{m_{Cl}^{\beta}}{\phi} = 0 \quad (10)$$

Multiplying each term by r_D^{Cl}/m_{Cl}^{β} yields:

$$1 + r_D^{Cl} \frac{m_A^{\alpha}}{m_{Cl}^{\beta}} - \frac{(r_D^{Cl})^2}{\phi} = 0 \quad (11)$$

Solving for the quadratic root of r_D^{Cl} yields:

$$r_D^{Cl} = \frac{m_A^{\alpha} \phi}{2m_{Cl}^{\beta}} + \left[\phi + \left(\frac{m_A^{\alpha} \phi}{2m_{Cl}^{\beta}} \right)^2 \right]^{1/2} \quad (12)$$

To simplify Eq. (12), $m_{Cl}^{\beta}/m_A^{\alpha}$ can be defined as Y (Babcock, 1960). Thus,

$$r_D^{Cl} = \frac{\phi}{2Y} + \left[\phi + \left(\frac{\phi}{2Y} \right)^2 \right]^{1/2} \quad (13)$$

For uni-univalent salts, the value ϕ can be defined as a function of the ratio of mean ionic activity coefficients in the interior and exterior solutions. The ratio of mean ionic activity coefficients has been denoted by Babcock (1960) as:

$$\theta = \left(\frac{\gamma_K^{\alpha} \gamma_{Cl}^{\alpha}}{\gamma_K^{\beta} \gamma_{Cl}^{\beta}} \right)^{1/2} = \frac{\gamma_{\pm KCl}^{\alpha}}{\gamma_{\pm KCl}^{\beta}} \quad (14)$$

From Eq. (4) it can be seen that $\phi = (\theta)^2$. Substitution of θ into Eq. (12) yields the following:

$$r_D^{Cl} = \frac{\theta^2}{2Y} + \left[\theta^2 + \left(\frac{\theta^2}{2Y} \right)^2 \right]^{1/2} \quad (15)$$

Examination of Eq. (15) indicates that the Donnan ratio, r_D^{Cl} , is a function of the concentration of equivalent negative charge on the macromolecule, m_A^α , and the equilibrium concentration of Cl^- in the exterior solution, m_{Cl}^β . As the concentration of equivalent negative charge on a colloid increases, the Donnan ratio increases. Eq. (15) also indicates that the Donnan ratio will decrease to a limiting value of θ^2 with an increase of I in the exterior solution, or equivalently m_{Cl}^β . If the activity coefficients for K^+ and Cl^- are equivalent in the interior and exterior phases, the limiting Donnan ratio at high I is one.

The derivation of Eq. (15) is based on the assumption that the concentrations of H^+ , OH^- , and carbonate species are negligible. Also, only a univalent salt is considered.

The theoretical treatment of anion exclusion by negatively charged colloids in light of Donnan theory has been covered by Babcock (1960, 1963). Considering a K^+ and Cl^- system, and by replacing ϕ in Eq. (6) with θ^2 , the following is obtained:

$$\frac{m_K^\alpha}{m_K^\beta} \theta^2 = \frac{m_{Cl}^\beta}{m_{Cl}^\alpha} \quad (16)$$

As with the derivation of Eq. (15), the electroneutrality constraints of the interior and exterior solutions must be considered, where:

$$m_K^\alpha = m_A^\alpha + m_{Cl}^\alpha ; \quad m_K^\beta = m_{Cl}^\beta \quad (17)$$

The assumptions are made that H^+ , OH^- , and carbonate species concentrations are negligible as was the case in the derivation of Eq. (13). Substitution of Eq. (15) into Eq. (16) and rearrangement yields:

$$\theta^2 (m_A^\alpha + m_{Cl}^\alpha) m_{Cl}^\alpha = (m_{Cl}^\beta)^2 \quad (18)$$

The negative adsorption of Cl^- , as defined by Babcock (1960), is:

$$\Gamma = \frac{m_{Cl}^\beta - m_{Cl}^\alpha}{m_A^\alpha} \quad (19)$$

where Γ is the Donnan coefficient. Solving for m_{Cl}^α in Eq. (18) and substituting in Eq. (19) yields:

$$\Gamma = \frac{2\theta m_{Cl}^\beta + \theta m_A^\alpha - (\theta^2 (m_A^\alpha)^2 + 4(m_{Cl}^\beta)^2)^{1/2}}{2 m_A^\alpha \theta} \quad (20)$$

From the definition of Y as $m_{Cl}^\beta / m_A^\alpha$, this equation can be simplified to:

$$\Gamma = \frac{2\theta Y + \theta - (\theta^2 + 4Y^2)^{1/2}}{2\theta} \quad (21)$$

In an ideal Donnan system, $\lambda_{\pm KCl}$ is the same in the suspension and bulk solution phase ($\theta=1$); in a non-ideal system, $\lambda_{\pm KCl}$ in the suspension phase will be less than that for the bulk solution phase ($\theta < 1$). A lower activity coefficient can arise from electrostatic interactions with the colloid.

In Babcock's (1960) definition of negative adsorption, difficulty arises in comparing anion exclusion among colloids of differing negative charges, since Γ is the moles of anion excluded per equivalent charge. Greater insight into the interactions of anions with negatively charged colloids would result if Cl^- exclusion were expressed on a unit weight basis. This can be accomplished by multiplying Γ by m_A^α / g_c at the pH of the system where:

$$\text{excluded Cl}^- = r \frac{a_M^\alpha}{g_c} \quad (22)$$

Excluded Cl^- is in units of meq g^{-1} and g_c refers to the concentration of the colloid on a gram basis.

Equations (15) and (21) are useful for studying the distribution of ions in a Donnan system. Although K^+ and Cl^- were used as specific examples, the distribution of cations and anions for any system containing a uni-univalent salt could be determined. A limitation of the two equations is that distributions for other systems cannot be determined. Another limitation is the absence of H^+ , OH^- , and carbonate species in electroneutrality Eqs. (7), (8), and (17). The assumption that these species are insignificant may not be valid at low or high pH where H^+ , OH^- , and carbonate species become significant. A more general equation is needed to study the interactions of ions with different valences at extreme pH conditions in a Donnan system.

The derivation of a completely general equation for ion distributions in a Donnan system must obey three constraints. The first follows from Eq. (1), where the true Donnan ratios should be equivalent for every ionic species in solution. Therefore,

$$r_D = \left(\frac{a_M^\alpha}{a_M^\beta} \right)^{1/x} = \left(\frac{a_N^\beta}{a_N^\alpha} \right)^{1/y} = \frac{a_H^\alpha}{a_H^\beta} = \frac{a_{\text{OH}}^\beta}{a_{\text{OH}}^\alpha} = \frac{a_{\text{HCO}_3}^\beta}{a_{\text{HCO}_3}^\alpha} = \left(\frac{a_{\text{CO}_3}^\beta}{a_{\text{CO}_3}^\alpha} \right)^{1/2} \quad (23)$$

For the relationship shown in Eq. (23), M and N refer to the cation and anion, respectively, in the system. The parameter x and y are the absolute charge of the ions. Carbonate species are represented since they can become significant at moderate to high pH values. The other

two constraints come directly from electroneutrality, which dictates that total positive charges must equal total negative charges in each phase:

$$m_A^\alpha + y(m_N^\alpha) + m_{\text{HCO}_3}^\alpha + 2(m_{\text{CO}_3}^\alpha) - x(m_M^\alpha) - m_H^\alpha + m_{\text{OH}}^\alpha = 0 \quad (24)$$

and

$$y(m_N^\beta) + m_{\text{HCO}_3}^\beta + 2(m_{\text{CO}_3}^\beta) - x(m_M^\beta) - m_H^\beta + m_{\text{OH}}^\beta = 0 \quad (25)$$

A general Donnan equation for use at all pH ranges must be derived from basic electroneutrality equations which consider m_H , m_{OH} , m_{HCO_3} , and m_{CO_3} . Carbonate species are considered in Eqs. (24) and (25) since they can become significant at higher pH levels.

The derivation of a general Donnan equation involves the manipulation of Eqs. (23), (24), and (25). To begin with, Eq. (23) must be expressed in terms of the concentrations of all ionic species:

$$r_D^N = \left(\frac{m_M^\alpha}{m_M^\beta} \right)^{1/x} \theta^2 = \left(\frac{m_N^\beta}{m_N^\alpha} \right)^{1/y} = \frac{m_H^\alpha}{m_H^\beta} = \frac{m_{\text{OH}}^\beta}{m_{\text{OH}}^\alpha} = \frac{m_{\text{HCO}_3}^\beta}{m_{\text{HCO}_3}^\alpha} = \left(\frac{m_{\text{CO}_3}^\beta}{m_{\text{CO}_3}^\alpha} \right)^{1/2} \quad (26)$$

The value r_D^N is the Donnan ratio for the molar concentration of anion N. The activity coefficient ratios for H^+ , OH^- , HCO_3^- , and CO_3^{2-} are assumed to equal one. Rearrangement of the Donnan ratios for M and N in Eq. (26) and substitution into (24) yields:

$$m_A^\alpha + y \frac{m_N^\beta}{(r_D^N)^y} + m_{\text{HCO}_3}^\alpha + 2(m_{\text{CO}_3}^\alpha) - x \frac{(r_D^N)^x m_M^\beta}{\theta^2} - m_H^\alpha + m_{\text{OH}}^\alpha = 0 \quad (27)$$

The carbonate equilibria must now be considered where:

If the condition of low I is imposed on the system, the activity coefficient ratios can be assumed to be equivalent to one. The parameters $m_{\text{HCO}_3^-}$ and $m_{\text{CO}_3^{2-}}$ can then be shown to be equivalent to:

$$m_{\text{HCO}_3^-} = \frac{K_a P_{\text{CO}_2}}{K_c a_{\text{H}}} \quad (31)$$

$$m_{\text{CO}_3^{2-}} = \frac{K_a K_b P_{\text{CO}_2}}{K_c (a_{\text{H}})^2} \quad (32)$$

The constants in Eqs. (31) and (32) can be defined as:

$$KAA = K_a / K_c = 10^{-7.82} \text{ M}^2 \quad (33)$$

and

$$KAB = (K_a K_b) / K_c = 10^{-18.15} \text{ M}^3 \quad (34)$$

Substitution of Eqs. (33) and (34) into Eqs. (31) and (32) yields:

$$m_{\text{HCO}_3^-} = (KAA P_{\text{CO}_2}) / a_{\text{H}} \quad (35)$$

and

$$m_{\text{CO}_3^{2-}} = (KAB P_{\text{CO}_2}) / (a_{\text{H}})^2 \quad (36)$$

It should be noted that the concentration of HCO_3^- will only be

significant at pH 6.3 or higher and at concentrations of 10^{-5} M and greater. Bicarbonate will only be significant at pH 8.3 or higher and at concentration of 10^{-5} M and greater. At pH levels below 6.0, the concentrations of all carbonate species are insignificant.

Substitution of Eqs. (35) and (36) into (27) yields:

$$m_A^\alpha + y \frac{m_N^\beta}{(r_D^N)^y} + \frac{(KAA) P_{CO_2}}{a_H^\alpha} + \frac{2(KAB) P_{CO_2}}{(a_H^\alpha)^2} - x \frac{(r_D^N)^x m_M^\beta}{\theta^2} - m_H^\alpha + m_{OH}^\alpha = 0 \quad (37)$$

Rearrangement of the electroneutrality equation for m_M^β in Eq. (25), substitution into Eq. (37), and substitution of $a_H^\alpha/\gamma_H^\alpha$ and $a_{OH}^\alpha/\gamma_{OH}^\alpha$ for m_H^α and m_{OH}^α , respectively, yields:

$$\begin{aligned} m_A^\alpha + y \frac{m_N^\beta}{(r_D^N)^y} + \frac{(KAA) P_{CO_2}}{a_H^\alpha} + \frac{2(KAB) P_{CO_2}}{(a_H^\alpha)^2} \\ - \frac{(r_D^N)^x}{\theta^2} \left[y(m_N^\beta) + \frac{r_D^N (KAA) P_{CO_2}}{a_H^\alpha} + \frac{(r_D^N)^2 2(KAB) P_{CO_2}}{(a_H^\alpha)^2} \right. \\ \left. - \frac{a_H^\alpha}{r_D^N \gamma_H^\alpha} + \frac{r_D^N a_{OH}^\alpha}{\gamma_{OH}^\alpha} \right] - \frac{a_H^\alpha}{\gamma_H^\alpha} + \frac{a_{OH}^\alpha}{\gamma_{OH}^\alpha} = 0 \end{aligned} \quad (38)$$

Equation (38) is the final Donnan equation that is completely general in nature. The equation can be used to predict Donnan distributions for ions of any valence at any pH level. Unlike Eq. (15), r_D^N cannot be easily identified as one function dependent on m_{Cl}^β and m_A^α . In order to determine r_D^N at given values of m_N^β , m_A^α , x , y , θ^2 , p_{CO_2} , and

pH, repetitive calculations can be performed to determine a value for r_D^N where the left hand side of Eq. (38) equals 0. Once r_D^N is determined, the concentration values for every ionic species in each phase can be easily calculated. A computer program was devised to determine r_D^N from Eq. (38) (Sikora, 1986).

The assumptions that were made in the derivation of Eq. (38) are:

(i) The Donnan system contains a restrained macromolecule in the interior phase and a salt $M_y N_x$ that is free to diffuse into both the interior and exterior phases.

(ii) The activity coefficients for H^+ , OH^- , HCO_3^- , and CO_3^{2-} are the same in the interior and exterior phases.

(iii) The activity coefficients of the carbonate species are equivalent:

$$\gamma_{HCO_3}^\alpha = \gamma_{H_2CO_3}^\alpha, \quad \gamma_{CO_3}^\alpha = \gamma_{H_2CO_3}^\alpha, \quad \gamma_{CO_2}^\alpha = \gamma_{H_2CO_3}^\alpha$$

Equivalent activity coefficient ratios in the interior and exterior phases for the anions OH^- , HCO_3^- and CO_3^{2-} are believed to be valid approximations since the activity coefficients for coions in the presence of a negatively charged colloid has been shown to be nearly the same as the activity coefficient in the absence of the colloid (Manning, 1974). In the case of H^+ , the low charge density of the humic colloid at low pH, where H^+ becomes significant in a Donnan distribution, is assumed not to alter the activity coefficient of the H^+ ion to any significant degree compared to H^+ in the absence of the colloid. As with assumption (ii), assumption (iii) was made to simplify the derivation. The assumption of equivalent activity coefficients are valid at low I but become less valid at higher I where $\gamma_{HCO_3}^\alpha$ and $\gamma_{CO_3}^\alpha$ decrease at a greater rate than $\gamma_{H_2CO_3}^\alpha$ with an increase in I.

In the pH range of 3.5 to 7.0, and at low I, assumption (iii) should be valid since the low pH range indicates negligible carbonate species concentrations and the low I dictate activity coefficient values that are nearly equivalent. If Eq. (38) were used for conditions at high pH and high I, the individual activity coefficients for the carbonate species would need to be considered.

Although Eq. (38) is more complete than Eqs. (15) and (21), its application is still limited because only one cation and one anion are considered. Since real systems would undoubtedly contain additional cations and anions, a more complete equation is required. The following derivation is for a Donnan system containing one cation and two anions.

For an extra anion, Eq. (23) can be rewritten as:

$$r'_D = \left(\frac{a_M^\alpha}{a_M^\beta} \right)^{1/x} = \left(\frac{a_N^\beta}{a_N^\alpha} \right)^{1/y} = \left(\frac{a_P^\beta}{a_P^\alpha} \right)^{1/z} = \frac{a_H^\alpha}{a_H^\beta} = \frac{a_{OH}^\beta}{a_{OH}^\alpha} = \frac{a_{HCO_3}^\beta}{a_{HCO_3}^\alpha} = \left(\frac{a_{CO_3}^\beta}{a_{CO_3}^\alpha} \right)^{1/2} \quad (23a)$$

where P is the extra anion with an absolute charge, z. The electroneutrality constraints from Eqs. (24) and (25) would need to include the extra anion as:

$$m_A^\alpha + y(m_N^\alpha) + z(m_P^\alpha) + m_{HCO_3}^\alpha + 2(m_{CO_3}^\alpha) - x(m_{OH}^\alpha) - x(m_M^\alpha) - m_H^\alpha = 0 \quad (38)$$

$$y(m_N^\beta) + z(m_P^\beta) + m_{HCO_3}^\beta + 2(m_{CO_3}^\beta) + m_{OH}^\beta - x(m_M^\beta) - m_H^\beta = 0 \quad (40)$$

The activities in Eq. (39) can be expressed in terms of concentrations as:

$$r_D^N = \left(\frac{m_M^\alpha}{m_M^\beta} \right)^{1/x} \theta^2 = \left(\frac{m_N^\beta}{m_N^\alpha} \right)^{1/y} = \left(\frac{m_P^\beta}{m_P^\alpha} \right)^{1/z} = \frac{m_H^\alpha}{m_H^\beta} = \frac{m_{OH}^\beta}{m_{OH}^\alpha} = \frac{m_{HCO_3}^\beta}{m_{HCO_3}^\alpha} = \left(\frac{m_{CO_3}^\beta}{m_{CO_3}^\alpha} \right)^{1/2} \quad (41)$$

As mentioned in the derivation of Eq. (38), the activity coefficient ratios for H^+ , OH^- , HCO_3^- , and CO_3^{2-} are assumed to be one. The symbol ω is defined as $(\gamma_P^\beta/\gamma_P^\alpha)^{1/z}$.

Rearrangement of the Donnan ratios for M, N, and P in Eq. (41) and substitution into Eq. (39) yields:

$$m_A^\alpha + y \frac{m_N^\beta}{(r_D^N)^y} + z \frac{\omega m_P^\beta}{(r_D^N)^z} + m_{HCO_3}^\alpha + 2m_{CO_3}^\alpha - x \frac{(r_D^N)^x m_M^\beta}{\theta^2} - m_H^\alpha + m_{OH}^\alpha = 0 \quad (42)$$

Substitution of Eqs. (35) and (36) into Eq. (42) for the carbonate species and rearranging Eq. (40) for m_M^β and substitution into (42) yields:

$$\begin{aligned} m_A^\alpha + y \frac{m_N^\beta}{(r_D^N)^y} + z \frac{\omega m_P^\beta}{(r_D^N)^z} + \frac{(KAA) P_{CO_2}}{a_H^\alpha} + \frac{2 (KAB) P_{CO_2}}{(a_H^\alpha)^2} \\ - \frac{(r_D^N)^x}{\theta^2} \left[y(m_N^\beta) + z(m_P^\beta) + \frac{r_D^N (KAA) P_{CO_2}}{a_H^\alpha} + \frac{(r_D^N)^2 2(KAB) P_{CO_2}}{(a_H^\alpha)^2} \right. \\ \left. - \frac{a_H^\alpha}{r_D^N \gamma_H^\alpha} + \frac{r_D^N a_{OH}^\alpha}{\gamma_{OH}^\alpha} \right] - \frac{a_H^\alpha}{\gamma_H^\alpha} + \frac{a_{OH}^\alpha}{\gamma_{OH}^\alpha} = 0 \end{aligned} \quad (43)$$

Equation (43) is very similar to Eq. (38). The addition of the second anion dictates that m_P^β , ω , and z must be inputs into the equation along with m_N^β , m_A^α , x , y , θ^2 , pCO_2 , and pH . A computer program for solving Eq. (43) has been devised by Sikora (1986).

The inclusion of additional anions and cations into Eq. (43) can be accomplished by following the same procedure for inclusion of the second

anion, P. If additional cations are input into the equation, the concentration of cations in the exterior solution, along with activity coefficient ratios and valences, become necessary input values to determine r_D^N .

With the potential to add additional cations and anions into Eq. (43), the expression can become extremely useful in determining Donnan ratios in real systems. Equation (43) can also find importance in the study of interactions between cation and anions in Donnan systems.

The fact must be emphasized that Eqs. (38) and (43) are useful for predicting cation and anion distributions in a Donnan system where only electrostatic attraction of cations and exclusion of anions occur. They do not predict the distribution of cations and anions where specific covalent bonding or counterion condensation occurs.

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3.2 Counterion condensation theory

3.2.1 Planner and polyelectrolyte model (F.J. Sikora and F.J. Stevenson)

Counterions can neutralize the negative charges of a weak acid polyelectrolyte in two different ways, first, by being adsorbed directly onto the macroion and second, by being present in a diffuse double layer away from the polyelectrolyte. According to theory, counterion condensation will occur when ξ is greater than one, where ξ is defined by:

$$\xi = e^2 / \epsilon k T b = 7.135 \text{ \AA} / b \quad (44)$$

where e is the electronic charge, ϵ is the dielectric constant, k is the Boltzmann's constant, T is temperature, and b is the charge spacing in Angstrom units (Manning, 1969). Thus, in order to satisfy the requirements for counterion condensation, the molecule must contain charge spacings that are 7.135 Å or less apart.

Counterions neutralize negative charges on the polyelectrolyte by being adsorbed directly onto the macroion as opposed to being present in a diffuse double layer away from the polyelectrolyte. The two populations are classified as "condensed counterions" and "double layer counterions", as shown below.

Condensed counterions that retain their hydrated state are free to move on the surface of the polyelectrolyte. This type of binding is referred to as delocalized binding, as opposed to localized site binding where the counterion loses hydration water and is bound to a specific site (Manning, 1978). Alkaline earth cation condensation on DNA was found to be primarily due to delocalized binding (Manning, 1978); about one-half of condensed Mn^{+2} and Co^{+2} on DNA has been attributed to localized binding (Spegt and Weill, 1976).

Counterion condensation onto a polyelectrolyte is balanced by two forces (Manning, 1978). The electrostatic repulsion between charged groups less than 7.135 Å distance from one another, as shown above, predicts condensation of counterions to reduce the repulsion energy. The greater entropy of counterions in solution, as opposed to those in the condensed state predicts dissociation of counterions from the polyelectrolyte surface. The two forces of repulsion energy reduction and increased entropy counterbalance one another to a point where counterions will condense until the charge spacings are effectively 7.135 Å apart.

The fraction of polyelectrolyte charge that is neutralized by condensation can be expressed as (Manning, 1978):

$$\text{FRACTION NEUTRALIZED} = x\theta = (1 - \epsilon^{-1}) \quad (45)$$

where x is the absolute value of the valence of the counterion and θ is

the ratio of the condensed counterions over the charge groups on the polyelectrolyte. If $b=1.427 \text{ \AA}$, then $\epsilon = 7.135 \text{ \AA} / 1.427 \text{ \AA} = 5$. From counterion condensation theory, counterions would condense on the polyelectrolyte until $\epsilon = 1$. For ϵ to equal one, counterions would condense and neutralize 4 out of 5 charge groups. The effective spacing would be $7.135 \text{ \AA}$ and the effective value for ϵ would be 1. For univalent counterions, Eq. (45) would be shown as:

$$\text{FRACTION NEUTRALIZED} = \theta = (1 - \xi^{-1}) = (1 - \frac{1}{5}) = \frac{4}{5} \quad (46)$$

For divalent counterions, the equation takes the form:

$$\text{FRACTION NEUTRALIZED} = 2\theta = (1 - \frac{1}{5}) = \frac{4}{5} \quad (47)$$

For univalent counterions, θ equals 4 counterions per 5 charged groups. For divalent counterions, θ equals 2 counterions per 5 charge groups.

The fraction of charge groups not neutralized is:

$$\text{FRACTION NOT NEUTRALIZED} = 1 - x\theta = \epsilon^{-1} \quad (48)$$

The molar concentration of condensed (m_{cc}) and uncondensed (m_{ucc}) counterions can then be calculated in a solution with one salt as:

$$m_{cc} = (1 - \xi^{-1}) m_A/x \quad (49)$$

$$m_{ucc} = \xi^{-1} m_A/x + y m_N/x \quad (50)$$

where m_A is the molar concentration of univalent negative charges on the polyelectrolyte and m_N is the molar concentration of coion with valence y .

Since the amount of condensed counterions is dependent on b , or ϵ as shown in Eq. (49), the quantity of condensed counterions is also a function of the end to end distance of the macromolecule, since:

$$b = L/\alpha'P \quad (51)$$

where L is the end to end length, P is the number of functional groups on the molecule, and α' is the degree of dissociation. Defining the end to end length at maximum extension as L' , the fraction, L'/P , is the distance between the functional groups. If the end to end length remains a distance of L' with a change in α' , b would increase exponentially with a decrease in α' . If the end to end length decreases with a decrease in α' , then the increase in b would not be so drastic.

If the charge spacing were known for a polyelectrolyte, the quantity of expected condensed counterions could be calculated from Eq. (49). For ill-defined polyelectrolytes whose charge spacings are not well known (such as humic substances), the charge spacing can be calculated if the quantity of condensed counterions were known through rearrangement of (49) to give

$$b = 7.135 \text{ \AA} \left(1 - \frac{xm_{cc}}{m_A} \right) \quad (52)$$

Important equations for the thermodynamic properties of ions associated with polyelectrolytes can be derived from counterion condensation theory (Manning, 1969). Equations derived from counterion

condensation theory are limiting laws that are valid only at low I . The mean ionic activity coefficient, $\gamma_{\pm}$, for a uni-univalent salt in the presence of a polyelectrolyte can be derived as:

$$\gamma_{\pm} = \exp \left[-\frac{1}{2} \xi X (X + 2)^{-1} \right], \quad \xi \leq 1 \quad (53)$$

$$\gamma_{\pm} = \frac{\xi^{-1} X + 1}{X + 1} \exp \left[\frac{-\xi^{-1} X}{2 (\xi^{-1} X + 2)} \right], \quad \xi > 1 \quad (54)$$

where X is equivalent to $m_A^{\alpha}/m_N^{\alpha}$.

The limiting laws shown in Eqs. (53) and (54) for polyelectrolyte solutions are completely different than the limiting laws for simple electrolyte solutions. As the salt concentration increases in a polyelectrolyte solution, X decreases and $\gamma_{\pm}$ increases. In simple electrolyte solutions, $\gamma_{\pm}$ decreases with increased salt concentration. From these observations, the general conclusion can be made that the activity levels of ions in polyelectrolyte solutions at low I are controlled more by the polyelectrolyte than the added salt.

The Donnan coefficient (Γ) for a uni-univalent salt can also be derived as a function of ϵ (Devore and Manning, 1974):

$$\Gamma = \sum_{i=1}^{\infty} A_i \left(\frac{1}{Y} \right)^{i-1} \quad (55)$$

where A_i are the following for $\epsilon \leq 1$:

$$\begin{aligned} A_1 &= \frac{1}{2} (1 - \frac{1}{2} \xi); & A_2 &= -\frac{1}{8} (1 - \frac{1}{4} \xi^2); & A_3 &= \xi (\xi - \frac{1}{6} \xi^3) \\ A_4 &= \frac{1}{128} (1 - \frac{9}{2} \xi^2 + \frac{9}{16} \xi^4); & A_5 &= -\frac{1}{16} (\frac{1}{4} \xi - \frac{1}{3} \xi^3 + \frac{1}{30} \xi^5) \end{aligned} \quad (56)$$

The values of A_i are the following for $\epsilon > 1$:

$$\begin{aligned} A_1 &= \frac{1}{4} \xi^{-1}; & A_2 &= -\frac{3}{32} \xi^{-2}; & A_3 &= \frac{5}{96} \xi^{-3} \\ A_4 &= -\frac{47}{2048} \xi^{-4}; & A_5 &= \frac{1}{320} \xi^{-5} \end{aligned} \quad (57)$$

Equation (57) is only valid for high values of Y .

Devore and Manning (1974) have derived another equation for low values of Y :

$$\Gamma = Y \left(1 - \sum_{i=1}^{\infty} a_i Y^i \right) \quad (58)$$

The values of a_i are the following for $\epsilon \leq 1$:

$$a_1 = e^{\xi}; \quad a_2 = 0; \quad a_3 = e^{2\xi} (1 + 2\xi) \quad (59)$$

The value of a_i are the following for $\epsilon > 1$:

$$a_1 = e\xi; \quad a_2 = 0; \quad a_3 = -3e^2 \xi^3 \quad (60)$$

Equations (53), (54), (55), and (58) are of practical significance in the study of counterion condensation on ill-defined humic substances. If the Donnan coefficient or mean ionic activity coefficient are known along with Y and X , ϵ can be calculated. The calculation of ϵ is straightforward from Eq. (53) where:

$$\xi = -((X + 2) / 2 \ln \gamma_{\pm}) / X, \quad \xi \leq 1 \quad (61)$$

Calculation of ϵ from Eqs. (54), (55), and (58) requires an iterative

technique based of successive approximations of ϵ until both sides of the equation are identical. The charge spacing, b , can then be determined from ϵ in Eq. (44).

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5.2 Apparent Radius of Humic Molecules from Acid-Base Titration and Nitrate Exclusion Data

P. Barak and Y. Chen

Humic substances are natural, organic substances ubiquitous in soil and sediments. Humic substances have molecular weights of 500 to 100,000 and are believed to be long-chain molecules which are fully expanded at neutral or alkaline conditions because of mutual repulsion of charged acidic groups (Stevenson, 1982). Unlike most other macromolecules of biological origin such as proteins and nucleic acids, these molecules are heterogenous in size and chemical composition, and so can usually be treated only in the aggregate by means of averages. Soil-derived humic substances are generally considered to have a high aromatic carbon content and a high content of weakly acidic (carboxylic) and very weakly acidic (phenolic and amine) functional groups. Humic substances are amenable to standard measurements conducted on macromolecules, such as potentiometric titration, viscometric measurements, and electron microscopy but interpretation of the results of such tests must consider the heterogenous nature of humic substances.

For a simple carboxylic acid, the equation for dissociation may be written as:

(1)

where:

(2)

$$K = (H^+) (RCOO^-) / (RCOOH) \quad \text{and} \quad pK = pH - \log[(RCOO^-) / (RCOOH)]$$

However, if a double electric layer is present, then the appropriate equation is:

(3)

$$K = (H^+)_s (RCOO^-) / (RCOOH) \quad \text{and} \quad pK = pH_s - \log[(RCOO^-) / (RCOOH)]$$

where $(H^+)_s$ is the hydrogen activity at the acidic site on the surface of the molecule and is given by:

$$(H^+)_s = (H^+) \exp(-\phi_s) \quad \text{and} \quad pH_s = pH + 0.434 \phi_s \quad (4)$$

where ϕ_s is the dimensionless surface electric potential ($e\phi_s/kT$) and $\log_{10}e = 0.434$. Combining the two expressions yields:

$$pK = pH + 0.434 \phi_s - \log[(RCOO^-) / (RCOOH)] \quad (5)$$

If the surface of the macromolecule is covered with acidic groups of the same type, then pK is an intrinsic property of the molecule and is not changed by the degree of ionization or ionic strength. Instead, as the surface potential ϕ_s changes to reflect ionic strength of the solution and charge density on the macromolecule surface, the pH of the bulk solution at a given degree of ionization changes as well to reflect the constancy of the intrinsic pK . The surface potential ϕ_s may be calculated from the Poisson-Boltzmann equation:

$$\frac{d^2\phi}{dx^2} + \frac{g}{x} \frac{d\phi}{dx} = \kappa^2 \sinh \phi \quad (6)$$

with the boundary conditions:

$$\left. \frac{d\phi}{dx} \right|_{x=s} = 4\pi\sigma e / \epsilon k T \kappa \quad \text{and} \quad \left. \frac{d\phi}{dx} \right|_{x=\infty} = \phi(\infty) = 0 \quad (7)$$

where x is distance from the charged surface s to infinity, g is a geometric factor equal to 0 for a charged plane, 1 for a cylinder, and 2 for a sphere, σ is the charge density, and $\kappa = (8\pi e^2 I N / 1000 \epsilon k T)^{1/2}$ for a 1:1 electrolyte where I is the solution concentration (mol/L) and N is Avogadro's number. Analytical solutions exist for the planar case and an analytical approximation exists for the cylindrical case; however, exact solutions for the cylindrical and spherical case are semianalytical or numerical solutions.

Experimental evidence collected to date strongly suggests that humic substances in solution have a strand-like "tertiary" structure, often described as a random coil (Hayes, 1986). Cameron et al. (1979) determined the molecular weight by ultracentrifuge sedimentation velocity of a number of humic acids fractionated by gel chromatography; on the same substances, diffusion coefficients were measured, permitting the calculation of intrinsic friction coefficients. The experimental relationship between the friction coefficients and molecular weight fitted the behavior expected of either a flexible random coil or a disk shape, ruling out spheres and oblate or prolate ellipsoids. Chen and Schnitzer (1976a) considered the problem of the dimension and geometry of humic molecules using viscometric techniques. The viscometric behavior of both humic and fulvic acid solutions was found to exclude

spherical geometry, while stereochemical considerations ruled out a disk shape, leaving a rod geometry as the most likely approximation. Scanning electron microscopy of dry preparations seems to support rod-like geometry (Chen and Schnitzer, 1976b), as do additional viscometric measurements (Ghosh and Schnitzer, 1980).

For purposes of calculation of phenomena related to the electric double layer, humic substances may be modelled as an infinite cylinder with charges arranged on the surface. The semi-infinite length is acceptable if the cylinder is suitably long and the radius is suitably small so that end effects are negligible. Ionized groups are hydrophilic and are likely to be located at the interface of the humic substance with the solution whereas hydrophobic portions of the molecule are likely to arrange themselves in the interior of the molecule. Although the uncharged molecule may be coiled, when ionized, electrostatic repulsion between ionized groups is likely to extend and straighten the molecule to open configurations.

As mentioned above, no analytical solution exists for the double electric layer of a charged cylinder. However, the use of a linearized version of the Poisson-Boltzmann equation (eq. 11), setting $\sinh \phi$ equal to ϕ , is common; this approximation is based upon the expansion:

$$\sinh \phi = \phi + \phi^3/3! + \phi^5/5! + \dots$$

and only holds for $\phi \ll 1$. The resulting equation is solved by a modified Bessel function, which is basic to the solution of many problems of heat diffusion (Carslaw and Jaeger, 1959) and diffusion (Crank, 1956) in cylindrical systems, as well as for their original use

by F.W. Bessel in the 1820s for calculating Keplerian laws of planetary systems. The solution is of the general form $\phi(x) \propto K_0(\kappa x)$ where $K_0(\kappa x)$ is a modified Bessel function of zero order (Dube, 1943). This solution in a cylindrical system is analogous to the approximation $\phi(x) \propto \exp(-\kappa x)$ in planar systems for $\phi \ll 1$, (van Olphen, 1977) especially considering that the polynomial expansion for the modified Bessel function is:

$$K_0(\kappa x) = \exp(-\kappa x) [1.253(\kappa x)^{-1/2} - 0.157(\kappa x)^{-3/2} + 0.219(\kappa x)^{-5/2} \dots]$$

where $\kappa x > 2$ (Olver, 1964). The Bessel function is the basis of approximations of the double electric layer of charged cylinders (Brenner and McQuarrie, 1973) and also serves as the basis for various semianalytical, numerical solutions of the Poisson-Boltzmann equation (Sugai and Nitta, 1973; Stigter, 1975)

The aim of this chapter is to characterize the acidic groups of a humic acid and to match the behavior of the weakly acidic group to that of an ideal cylinder of infinite length, specified radius, and an ideal double electric layer. After the apparent radius of the humic acid is determined from acid-base titration, the ideal predicted anion exclusion will be compared to the nitrate exclusion data collected in the previous section.

MATERIALS AND METHODS

Humic acid:

Humic acid was extracted according to the procedure recommended by the International Humic Substance Society (1982) from a peat soil from the Hula Valley, Israel (pH 3.8 in 1 M KCl, 69% organic matter). The soil was shaken overnight with 0.1 M NaOH under N_2 with a 10:1 extractant:soil ratio. The extract was centrifuged to remove suspended matter. The pH of the extract was adjusted to 2 with HCl and the humic acid precipitate was collected by centrifugation. The humic acid was redissolved in 0.1 M KOH + 0.3 M KCl, followed by reprecipitation with HCl. The humic acid was then treated with 0.1 M HCl + 0.3 M HF for 7 days at which time the ash content was less than 1%. The purified humic acid was then dialyzed against deionized water to remove free acid and salt, and lyophilized.

The particle density of humic acid was measured by pycnometer (Chen and Schnitzer, 1976a).

Titration procedure:

A stock solution of 0.30% w/w humic acid was prepared by dissolving humic acid in NaOH followed by dialysis against an exterior solution of 2.5 mM HCl. A series of titrations of humic acid was conducted at ionic strengths varying from approximately 2 mM to 100 mM NaCl. Fixed quantities of NaCl and HCl solutions were added to the humic acid stock solution to produce 0.25% w/w humic acid solutions with initial pH values between 2.5 and 3.0; no coagulation was apparent. Eight mL aliquots of the solutions were titrated with 0.05 ml increments of 0.100

M NaOH using a Metrohm 665 Dosimat. Measurements of pH were made with a Metrohm 6.0203.000 combined pH glass electrode. When the reading did not vary by 0.01 pH unit over 3 minutes, the reading was taken as final and the next increment of base added.

Titration analysis:

Titration curves were analyzed by the procedure of Takamatsu and Yoshida (1978). Gran's plot calculations were performed to determine the strong (free) acid equivalence point, that point at which the strong acid residual from sample preparation has been titrated and at which the titration of the humic acid has begun, as follows:

$$(V_0 + V)[H^+] \approx m(V_S - V) \quad (8)$$

where V_0 = initial volume of sample

V = volume of titrant base

m = concentration of titrant base

V_S = volume of titrant base equivalent to the strong acid

The strong acid equivalent was obtained by plotting $(V_0 + V)[10^{-pH}]$ against V .

The total acid equivalent point was determined using the Gran plot for the high pH region, as follows:

$$(V_0 + V)[OH^-] \approx m(V - V_e) \quad (9)$$

where V_e = volume of titrant base equivalent to total acidity. Therefore, $(V_0 + V)[10^{pH-14}]$ was plotted against V to obtain total acidity.

Differentiation between weak and very weak acidic groups was made by determination of the point of maximum inflection of the titration curve; the volume of titrant base equivalent to the strong + weak acid equivalency point is V_w .

Henderson-Hasselbach plots of the weakly acidic functional group were calculated from the titration data as follows:

$$pH = pK + n \log [(RCOOH)/(RCOO^-)] \quad \text{or}$$

$$pH = pK + n \log \left[\frac{m(V_w - V) - ([H^+] - [OH^-])(V_0 + V)}{m(V - V_s) + ([H^+] - [OH^-])(V_0 + V)} \right] \quad (10)$$

This form of the Henderson-Hasselbach equation considers explicitly the presence of free strong acid at the beginning of the titration and volume changes during the course of the titration due to addition of base. In the case of a simple monobasic acid, n reduces to one; in the case of no weak acid titrated at all, the equation describes the course of titration of strong acid with strong base (Ricci, 1952).

Surface Potential Calculations:

The Poisson-Boltzmann equation for distribution of electric potential about an infinitely long charged cylinder was solved with either the semianalytical solution of Sugai and Nitta (1973) or numerically using the Adams method (Hildebrand, 1956) if the

semianalytical solution failed to converge within the first nine terms. The semianalytical solution was of the form:

$$\phi(Kx) = \sum f_{2n-1} (C * K_0(Kx))^{2n-1} \quad (11)$$

where $n = 0, 1, 2, \dots, 9$, f_{2n-1} is a numerically integrated constant, $K_0(Kx)$ is a modified Bessel function of zero order, and C is a constant whose value is determined iteratively by the Newton-Raphson method using the boundary condition at the surface.

The Adams predictor-corrector method used to numerically solve the differential equation replaces the second-order differential equation with a set of two first-order equations in ϕ and ϕ' ($d\phi/dx$) using ϕ' and ϕ'' ($d^2\phi/dx^2$), as follows:

$$\phi(I) = \phi(I-1) + \Delta x * [9\phi'(I-1) - 5\phi'(I-2) + \phi'(I-3)]/24 \quad (12)$$

$$\phi'(I) = \phi'(I-1) + \Delta x * [9\phi''(I-1) - 5\phi''(I-2) + \phi''(I-3)]/24 \quad (13)$$

where $I = 0, 1, 2, \dots$, Δx is the size of the x increment, and $x = I * \Delta x$. The equations are solved together first in open form based on previous values of $\phi(x)$ and $\phi'(x)$ and then solved iteratively in closed form based on current and previous values, using the formulas of Table 1.

The Adams predictor-corrector formulas are not self-starting. The Taylor series up to $\phi^{(4)}(a)$ was used to generate the first step. The Adams formulas were started at the second step using a first difference predictor and second difference corrector. The third step used a second difference predictor and third difference corrector. The fourth and subsequent steps used a third difference predictor and corrector,

yielding fourth-order accuracy. Truncation error is due only to terms including $\phi^{(5)}$ and higher derivatives.

The Adams predictor-corrector method actually solves initial value problems, i.e., $\phi(a)$ and $\phi'(a)$ are known. Since the differential equation is to be solved as a boundary value problem with one criterion at $x = a$ and $u = \infty$ each, a value of $\phi(a)$ was chosen and the solution was continued until either $\phi(x)$ or $\phi'(x)$ reversed sign. The first estimate was found to be conveniently taken as the surface potential calculated analytically for the planar case. If $\phi(x)$ reversed sign, this was taken as indicative of an overly small $\phi(a)$ and the calculations were repeated with a larger $\phi(a)$. If $\phi'(x)$ reversed sign, $\phi(a)$ was decreased and calculations repeated. The optimal value of $\phi(a)$ was considered that value which permitted the calculation of $\phi(x)$ to the greatest distance from the surface before $\phi(x)$ or $\phi'(x)$ changed sign. A search algorithm was written to automatically choose new values of $\phi(a)$ until changes in $\phi(a)$ were below the desired level of accuracy and iterations stopped.

As a check, the excess charge at each increment was calculated as a function of potential and integrated numerically as a cylindrical shell from $x = a$ to the point at which either $\phi(x)$ or $\phi'(x)$ reversed sign using Simpson's rule. Calculations were performed in Microsoft Basic 2.0 on an IBM-PC. A program listing and tests of algorithm accuracy are included as an appendix.

Anion Exclusion Calculations:

The electric potential as a function of distance from the humic surface was calculated for a number of discrete degrees of ionization using the semianalytical equation of Sugai and Nitta (1973) for a range

of ionic strengths from 1 mM to 100 mM; humic radii were taken as those producing the best fit of dependence of measured acid strength and theoretical surface potential on ionic strength (see Results and Discussion, Cation Condensation). The anion deficit relative to the equilibrium solution was calculated and integrated as follows:

$$A_{ex} = 2\pi a \int_0^{\infty} (1 - \exp(\phi(x))) \times \Delta x$$

$$L = 1 / \pi a^2 p$$

$$V_{ex} = A_{ex} L = \frac{2}{a^2 p} \int_0^{\infty} (1 - \exp(\phi(x))) \times \Delta x$$

where : A_{ex} = anion exclusion volume per cross-sectional area

x = distance from cylinder axis (cm)

$\phi(x)$ = dimensionless potential at x

L = cylinder length per unit weight

a = radius of cylinder (cm)

p = density of cylinder ($\text{cm}^3 \text{g}^{-1}$)

V_{ex} = anion exclusion volume ($\text{cm}^3 \text{g}^{-1} \text{HA}$)

Integration of $(1 - \exp(\phi(x)))x$ from the cylinder radius a to a distance of $12/K$ was performed numerically using the cubic spline algorithm described by de Boor (1978); at this distance, the reduced potential was of the order of 10^{-6} , and the anion deficit $(1 - \exp(\phi(x)))$ was of the same order of magnitude; calculations were therefore terminated with negligible truncation error.

Table 1. Formulas used for Adams predictor-corrector method, where $y = \phi$ or ϕ' , as appropriate. For $I \geq 2$, the equivalent formulas are constructed for $y''(I)$. The predictor formulas (P) are solved once for each I whereas the corrector formulas (C) are solved iteratively.

$I = 1:$

$$y(I) = y(a) + y'(a)*\Delta x + y''(a)*\Delta x^2/2! + y^{(3)}(a)*\Delta x^3/3! + y^{(4)}(a)*\Delta x^4/4!$$

$$y''(a) = \sinh(y_a) - y'(a)*(Ka)^{-1}$$

$$y^{(3)}(a) = y'(a) * (\cosh(y_a) + (Ka)^{-2}) - y''(a)*(Ka)^{-1}$$

$$y^{(4)}(a) = y'(a) * (\sinh(y_a)*y'(a) - 2*(Ka)^{-3}) + y''(0) * (\cosh(y_a) + 2*(Ka)^{-2}) - y^{(3)}(a)*(Ka)^{-1}$$

$I = 2:$

$$P: y(I) = y(I-1) + \Delta x*[36y'(I-1) - 12y'(I-2)]/24$$

$$C: y(I) = y(I-1) + \Delta x*[10y'(I) + 16y'(I-1) - 2y'(I-2)]/24$$

$I = 3:$

$$P: y(I) = y(I-1) + \Delta x*[46y'(I-1) - 32y'(I-2) + 10y'(I-3)]/24$$

$$C: y(I) = y(I-1) + \Delta x*[9y'(I) + 19y'(I-1) - 5y'(I-2) + y'(I-3)]/24$$

$I = 4:$

$$P: y(I) = y(I-1) + \Delta x*[55y'(I-1) - 59y'(I-2) + 37y'(I-3) - 9y'(I-4)]/24$$

$$C: y(I) = y(I-1) + \Delta x*[9y'(I) + 19y'(I-1) - 5y'(I-2) + y'(I-3)]/24$$

RESULTS AND DISCUSSION

Characterization of Acidic Groups:

The strong acid equivalence point, at which the titration of humic acid commences, was determined by Gran plot calculations and the end of the titration of the weakly acidic groups (and the beginning of the titration of the very weakly acidic groups) was determined by the point of maximum pH inflection of fitted cubic splines. The difference between these two points was taken as the weak acid content of the humic acid, and is generally regarded as due to carboxylic functional groups.

Titration curves of Hula Valley humic acid were similar for all ionic strengths measured and no unusual features were made apparent by varying the ionic strength (Fig. 1). Gran plot calculations permitted unambiguous extrapolation of the strong (free) acid and total acid equivalent points (Fig. 2). The measured contents of weakly acidic groups, very weakly acidic groups, and total acidic groups were not affected by the ionic strength during the course of the titration (Table 1). For a group of 11 soil-derived humic acids, Takamatsu and Yoshida (1978) found that weakly acidic groups were in the range of 1.42-3.23 meq g⁻¹, very weakly acidic groups 0.59-1.29 meq g⁻¹, and total acidity 2.41-4.51 meq g⁻¹. The Hula Valley humic acid has 2.39 meq g⁻¹ weakly acidic groups, 1.11 meq g⁻¹ very weakly acidic groups, and 3.51 meq g⁻¹ total acidity, and is therefore fairly typical of soil-derived humic acids.

In contrast to the acidic group content of the humic acid, the acidity of the weakly acidic groups, as shown by the Henderson-Hasselbach plot (Fig. 3), is clearly influenced by ionic strength. The

Fig. 1. Titration of Hula humic acid at several ionic strengths, 0.0927 M (1), 0.0266 M (2), 0.0135 M (3), 0.0046 M (4), 0.0018 M (5), and 0.0017 M (6). Curves have been displaced for purposes of display.

Fig. 2. Gran plot calculations for Hula humic acid. Sample labels as in Fig. 1. Curves have been displaced for purposes of display.

Fig. 3. Henderson-Hasselbalch plots as a function of ionic strength for six titrations of Hula humic acid.

apparent pK_a values decline from 5.6 to 4.9 as ionic strength increases from 2 mM to 93 mM (Table 2 and Fig. 4), but the slope of the Henderson-Hasselbach n was not appreciably affected by ionic strength. The pK_a values at 0.100 M ionic strength and n values for 11 soil-derived humic acids (Takamatsu and Yoshida, 1978) ranged from 4.63 to 5.10 and from 1.80 to 2.85, respectively; the values measured for Hula humic acid at 0.093 M, $pK_a = 4.93$ and $n = 2.07$, are in the middle of these ranges. The change in measured pK_a with ionic strength exceeds simple activity corrections which are approximately proportional to the square root of ionic strength. The slope indicated by Fig. 4 between 2 and 25 mM is -5.03, compared to -0.51 for a simple carboxylic acid or -1.53 for a simple dicarboxylic acid (Fig. 4, slopes labelled A and B, respectively; Albert and Sergeant, 1971). Posner (1964) found a slope of -4.01 for a soil-derived humic acid. Furthermore, using the simple carboxylic acid model, the intrinsic pK , in the absence of activity effects, by extrapolation to zero ionic strength indicates a pK of 5.9 for the humic acid; this value is exceptional for known simple carboxylic acids, 75% of which have a pK in the range of 2.5 to 5.5 (Perdue, 1985). This discrepancy clearly indicates that electrostatic phenomena related to the macromolecular nature of humic substances are present.

Cation Condensation:

The apparent radius of the humic macromolecule was calculated by assuming pK to be a function of the degree of ionization alone and independent of ionic strength effects; this concept considers pK to be an intrinsic characteristic of the molecule. For discrete degrees of

Fig. 4. Apparent pK at 50% ionization as a function of square root of ionic strength. Lines A and B show slopes predicted by Debye-Huckel activity equations for a monocarboxylic and dicarboxylic acid, respectively.

ionization of the carboxylic acid groups of Hula humic acid, a number of hypothetical radii for cylinders of infinite length were tested for the surface potential upon solution of equations 5, 6, 7, and 11. The sum $\text{pH} + 0.434 \phi_s$, where pH is the measured pH at the given degree of ionization and ionic strength, was tested for independence from ionic strength phenomena by plotting it against the square root of ionic strength (Fig. 5) and by calculating the variance of $\text{pH} + 0.434 \phi_s$ about a mean for all six ionic strengths measured (Fig. 6).

As seen in Fig. 5, an ideal cylinder with a radius of 0.45 nm or more and with the exact charge and particle density of Hula humic acid at 50% ionization of carboxylic acidity has a negative dependence of $\text{pH} + 0.434 \phi_s$ on ionic strength, whereas for a radius of 0.40 nm or less, the dependence on ionic strength is positive. However, as shown in Figs. 5 and 6, an ideal cylinder of 0.42 nm radius would possess a double electric layer, the surface potential of which changes with ionic strength to an extent which balances measured pH changes over the same ionic strength range within 0.02 pH units. Radii determined by the above procedure may be considered the best fit of measured pH values at different ionic strengths and degrees of ionization with ideal charged cylinders; similarly, the pK and $\text{pH} + 0.434 \phi_s$ values so derived may be considered to be intrinsic to the macromolecule itself since they are independent of ionic strength variations.

Best fit radii and intrinsic $\text{pH} + 0.434 \phi_s$ are summarized in Table 3 and Fig. 7 for ionization ranging from 12.5 to 75%. Of particular interest is that the apparent radii are similar but not identical over the range of ionization considered. The convergence of apparent radii to a narrow range over a wide range of acid ionization

Fig. 5. Effect of assumed apparent radius on $\text{pH} + 0.434 \phi_S$ at 50% ionization for the range of ionic strengths measured.

Fig. 6. Effect of choice of radius on variance of $\text{pH} + 0.434 \phi_S$ for 25, 50, and 75% ionization.

strengthens the validity of the treatment of humic substances as a charged cylinder. The apparent radius decreases from 0.85 nm to 0.35 nm as acid ionization increases from 12.5% to 75%, which seems to be the result of configurational changes in humic acid resulting from increasing the quantity of negative charge and the extension and subsequent thinning of the humic macromolecule resulting from the electrostatic repulsion between ionized acidic groups.

When the intrinsic $\text{pH} + 0.434 \log c_s$ is plotted against $\log[(\text{RCOOH}/\text{RCOO}^-)]$ (see eq. 11) in Fig. 8, the slope of the resulting Henderson-Hasselbach plot, even in its intrinsic form, shows that the carboxylic functional group is not of a single type since the slope is not equal to unity. This finding agrees with numerous chemical degradation studies which show a variety of types of carboxylic acids in humic acids, each of which is likely to have its own pK , regardless of macromolecular behavior. Therefore only the average or aggregate behavior is measured during titration. For illustrative purposes, the data in the intrinsic Henderson-Hasselbach plot was resolved mathematically into two ideal carboxylic acids. The titration curve of two weak acids titrated by strong base is described mathematically as follows:

$$\text{H}^+ - \text{OH}^- = \frac{a_1 K_1}{K_1 + \text{H}^+} + \frac{a_2 K_2}{K_2 + \text{H}^+} - b_s \quad (14)$$

where a_i and K_i are the concentration and dissociation constant of weak acid i and b_s is the concentration of strong base added (Ricci, 1952).

Rearrangement of the above equation gives a quartic equation for H^+ :

Fig. 7. Apparent radius and $\text{pH} + 0.434\phi_s$ as a function of degree of ionization.

Fig. 8. Intrinsic Henderson-Hasselbalch plot (symbols) and resolution by a mixture of two weak acids, 1.22 meq g^{-1} and $\text{pK} = 3.59$ and 1.17 meq g^{-1} and $\text{pK} = 5.32$ (solid line).

$$\begin{aligned}
 & H^4 + H^3 [K_1 + K_2 + b_5] + H^2 [K_1 K_2 - W + (K_1 + K_2) b_5 - a_1 K_1 - a_2 K_2] \\
 & - H [(K_1 K_2 (a_1 + a_2 - b_5) + W (K_1 + K_2))] - K_1 K_2 W = 0
 \end{aligned}
 \tag{15}$$

where W is the dissociation constant of water. The equation was solved for $H^+(b_5)$ by the Newton-Raphson method and pH_{calc} was fitted by least squares to pH_{int} by adjusting a_1 , K_1 and K_2 (a_2 by difference from total carboxylic acidity). The results of curve-fitting indicate 1.22 meq g^{-1} of a type I acid with pK 3.59 and 1.17 meq g^{-1} of a type II acid with pK 5.32. The resulting behavior is superimposed on the intrinsic Hasselbach-Henderson plot in Fig. 8. No evidence exists that only two types of discrete carboxylic acids do indeed compose humic acid; the intrinsic pH approach however produces no problem of unresolvable data since any model with two or more types of acidic group or other assumptions regarding acid type distribution will be able to fit the data.

Anion Exclusion:

In a planar system, the exclusion volume is proportional to $1/\kappa$, the Debye-Huckel length; the coefficient of proportionality is equal to the particle surface area multiplied by a coefficient dependent upon the valencies of the cations and anions in solution (Schofield, 1947; Bolt and de Haan, 1979).

$$V_{ex,a} = S (Q / \sqrt{\beta} C_0 - \delta)$$

where $V_{ex,a}$ = experimental exclusion volume of ion a

S = specific surface area

Q = a constant derived from the solution of an exact integral, 2 for a 1:1 electrolyte solution, and 1.04, 2.45, and 1.41 for a 2:1, 1:2, and 2:2 electrolyte solutions, respectively

$\sqrt{\beta C_0}$ = $\sqrt{(8\pi e^2/\epsilon kT \times \text{total electrolyte concentration in equilibrium solution (eq/L)})}$

δ = a cutoff distance, usually between 0.2-0.4 nm from the surface.

Of interest is that $\sqrt{\beta C_0}$ is related to $\sqrt{\beta I_0} \equiv \kappa$, where I_0 is the ionic strength of the equilibrium solution. As mentioned above, there is no analytical solution of the double electric layer in a cylindrical system and the calculation of Q as a single-valued parameter Q for each type of electrolyte is not possible.

When the theoretical exclusion volumes calculated from the proton condensation values are plotted against $1/\kappa$, a curvilinear function is found for each radius and degree of dissociation (Fig. 9). When theoretical exclusion volumes are expressed as a function of $1/\kappa^2$ (units of distance²), linear functions are obtained with zero intercepts, with a slope characteristic of each degree of ionization (charge density) and radius (Fig. 10). This linear relationship is in retrospect not surprising since in the planar system, $V_{ex} \propto 1/\kappa$ and the exclusion distance from the plane d_{ex} is proportional to $1/\kappa$. Therefore in a cylindrical system where $V = \pi r^2$, it would be expected that $V_{ex} \propto 1/\kappa^2$. The proportionality factor of V_{ex} against $1/\kappa^2$ has units of length and in geometric terms represents the length of the cylinder with the excluded cross-sectional area proportional to $1/\kappa^2$. Since the length per unit weight of the cylinder is equal to $1/p\pi a^2$, where p is the

Fig. 9. Predicted anion exclusion volume ($\text{cm}^3 \text{g}^{-1}$) plotted against Debye-Huckel distance $1/K$, based on acid-base behavior.

Fig. 10. Predicted anion exclusion volume ($\text{cm}^3 \text{g}^{-1}$) plotted against $1/K^2$

density, it would be expected that V_{ex} is also proportional to $1/a^2$. If, indeed, the theoretical exclusion distance is plotted against $1/(\kappa a)^2$, a dimensionless quantity, all predicted exclusion volumes fall on approximately the same line (Fig. 11). From this relationship, it is clear that within the limits considered here for the cylindrical case, as for the planar case (Bolt and de Haan, 1979), charge density has little role in the dependence of anion exclusion volume upon surface area.

Apparent radii based on nitrate exclusion can be extracted from the experimental data based on the linear function in Fig. 11, and are presented in Fig. 12. The data show greater scatter than the radii from the acid-base calculations but the agreement is surprisingly close. No assumptions were made a priori regarding the size of the radius of humic molecules treated mathematically as an infinite cylinder either for the case of cation condensation or anion exclusion, but both types of experimental data converge on an apparent radius of 0.9 - 1.3 nm at low levels of dissociation (low pH) declining gradually to 0.3 - 0.6 nm at high levels of dissociation of the carboxylic functional group (neutral pH).

The dimensions of the humic macromolecule fall in the range of those of a number of other fibrous biomacromolecules. For example, the double helix of DNA has a radius of 1.0 nm. Of the fibrous proteins, tropocollagen, the subunit of collagen fibrils, is a triple helix whose overall length is about 300 nm with a radius of 0.75 nm (Lehninger, 1982). The ubiquitous α -helix, a structural constituent of numerous proteins, has a radius of 0.23 nm, not including residues (Schultz and Schirmer, 1979). It should be born in mind that the biomolecules of

Fig. 11. Predicted anion exclusion volume ($\text{cm}^3 \text{g}^{-1}$) plotted against $1/(\text{Ka})^2$.

Fig. 12. Apparent radius of humic molecules based on nitrate exclusion (crosses and squares) compared with radii measured by acid-base behavior. Cation condensation results extrapolated (---) for illustrative purposes.

proteins and DNA differ essentially from those of humic substances because the proteins and DNA have a well-defined, repeating structure whereas the humic substances are heterogenous.

For the sake of simplicity, the above calculations, both of cation condensation and anion exclusion, neglect the existence of discrete charges on the cylinder surface and in solution. Activity of ions is approximated by concentration. No conformational changes in the humic acid are modelled and density is considered to be constant over the range of pH and ion strengths considered. No information about the ends of the cylinder has been introduced into the model, which therefore assumes that the cylinder is infinite. Expanded double electric layers would be expected to cause some degree of truncation of the electric layers of adjacent particles. Some of these assumptions may be obviated by further refinements of numerical modelling.

Similarly, all calculations have been conducted on the carboxylic portion of the titration curve since the titration curve of the phenolic groups is often slowly attained and is open to some question (Tanford, 1962). The existence of cation binding to the humic macromolecule other than that by H^+ cannot be discounted out of hand, but Posner (1964) measured the pK (50% ionization) at 0.091 M for three alkali metals for a humic acid and reported values of 4.50, 4.47, and 4.50 for Li, Na, and K, respectively. This data would indicate that all three cations have almost identical binding constants or that all three cations are weakly bound to the carboxylic sites compared to H^+ in this pH range. Treatment has not been extended to divalent or trivalent cations, which are of great interest due to the reduction of charge density by cation adsorption, because the coagulation which occurs at low concentrations

of divalent and trivalent cations (Ong and Bisque, 1968) certainly reflects conformational changes which negate the assumption of cylindrical geometry.

CONCLUSIONS:

Acid-base titration data for a humic acid extracted from peat soil is not consistent with a model of a simple organic acid behaving in accordance with simple activity considerations. When the humic acid was considered to be a linear polyelectrolyte with cylindrical geometry and charge evenly distributed on the cylinder surface, solutions of the relevant Boltzmann-Poisson equations showed that cylinders of radius from 0.85 to 0.35 nm would show the same pH/ionic strength response as measured. The apparent radius measured by this method agrees closely with the apparent viscometric radius reported for other humic substances. The carboxylic groups, when cleared on behavior related to macromolecular nature, may be well-described as the result of two or more types of carboxylic groups.

Nitrate exclusion data for the same humic acid shows that the radius of a charged cylinder which would cause nitrate exclusion identical to that actually measured is strikingly similar to that measured for cation condensation. Radii measured are 0.9 - 1.3 nm at low degrees of ionization (low pH), gradually declining to 0.3 - 0.6 nm at near total ionization of the carboxylic functional group (neutral pH). The degree of convergence of results of two complementary phenomena may be regarded as enhancing the credibility of both the model

and the data for the humic acid employed herein. The thinning of the humic molecule with increasing ionization is consistent with prevailing views of humic substances as randomly coiled polyelectrolytes which extend due to ionic repulsion.

Based on viscometric behavior of humic substances analyzed with the Simha equation, Chen and Schnitzer (1976a) found that the measured particle radius of a humic acid and a fulvic acid was between 0.30 and 0.34 nm over the range of pH 3 to 10, while the particle length over the same range was 3.06 - 4.43 nm for the fulvic acid and 7.86 - 9.91 nm for the humic acid. The particle radii measured viscometrically were virtually identical for humic and fulvic acids and agree well with the apparent radius determined here potentiometrically (cation condensation) and chromatographically (anion exclusion); furthermore, the viscometric measurements show the particles to be sufficiently long that the assumption of infinitely long rods, and therefore negligible end effects, to be a reasonable approximation.

The dimensions of the humic macromolecule fall in the range of those of a number of other fibrous biomacromolecules, such as the DNA double helix (1.0 nm radius), tropocollagen triple helix (0.75 nm radius), and the α -helix (0.23 nm radius). It should be born in mind that the biomolecules of proteins and DNA differ essentially from those of humic substances because the proteins and DNA have a well-defined, repeating structure whereas the humic substances are heterogenous.

The dimensions of the peat soil-derived humic acid examined herein further suits several of the structural models which have been developed over the years by veteran researchers of humic substances. Given that the width of a benzene ring is about 0.25 nm, the experimental

dimensions are consistent with structural models of Dragunov (Kononova, 1966), Flaig (1960), Buffle (1977) and Stevenson (1982) [Fig. 13], especially when permitting strands to condense lengthwise at low pH. The small apparent radius of humic substances have thwarted but numerous attempts to prepare single-particle prepartes for electron microscopy and to resolve such particles once prepared. Additionally, the small radius must contribute significantly to the great flexibility of humic electrolytes and their ability to undergo conformational changes in the presence of high salt concentrations and even low concentration of di- and trivalent metals.

Fig. 13. Structural models of humic substances proposed by Dragunov (Kononova, 1966), Flaig (1960), Buffle (1977), and Stevenson (1982).

Table 2. Characteristics of Hula Valley humic acid.

Ionic Strength mol L ⁻¹	Weakly acidic group			Very weakly acidic group meq g ⁻¹	Total Acidity meq g ⁻¹
	meq g ⁻¹	pK _a	n		
0.0927	2.41	4.931	2.01	1.20	3.61
0.0266	2.37	5.114	2.04	1.16	3.53
0.0135	2.34	5.245	2.02	1.10	3.44
0.0046	2.39	5.485	2.15	1.04	3.43
0.0018	2.40	5.611	2.00	1.07	3.47
0.0017	2.46	5.620	2.20	1.10	3.56
average	2.39		2.07	1.11	3.51
standard deviation	0.04		0.08	0.06	0.07

Table 3. Results of application of double electric layer theory to humic acid.

Degree of Ionization	Apparent Radius (nm)	pH + 0.434p _s	Specific Surface Area (m ² g ⁻¹)	Area per Charge (nm ²)
		average ± st.dev.		
12.5%	0.85	3.336 ± 0.019	1460	7.8
25%	0.63	3.636 ± 0.020	1970	5.5
37.5%	0.50	4.067 ± 0.016	2480	4.6
50%	0.42	4.478 ± 0.021	2966	4.1
62.5%	0.40	4.823 ± 0.029	3105	3.4
75%	0.35	5.398 ± 0.033	3549	3.3

SYMBOLS AND VALUES

n	local ion concentration in number of ions/cm ³
σ	charge density in esu/cm ²
e	elementary charge = $4.77 \cdot 10^{-10}$ esu.
k	Boltzmann constant = $1.381 \cdot 10^{-16}$ erg °K ⁻¹
T	absolute temperature taken as 298°K
ϵ	dielectric constant of water at 298°K = 78.5
κ	$(8\pi n e^2 / \epsilon k T)^{1/2} = 3.29 \cdot 10^7$ JI for aqueous solutions at 25°
I_0	ionic strength of equilibrium solution (mol/l)
C_0	concentration of equilibrium solution (eq/L)
ϕ	reduced potential = $\phi e / k T$
x	distance from axis of cylinder
a	distance of cylinder surface from axis
ρ	density of water (w) or humic acid (HA)

5.2 APPENDIX

Numerical solution of the Poisson-Boltzmann equation for cylinders using the Adams algorithm for solution and Simpsons Rule for check.

```

10 NPTS=2000
20 DELX=.1/8
30 DEL=DELX/24
40 DIM Y(NPTS), Y1(NPTS), Y2(NPTS), Y3(10), Y4(10)
50 CLS
60 REM Program to extract K1 from equation for distribution
70 REM of electrical potential about a charged cylinder of
80 REM radius a and charge density sigma in environment of
90 REM kappa. Calculations in cgs and esu.
100 DEF FNSINH(Q)=(Q-1/Q)/2: REM q must be exp of y(I)
110 DEF FNCOSH(Q)=(Q+1/Q)/2: REM q must be exp of y(I)
120 REM PHYSICAL CONSTANTS
130 AVOGADRO=6.02E+23
140 E=4.77E-10
150 K=1.38E-16
160 T=298
170 EKT=E/(K*T)
180 EPSILON=78.3
190 PI=3.141592
200 REM CYLINDER PARAMETERS
210 A=.0000001: REM radius of cylinder
220 CEC=2.39/2: REM meq/g at half ionization
230 DENSITY = 1.61: REM g/cm3
240 SIGMA=A*CEC*DENSITY*AVOGADRO*E/2000
250 FACTOR =.95
260 FOR CONCOUNT = 1 TO 7
270 SQCONC= CONCOUNT*SQR(.1)/7
280 KAPPA= SQCONC/3.04E-08
290 N=KAPPA^2*K*T*EPSILON/(8*PI*E^2)
300 RADIUS=A*KAPPA
310 SIMP=DELX*E*N/(3*A*KAPPA*KAPPA)
320 I=0
330 Y1(0)=4*PI*SIGMA*EKT/(KAPPA*EPSILON)
340 QQ=Y1(0)/-2: Y0=2*LOG(QQ+SQR(QQ^2+1))
350 Y(0)=Y0*FACTOR
360 REM ENTRY POINT FOR NEW Y(0)
370 SINHY0 = FNSINH(EXP(Y(0))): COSHY0 = FNCOSH(EXP(Y(0)))
380 Y2(0)=SINHY0-Y1(0)/RADIUS
390 Y3(0)=Y1(0)*(COSHY0+1/RADIUS^2)-Y2(0)/RADIUS
400 Y4(0)=Y1(0)*(SINHY0*Y1(0)-2/RADIUS^3)-Y3(0)/RADIUS+Y2(0)/RADIUS^2
      +Y2(0)*(COSHY0+1/RADIUS^2)
410 U=EXP(-Y(0)): SUM=RADIUS*(U-1/U)*SIMP
420 REM PRINT RADIUS, Y(0), Y1(0), Y2(0)
430 REM
440 REM Taylor series approximation of first two new lines

```

```

450 REM
460 I=1
470 X=RADIUS+DELX
480 Y(1)= Y(0)+Y1(0)*DELX+Y2(0)*DELX^2/2+Y3(0)*DELX^3/6+Y4(0)*DELX^4/24
490 Y1(1)= Y1(0)+Y2(0)*DELX+Y3(0)*DELX^2/2+Y4(0)*DELX^3/6
500 Y2(1)=FNSINH(EXP(Y(1)))-Y1(1)/X
510 U=EXP(-Y(1)): SUM=SUM+4*X*(U-1/U)*SIMP
520 REM PRINT RADIUS+DELX,Y(1),Y1(1),Y2(1)
530 REM
540 REM     START ADAMS FORMULA
550 REM
560 REM     I = 2
570 REM     First difference Predictor and Second diff Corrector
580 REM
590 I = 2
600 X=RADIUS+2*DELX
610 REM
620 REM     Open formula (predictor)
630 REM
640 Y(I)=Y(I-1)+DEL*(36*Y1(I-1)-12*Y1(I-2))
650 Y1(I)=Y1(I-1)+DEL*(36*Y2(I-1)-12*Y2(I-2))
660 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
670 REM
680 REM     Closed formula (corrector)
690 REM
700 CY1=16*Y1(I-1)-2*Y1(I-2)
710 CY2=16*Y2(I-1)-2*Y2(I-2)
720 J=1
730 Y(I)=Y(I-1)+DEL*(10*Y1(I)+CY1)
740 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
750 Y1(I)=Y1(I-1)+DEL*(10*Y2(I)+CY2)
760 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
770 REM PRINT Y(I),Y1(I),Y2(I): STOP
780 IF J<5 THEN J=J+1: GOTO 730
790 U=EXP(-Y(2)): SUM=SUM+2*X*(U-1/U)*SIMP
800 REM PRINT X,Y(I),Y1(I),Y2(I)
810 REM
820 REM     I = 3
830 REM     Second diff Predictor and Third diff Corrector
840 REM
850 I = 3
860 X=RADIUS+3*DELX
870 REM
880 REM     Open formula (predictor)
890 REM
900 Y(I)=Y(I-1)+DEL*(46*Y1(I-1)-32*Y1(I-2)+10*Y1(I-3))
910 Y1(I)=Y1(I-1)+DEL*(46*Y2(I-1)-32*Y2(I-2)+10*Y2(I-3))
920 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
930 REM
940 REM     Closed formula (corrector)
950 REM
960 CY1=19*Y1(I-1)-5*Y1(I-2)+1*Y1(I-3)
970 CY2=19*Y2(I-1)-5*Y2(I-2)+1*Y2(I-3)
980 J=1

```

```

990 Y(I)=Y(I-1)+DEL*(9*Y1(I)+CY1)
1000 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
1010 Y1(I)=Y1(I-1)+DEL*(9*Y2(I)+CY2)
1020 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
1030 IF J<5 THEN J=J+1: GOTO 990
1040 U=EXP(-Y(3)): SUM=SUM+4*X*(U-1/U)*SIMP
1050 REM PRINT X,Y(I),Y1(I),Y2(I)
1060 REM
1070 REM      I > 3
1080 REM      Third diff Predictor and Corrector
1090 REM
1100 I=I+1
1110 X=RADIUS+I*DELX
1120 REM
1130 REM      Open formula (predictor)
1140 REM
1150 Y(I)=Y(I-1)+DEL*(55*Y1(I-1)-59*Y1(I-2)+37*Y1(I-3)-9*Y1(I-4))
1160 Y1(I)=Y1(I-1)+DEL*(55*Y2(I-1)-59*Y2(I-2)+37*Y2(I-3)-9*Y1(I-4))
1170 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
1180 REM
1190 REM      Closed formula (corrector)
1200 REM
1210 CY1=19*Y1(I-1)-5*Y1(I-2)+1*Y1(I-3)
1220 CY2=19*Y2(I-1)-5*Y2(I-2)+1*Y2(I-3)
1230 J=1
1240 Y(I)=Y(I-1)+DEL*(9*Y1(I)+CY1)
1250 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
1260 Y1(I)=Y1(I-1)+DEL*(9*Y2(I)+CY2)
1270 Y2(I)=FNSINH(EXP(Y(I)))-Y1(I)/X
1280 IF J<5 THEN J=J+1:GOTO 1240
1290 U=EXP(-Y(I)):IF INT(I/2)=INT((I-1)/2) THEN SUM=SUM+4*X*(U-1/U)
      *SIMP ELSE SUM=SUM+2*X*(U-1/U)*SIMP
1300 IF SGN(Y(I))=SGN(Y1(I)) GOTO 1370
1310 REM PRINT X,Y(I),Y1(I),Y2(I)
1320 GOTO 1100
1330 END
1340 REM
1350 REM      Upon sign reversal in y or y1, stops program
1360 REM
1370 IF SGN(Y(I))<>SGN(Y(0)) THEN PRINT:PRINT "Sign Change in y":
      GOTO 1500
1380 IF SGN(Y1(I))<>SGN(Y1(0)) THEN PRINT:PRINT "Sign Change in y'":
      GOTO 1490
1390 REM
1400 REM      This is a search routine for optimizing the potential at
1410 REM      at the b.c. through modification of the FACTOR
1420 REM
1430 XMAX=X: YMAX=Y(0):SUMMAX=SUM:GOTO 1450
1440 XMIN=X: YMIN=Y(0):SUMMIN=SUM:GOTO 1450
1450 IF ABS((YMAX-YMIN)/YMAX)<.0000002 GOTO 1530
1460 IF ABS(YMAX-YMIN)<.0000003 THEN Y(0)=YMIN+.0000001:GOTO 1500
1470 IF XMIN=0 THEN Y(0)=Y(0)*.95: GOTO 1500
1480 IF XMAX=0 THEN Y(0)=Y(0)*1.05: GOTO 1500
1490 Y(0)=(1#*YMAX*XMAX^4+YMIN*XMIN^4)/(XMAX^4+XMIN^4): REM double-prec

```

```

1500 PRINT "new y0 = "; Y(0),XMAX,YMAX,XMIN,YMIN
1510 I=0: SUM =0
1520 GOTO 360
1530 IF XMAX>XMIN THEN XREP=XMAX:SUMREP=SUMMAX:YREP=YMAX ELSE
      XREP=XMIN:SUMREP=SUMMIN:YREP=YMIN
1540 LPRINT A,SIGMA,KAPPA,YREP,XREP,SUMREP
1550 XMIN=0:XMAX=0:YMAX=0:YMIN=0
1560 NEXT CONCOUNT
1570 END

```

The above algorithm for calculation of the double electric layer potentials begins with an initial estimate of the surface potential ϕ_s based on the planar case. The calculation is continued until the $d\phi/dx$ reverses sign, whereupon the estimate of ϕ_s is reduced and calculations are restarted. If, during the course of calculations, ϕ or $d\phi/dx$ reverses sign then a new ϕ_s is calculated based on a weighted average of the latest values of ϕ_s which caused sign reversals of both types. This calculation extends further away from the charged surface as the value of ϕ_s is refined, approaching the true boundary condition at infinity where $\phi = d\phi/dx = 0$. As a check of the boundary condition at the cylinder surface, the countercharge in the double electric layer is numerically integrated and compared to the charge density specified by the model. Two cases are presented below in Figs. 1 and 2 to show the ability of the model to numerically maintain the boundary conditions while solving the differential equation. The inapplicability of the planar case for modelling the double electric layers of cylinders with small radii is apparent in both cases by the difference between the initial estimate and the final result.

Fig. 1. Results of the algorithm for calculation of the double electric layer for an infinite cylinder of 0.5 nm radius and a charge density of 13811.75 esu (equivalent to 2.39/2 meq/g charge and 1.61 g/cm³ density) in the presence of 0.1 mol/L 1:1 electrolyte. The best dimensionless surface potential ϕ_s was -1.351508, which permitted calculation to a distance of 9.4/ κ (9.0 nm) (A) with a charge balance deviation of 0.0006% (B).

Fig. 2. Results of the algorithm for calculation of the double electric layer for an infinite cylinder of 0.5 nm radius and a charge density of 13811.75 esu (equivalent to 2.39/2 meq/g charge and 1.61 g/cm³ density) in the presence of 0.001 mol/L 1:1 electrolyte. The best dimensionless surface potential ϕ_s was -3.680782, which permitted calculation to a distance of 8.5/ κ (81 nm) (A) with a charge balance deviation of 0.45% (B).

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6. ADSORPTION AND DESORPTION OF BORON ON HUMIC SUBSTANCES AND ITS EFFECTS
ON PLANT GROWTH

6.1 BORON ADSORPTION ON COMPOSTED ORGANIC MATTER

U. Yermiaho¹, R. Keren¹ and Y. Chen²ABSTRACT

Boron (B) adsorption and desorption on composted organic matter at various pH levels has been determined. The compost consisted of 158 mg g⁻¹ and 56 mg g⁻¹ humic acid and fulvic acid fractions, respectively, totaling 21.4% of the organic matter. At pH 8, the amount of adsorbed B increased by about 57% with an ionic strength increase up to 0.15 M. For solutions of CaCl₂ at concentrations lower than 5x10⁻³ M, the effect of CaCl₂ on B adsorption was negligible. Adsorption isotherms of B on organic matter were determined at pH levels of 7.0, 7.9, and 8.9. Adsorption increases with increasing B concentration in solution and pH.

A comparison of literature data for clays to those measured for the organic matter shows that adsorption on the latter at similar pH levels and total B concentration is at least times higher. A phenomenological equation based on a modified Langmuir equation was successfully used to fit the measured data. Based on the isotherms, adsorption coefficients were derived and used to calculate the predicted dependence on pH of B adsorption on organic matter at various total B levels. Desorption studies of B from organic matter at pH 8 and increasing levels of adsorbed B exhibited strong hysteresis effects and the adsorbed B was removed to a partial extent only. The formation of di-hydroxy and/or hydroxy-carboxy bonds of borate to humic substances and/or carbohydrates is proposed as an adsorption mechanism that agrees with the irreversibility of the adsorption process.

Additional Key Words: Boron adsorption, boron desorption, compost, organic matter.

1

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2

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Boron (B) is a toxic element to many plant species when present in soil solutions at concentrations that are only slightly greater than the optimal concentration required for plant nutrition. Therefore, it is necessary to maintain non-toxic B levels in soils irrigated with water exhibiting high B concentration or in soils containing high natural B levels.

Substantial work on B uptake by plants was conducted in sand cultures (Eaton, 1944; Eaton and Wilcox, 1939) and criteria for acceptable B in irrigation water were established. However, when assessing water quality for irrigation with respect to B the physico-chemical characteristics of the soil must be considered because interactions between B and the soil will most likely take place. Adsorption sites in soils act as a pool from which B is supplied to the soil solution or where B is adsorbed, depending on the changes in B concentration in the soil solution and on the soil affinity for B. The amount of B adsorbed by soils varies greatly with changes in soil constituents. Existing criteria, however, do not refer to soil type nor to organic matter (Keren and Bingham, 1985).

In semi-arid and arid soils the organic matter content is relatively low and therefore its contribution to B adsorption was considered small in comparison to that of clay minerals and hydrous oxides (Mezuman and Keren, 1981; Keren and Bingham, 1985). However, B retention by organic matter may be very important in sandy soils irrigated with water high in B or in soils with naturally high B content. One may hypothesize that amendment of such soils with organic matter may significantly magnify the importance of this soil constituent in maintaining low B concentration in the soil solution.

This hypothesis is based on a number of literature reports suggesting

B binding to soil organic materials. Most of these reports include qualitative information on the binding reactions. The importance of organic matter to B retention was firstly noted by Parks and White (1952). According to their observations, soil humus exhibits a chemical affinity for B and it seems to play an important role in the retention of B by soils. Berger and Pratt (1963) stated that a large part of the total B in soils is tightly bound to organic matter and that this fraction of B can be released to the soil solution in forms that are available to plants. The reactions between soil organic matter and anionic borate species are probably of a di-hydroxy or hydroxy-carboxy complex nature as proposed for manitol, tartaric acid, and for sugars (Kustin and Pizer, 1969; Huettl, 1976). These reactions are of the following nature:

Stevenson et al. (1952) report on the presence of various sugars such as galacturonic acid, xylose, mannose, arabinose, glucose and galactose in the hydrolysate of soil organic matter. These compounds meet the requirements for di-hydroxy complex formation with B. Most of these sugars have been demonstrated to react with boric acid (Boesehan, 1949). Other polymeric sugars are well-known to constitute a fraction of soil organic matter (Stevenson, 1982). It has been suggested (Huettl, 1976) that the Langmuir adsorption equation applies to B adsorption on organic matter.

Boron adsorption by clays and soils has been modeled by Keren et al. (1981). This model is based on the assumption that two B species - $B(OH)_3$ and $B(OH)_4^-$ of different affinities to the adsorbent are involved, their proportion in the equilibrium solution varies with pH, and that the affinity and the capacity of the adsorbent to the adsorbed species are pH independent. Since ligand exchange is a possible mechanism for B adsorption by organic matter, it is hypothesized that this model may describe B adsorption by organic matter as well. A brief description of the model is therefore presented.

Boron adsorption model

A phenomenological equation that assumes that $B(OH)_3$, $B(OH)_4^-$ and OH^- compete for the same adsorption site (Keren and Bingham, 1985). The adsorption coefficients K_{HB} , K_B , and K_{OH} for the three species $B(OH)_3$, $B(OH)_4^-$, and OH^- , respectively, were estimated using experimentally determined values for total amount of adsorbed boron, Q_{BT} (mol g⁻¹ organic matter) vs. equilibrium boron concentration (mol L⁻¹). The following form of the equation was used to estimate boron adsorption:

$$Q_{BT} = \frac{T [K_{HB}(HB) + K_B(B)]}{1 + K_{HB}(HB) + K_B(B) + K_{OH}(OH)} \quad (1)$$

where T is the maximum boron adsorption (mol g^{-1}), and (HB), (B), and (OH) are the solution activities of the species $B(OH)_3$, $B(OH)_4^-$, and $(OH)^-$, respectively.

Equation (1) can be rearranged to relate total amount of adsorbed boron, Q_{BT} , to total boron in the suspension, Q_T (adsorbed boron + boron solution), and to solution-to-adsorbent ratio, R:

$$Q_{BT} = T_i \left\{ 1 + \frac{PR}{F(Q_T - Q_{BT})} [1 + K_{OH}(OH)] \right\}^{-1} \quad (2)$$

Here $P = 1 + K_h \times 10^{14} (OH)$; K_h is the hydrolysis constant of the reaction

and $F = K_{HB} + K_B (P - 1)$.

Objectives of the present study were to determine the effect of pH and B concentration on B adsorption and desorption by organic matter and to test the adsorption model for organic matter.

MATERIALS AND METHODS

The organic matter used in this study was a matured compost produced from the solid fraction of separated straw-containing cattle manure that served as a simulation of soil organic matter. The chemical composition of a 1:10 compost:water extract is presented in Table 1. The ash content of the compost was 29%. Humic acid and fulvic acid fractions were extracted by four sequential extractions with 0.1N NaOH. The concentration of humic acid fraction based on total dry weight and on organic matter dry weight

Table 1. Some physical and chemical properties of the composted organic matter determined in a 1:10 (solids:water) extract.

Electrical conductivity	pH	Na ⁺	K ⁺	Ca ²⁺ +Mg ²⁺	Cl ⁻	SO ₄ ²⁻	NO ₃ ⁻	P
ds · m ⁻¹				mmol L ⁻¹				µg g ⁻¹
5.0±0.2	7.2±0.1	14.3±0.5	25.7±1.3	4.5±1.2	19.5±2.1	10.8±0.9	11.1±1.5	213.3±5

was 112 mg g⁻¹ and 158 mg g⁻¹ , respectively. The concentration of extracted fulvic acid fraction based on total dry weight and on organic matter dry weight was 40 mg g⁻¹ and 56 mg g⁻¹ , respectively. The total concentration of the humic substances fraction was therefore 21.4% of the organic matter. Further chemical details on the compost have been described by Inbar et al., (1986). The organic matter was mixed and stored at a temperature of 4°C in order to minimize chemical changes during the experiments.

To remove free electrolytes, the organic matter was leached 4 sequential times with deionized water at a solid to liquid ratio of 15, using a high speed centrifuge. After leaching, negligible concentrations of B were found in the equilibrium solution.

Boron adsorption was studied by shaking 2 g of washed organic matter in 50 ml polypropylene centrifuge tubes containing 30 ml of the experimental solution at 24° ± 2 °C. The suspensions were pH adjusted prior to B additions with HCl or NaOH until the pH was constant for at least 12 hrs. The suspensions were then equilibrated with CaCl₂ by successive washings with 5 x 10⁻³ M CaCl₂ solution at the appropriate pH. Washing was repeated until a steady state with regard to CaCl₂ concentration was achieved. The B concentration of the initial solutions ranged from 9.25x10⁻³ to 1.39x10⁻³ M. The suspensions were then brought to the final volume by the addition of an appropriate amount of 5x10⁻³ M CaCl₂ solution with the desired pH. The volume of solution that remained in the organic matter after centrifugation was corrected for, in the calculations. The measurements were performed in triplicate.

Preliminary adsorption experiments performed as long as 48 hrs indicated that adsorption and desorption reactions were practically

complete after 3 and 24 hrs, respectively. After equilibration time, the suspensions were centrifuged and active carbon was added to an aliquot of the supernatant to remove soluble organic molecules from the solution. The concentration of soluble organic matter at the tested ionic strength and pH was negligible. Preliminary tests showed that B was not adsorbed on the active carbon. Boron was determined colorimetrically using the Azomethine-H method (Gupta and Stewart, 1975). The amount of adsorbed B was calculated by the difference between the amount added and that found in solution at equilibrium.

Desorption was initiated from samples formerly used for adsorption studies at pH 8 by replacing 20 ml of a removed aliquot with 20 ml of B-free 5×10^{-3} M CaCl_2 solution. The mixtures were resuspended by vigorous agitation and equilibrated on a shaker for 24 hrs. The suspension was centrifuged and 20 ml of the supernatant was removed for B analysis. This procedure was repeated three times thus resulting in three desorption steps for each sample.

RESULTS AND DISCUSSION

Boron adsorption on composted organic matter in CaCl_2 solution at various levels of ionic strength, pH 8 and at a given B content (Q_T) of 8.32×10^{-2} mol Kg^{-1} organic matter is presented in Fig. 1. The results indicate that the amount of adsorbed B increases by about 57% with an ionic strength increase from 0 up to 0.15 M. For solutions of CaCl_2 concentrations lower than 5×10^{-3} M, however, the effect of CaCl_2 on B adsorption is negligible. Reardon (1976) reported that borate ions form soluble complexes with alkali and alkaline earth cations. Thus, the formation of borate complexes in solution may affect the adsorption of B on organic matter. It is possible that the affinity of the organic matter to

Fig. 1. Adsorbed B on composted organic matter vs. ionic strength (CaCl_2) at pH 8 for a given B content ($8.32 \times 10^{-2} \text{ mol Kg}^{-1}$). Bar indicates the largest standard deviation.

$\text{CaB}(\text{OH})_4^+$ ion pair species is stronger than that for $\text{B}(\text{OH})_4^-$ at this pH and therefore the amount of adsorbed B increases as the CaCl_2 concentration in solution increases.

Boron adsorption isotherms on organic matter at various pH values and 5×10^{-3} M CaCl_2 solutions are presented in Fig. 2. The measured curves and trends resemble earlier observations on clays and soils (Bingham et al. 1971; Hingston, 1964; Keren et al. 1981). Boron adsorption on organic matter seems to depend both on equilibrium B concentration and solution pH. It is evident that the amount of adsorbed B increases with pH, at a given boron concentration in solution. On a weight basis, the amounts of adsorbed B on the organic matter are much higher than those measured for Ca-clays and Ca-soils (Keren and Mezuman, 1981; Mezuman and Keren, 1981). These observations suggest that small amounts of organic matter in soils may change significantly the B distribution between the solid and liquid phases. Parks and White (1952) found that humus has a chemical affinity for B and stated that humus appears to have an important role in the retention of boron by soils. These observations do not agree with reports by Mezuman and Keren (1981) who studied the effect of organic matter on B adsorption by a semi-arid soil containing 1.2% organic matter. These investigators used H_2O_2 oxidation for the destruction of the organic matter and found the effect to be small. This disagreement could possibly be explained by an incomplete destruction and/or by the formation of secondary compounds such as oxalate (known to be formed under these conditions) that may have B adsorption capacity.

The adsorption isotherms of B on the organic matter that are presented in Fig. 2 were determined at pH 7.0, 7.9 and 8.9. These measurements were used to calculate the coefficients that are required for calculating B adsorption according to the model that was described earlier. The lines in

Fig. 2. Boron adsorption isotherms for composted organic matter in $5 \times 10^{-3} \text{M}$ CaCl_2 solutions, adjusted to three pH values. Experimental points and calculated lines are presented. Bars indicates the largest standard deviation.

Fig. 2 were calculated according to equation 1 of this model. The agreement between the calculated lines and the experimental results indicates that the adsorption model describes reasonably well B adsorption by organic matter.

The adsorption coefficients T , K_{HB} , K_B and K_{OH} for the organic matter were estimated from the experimental data according to the model and they are listed in Table 2, along with the values obtained for some clay minerals and soils. It is evident that the adsorption capacity of the organic matter for B (on a weight basis) is at least 4 times higher than that of clays and soils. The results also indicate that the affinity of the organic matter to OH^- is stronger than that of montmorillonite whereas there is a similarity between the two adsorbants with respect to $B(OH)_3$ and $B(OH)_4^-$ species. This suggests that the higher capability of the organic matter to adsorbed B is due to the higher number of sites available for B adsorption. It is difficult at this point to attribute any physical significance to the absolute values of K_{HB} , K_B and K_{OH} .

Calculated lines describing the dependence of B adsorption on pH at a given total amount of B in the system are presented in Fig. 3. The lines in this figure were calculated using the adsorption model and the coefficients listed in Table 2. Boron adsorption increases with pH at the pH range 7.0 to 8.7, whereas a sharp decrease was observed at the pH range of 9.2 to 11. The highest adsorption was obtained at a pH range of 8.7-9.2 similarly to pH values found for clay minerals and hydroxy-aluminum polymers (Keren and Gast, 1983). The response of the systems to variations in pH can be explained as follows: below pH 7, $B(OH)_3$ predominates, but since the affinity of the organic matter for this species is relatively low, the amount of B adsorbed is relatively small. As the pH increases,

Table 2 Adsorption coefficients of the adsorption equations for clays and soils (Keren et al., 1981) and composted organic matter (this research) saturated with Ca^{2+} ions.

Adsorbent	Maximum B adsorption	Affinity coefficients		
		K_{HB}	K_{B}	K_{OH}
	mol Kg^{-1}		L mol^{-1}	
montmorillonite ¹	1.2×10^{-2}	1.0×10^2	1.0×10^3	9.2×10^3
illite ¹	1.5×10^{-2}	3.5×10^2	8.7×10^3	4.0×10^4
soil (Tel Rosef) ²	8.8×10^{-3}	2.2×10^2	8.0×10^3	2.0×10^5
soil (Golan) ²	1.4×10^{-2}	1.3×10^2	7.0×10^3	2.5×10^5
composted organic ³ matter	5.4×10^{-2}	0.9×10^2	1.6×10^3	1.4×10^5

¹ After Keren and Mezuman, 1981.

² After Mezuman and Keren, 1981.

³ This study.

Fig. 3. Curves calculated according to equation 1 for B adsorption by organic matter vs. pH (in 5×10^{-3} M CaCl_2 solutions) at three levels of total B content (Q_T). The parameters used for the calculations were: $V = 15 \text{ mL g}^{-1}$; $T = 5.4 \times 10^{-2} \text{ mmol g}^{-1}$; $K_{HB} = 91 \text{ M}^{-1}$; $K_B = 1.62 \times 10^3 \text{ M}^{-1}$; $K_{OH} = 1.42 \times 10^5 \text{ M}^{-1}$.

the concentration of $B(OH)_4^-$ increases rapidly and the amount of boron adsorbed increases due to the relatively strong affinity of the organic matter for $B(OH)_4^-$. Since the OH^- concentration is still low at pH 9, its effect on boron adsorption is small. Further increase in pH resulted in an enhanced OH^- concentration relative to $B(OH)_4^-$. In clays this results in a rapid decrease in B adsorption, as described by the model and by experimental data (Keren and Bingham, 1985). The validity of the model predictions for B adsorption on organic matter at pH values higher than 9 needs experimental verification that could not be performed by the techniques used in this study because of relatively high solubility of the organic matter at these pH values. Since these values are rare in soils this information is not essential. Further research is required to explain the high affinity of the organic matter to the negatively charged $B(OH)_4^-$ ion.

The results of B desorption from the organic matter at pH 8 are presented in Fig. 4. A hysteresis effect is evident when the adsorption and desorption are compared. Hysteresis tended to be greater at higher B concentration. The formation of polynuclear B species at high soluble-B concentrations could explain incomplete B release during desorption if the species were difficult to dissociate. Ingri et al., (1957) reported, however, insignificant amounts of polynuclear species at total B concentration <0.025 M. Therefore, it is unlikely that hysteresis was due to formation of these B species, since the initial B concentration in this investigation (before adsorption took place) was lower than 0.025 M. Stevenson et al., (1952) report that hydrolysis products of soil organic matter included galacturonic acid, xylose and mixture of other sugar compounds which all meet the requirements for complex formation with boric acid (Boegeken, 1949; Kustin and Pizer, 1969). Many other carbohydrates

Fig. 4. Boron adsorption and desorption isotherms for composted organic matter in 5×10^{-3} M CaCl_2 solutions at pH 8. Bars indicate the largest standard deviation.

were also reported to be present in soil organic matter (Stevenson, 1982). Thus, it is possible that the observed irreversibility is due to a chemical association between B and organic molecules, particularly carbohydrates and humic substances.

More work is necessary to fully understand B desorption characteristics by organic matter. Ignoring irreversibility of B adsorption on organic matter in soils will result in overestimates of B concentrations in the soil solution and of rates of B removal from soils.

Acknowledgment

This research was supported by the U.S.-Israel Binational Agricultural Research and Development Fund (BARD).

The authors wish to thank Miss Eva Klein for her technical assistance.

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6.2 Boron adsorption on soil organic matter in relation to pepper plants growth

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ABSTRACT

Effects of increasing application rates of boron (B) to a soil mixed with increasing levels (0-10%) of composted organic matter on B concentrations in pepper leaves and plant growth response was studied. Boron application levels were 0, 1, 5 and 10 mmol kg⁻¹ soil mixture, and organic matter (OM) levels were 0, 1, 3, 6 and 10%. Adsorption isotherms were determined on soil:OM mixtures, and the data were modeled and calculated according to a modified Langmuir equation. Adsorption increased with pH and OM levels. Calculated data for the adsorption curves agreed well with the measured ones. Calculated values for the soil solution in the plant growth experiment correlated well with the measured ones but were somewhat higher. Boron application levels effected plant yield at 0% OM only where high B levels resulted in a yield decrease. At higher OM levels plant yield responded positively to OM and the yields were similar for increasing B applications. However, B concentrations in the leaves were strongly dependent upon OM levels: at low OM and high B application rates B in leaves approached toxic levels and at 0% OM exceeded the toxicity limit (330 mg kg⁻¹). Increasing OM levels to 1% or more prevented this excessive uptake of B by adsorption thereby reducing B concentration in the soil solution. Measured and calculated B concentrations in pepper leaves correlated well with B concentration in the soil solution suggesting an optimal concentration of 40-60 mg kg⁻¹ in the leaves when soil solution concentrations are about 1 mg L⁻¹, and a linear increase of about 30 mg B

kg⁻¹ dry weight of leaves for further increase of 1 mg B L⁻¹ soil solution. Application of the findings to other plants requires further testing.

INTRODUCTION

Boron (B) is an essential element to plant growth. However, beneficial effects are found at a very narrow range of concentrations in irrigation waters of 0.5 to 2.0 mg L⁻¹. Higher concentrations are toxic whereas deficiency symptoms were reported at concentrations lower than 0.5 mg L⁻¹. Eaton (1935, 1944) has shown that concentrations higher than 2.0 to 3.0 mg L⁻¹ were deleterious to a number of plants. Information provided in the U.S. Salinity Laboratory, Riverside, Handbook 60 (1954) has set the criteria for B toxicity that still hold today. These criteria were based on sandy soils with low adsorbing capacity. One should expect, however, that soils containing clays and/or organic matter may effect B concentration in the soil solution and plant response to B in irrigation waters.

Adsorption of B on clays, free oxides and hydroxides were studied extensively in the last two decades (Biggar and Firemen, 1960; Hingston, 1964; Kerene and Mezuman, 1981; Keren and Gast, 1981; Keren and O'Connor, 1982; Parks and White, 1952; Sims and Bingham, 1967; Sims and Bingham, 1968). Effects of ionic strength, pH and drying and wetting processes have been studied. Adsorption models based on a modified Langmuir equation were developed and found to describe well experimentally measured isotherms (e.g. Keren and Gast, 1981). In general, adsorption significantly increased with pH whereas increasing ionic strength had a much smaller effect.

In contrast to the extensive interest in B adsorption on inorganic soil components the information available in the literature on B interactions with soil organic matter is very limited.

In semi-arid and arid soils organic matter content is relatively low and therefore its contribution to B adsorption was considered small in comparison to that of clay minerals and hydrous oxides (Mezuman and Keren, 1981; Keren and Bingham, 1985). However, B retention by organic matter may be very important in sandy soils irrigated with water high in B or in soils with naturally high B content. One may hypothesize that amendment of such soils with organic matter may significantly magnify the importance of this soil constituent in maintaining low B concentration in the soil solution.

This hypothesis is based on a number of literature reports suggesting B binding to soil organic materials. Most of these reports include qualitative information on the binding reactions. The importance of organic matter to B retention was firstly noted by Parks and White (1952). According to their observations, soil humus exhibits a chemical affinity for B and it seems to play an important role in the retention of B by soils. Berger and Pratt (1963) stated that a large part of the total B in soils is tightly bound to organic matter and that this fraction of B can be released to the soil solution in forms that are available to plants. The reactions between soil organic matter and anionic borate species are probably of a di-hydroxy or hydroxy-carboxy complex nature as proposed for manitol, tartaric acid, and for sugars (Kustin and Pizer, 1969; Huettl, 1976; Yermiahu et al., 1987).

Stevenson et al. (1952) report on the presence of various sugars such as galacturonic acid, xylose, mannose, arabinose, glucose and galactose in the hydrolysate of soil organic matter. These compounds meet the requirements for di-hydroxy complex formation with B. Most of these sugars have been demonstrated to react with boric acid (Boesehan, 1949). Other polymeric sugars are well-known to constitute a fraction of soil organic matter (Stevenson, 1982). It has been suggested (Huettl, 1976) that the Langmuir adsorption equation applies to B adsorption on organic matter.

Boron adsorption by clays and soils has been modeled by Keren et al. (1981). This model is based on the assumption that two B species - $B(OH)_3^0$ and $B(OH)_4^-$ of different affinities to the adsorbent are involved, their proportion in the equilibrium solution varies with pH, and that the affinity and the capacity of the adsorbent to the adsorbed species are pH independent.

Adsorption of B on organic matter was studied in the laboratory and the data were tested according to the model in an earlier report by Yermiahu, Keren and Chen (1987). Their study shows that adsorption of B on composted manure that served as a simulation of soil organic matter is at least 3 times higher than on montmorillonite. As for clays, adsorption significantly increased with pH, slightly increased with ionic strength, and could be described by the same modified Langmuir equation with appropriate parameters for organic matter. Desorption studies from organic matter showed that the adsorption is not reversible and only a small fraction of the adsorbed B is released.

In arid and semi-arid soils, B toxicity due to high levels of B in the soil or in irrigation waters is a common phenomenon. Based on the results summarized here, we hypothesize that addition of organic matter to arid soils containing high levels of B may result in a decrease in soluble B thereby preventing its toxic effects. The experiments conducted in this research were designed to investigate this hypothesis.

MATERIALS AND METHODS

Soil and organic matter

Plants were grown on a loessial soil from Alumim in the Western Negev, Israel. The soil was sampled from a 0-20 cm layer of an uncultivated field. Properties of the soil are listed in Table 1. The

soils consisted of 25% clay, 1.1% organic matter, 4.5% CaCO_3 and a low concentration (0.3 mg L^{-1}) of soluble B. The organic matter (OM) used was a composted manure. Properties of the OM organic matter and its adsorption-desorption reactions with B were described by Yermiah, Keren and Chen (1987). Quartz sand ranging in size from 0.1-0.6 mm was mixed with the soil to improve the hydraulic conductivity. The sand was prewashed with distilled water to elute salts and then dried.

Adsorption studies

To remove free electrolytes, the organic matter was leached 4 sequential times with deionized water at a solid to liquid ratio of 15, using a high speed centrifuge. After leaching, negligible concentrations of B were found in the equilibrium solution.

Boron adsorption was studied by shaking 2 g of washed organic matter in 50 ml polypropylene centrifuge tubes containing 30 ml of the experimental solution at $24 \pm 2^\circ\text{C}$. The suspensions were pH adjusted prior to B additions with HCl or NaOH until the pH was constant for at least 12 hrs. The suspensions were then equilibrated with CaCl_2 by successive washings with $5 \times 10^{-3} \text{ M CaCl}_2$ solution at the appropriate pH. Washing was repeated until a steady state with regard to CaCl_2 concentration was achieved. Boron concentration in the initial solutions ranged from 9.25×10^{-3} to $1.39 \times 10^{-3} \text{ M}$. The suspensions were then brought to the final volume by the addition of an appropriate amount of $5 \times 10^{-3} \text{ M CaCl}_2$ solution with the desired pH. The volume of solution that remained in the organic matter after centrifugation was corrected for, in the calculations. The measurements were performed in triplicate.

Preliminary adsorption experiments performed as long as 48 hrs indicated that adsorption was practically complete after 3 hrs. After equilibration time, the suspensions were centrifuged and active carbon was

added to an aliquot of the supernatant to remove soluble organic molecules from the solution. The concentration of soluble organic matter at the tested ionic strength and pH was negligible. Preliminary tests showed that B was not adsorbed on the active carbon. Boron was determined colorimetrically using the Azomethine-H method (Gupta and Stewart, 1975). The amount of adsorbed B was calculated by the difference between the amount added and that found in solution at equilibrium.

Boron adsorption on soil:sand:OM mixtures were performed following the same procedure. Mixtures were prepared as follows: levels of OM were 0, 1, 3, 6, and 10% by weight of the mixture. Levels of sand plus OM were complementary to 10% for all the mixture, thus the levels of sand were 10, 9, 7, 4, and 0%, respectively. The final soil mixtures consisted of 90% w/w of the Alumim soil plus 10% of the sand:OM mixture. These mixtures were used for adsorption studies and for the major experiment with plants. In a preliminary experiment, the levels of OM were 0, 5, and 10% and those of the sand 10, 5, and 0%, respectively. The sand and OM were prewashed with distilled water so that salts and free B were leached.

Five g of oven-dried mixture were equilibrated in polyethylene tubes with 20 mL of solution at the desired pH values. After 48 hrs the pH was readjusted and B was added to the desired levels. Initial B concentrations ranged from 5 to 30 mg L⁻¹. The tubes were shaken for additional 24 hrs until equilibrium was reached. From this point, the technique was the same as employed on adsorption studies on OM.

Plant growth experiment

(a) Preliminary experiment

The OM was leached with distilled water until the electrical conductivity was 1.0 dSim m⁻¹, then dried. Pots (5 replicates) were filled with 2 kg of soil:sand:OM mixtures containing 0, 5 and 10% OM.

Boron was added in solution to levels of 0.5×10^{-3} mol kg⁻¹ soil. The water capacity of the pots was 35% at a matric potential of 1/3 atmosphere. Five pepper seedlings, 2 weeks old, were planted in each pot. A mulch of polystyrene spheres was used to reduce evaporation. Plants were irrigated daily to the weight determined at 1/3 atmospheres.

Fertilization consisted of 200 mg kg⁻¹ N-NO₃, 500 mg kg⁻¹ P, 1 mg L⁻¹ FeEDDHA, 0.5mg L⁻¹ Zn, 0.3 mg L⁻¹Mn and 0.1 mg L⁻¹Cu in the irrigation waters. Zinc, Mn and Cu were added chelated with EDTA. One plant from each pot was harvested after 35, 49 (flowering) and 63 days.

Leaves were separated from stems, dried at 65°C, weighed, ground and stored in a dessicator. A 0.25 g sample was placed in a furnace at 550°C for 4 hrs. Five mL of 1N HCl were added to the ash after cooling, then the solution was filtered. An aliquot of 3 ml was taken for B analysis by the azomethin-H procedure (Gupta and Stewart, 1975). The leaves were also analyzed for N and P. After the last harvest, the soil of each pot was mixed and a saturated paste was prepared and tested for B, electrical conductivity (EC) and pH.

(b) Major plant experiment

In general, the procedures were the same as in the preliminary experiment. Five organic matter concentrations (0, 1, 3, 6 and 10%) of the soil weight were tested at four B levels (0, 0.1, 0.5, and 1.0 mmol kg⁻¹). Each treatment consisted of 20 pots set in blocks in an arbitrary manner and including one pot from all the treatments each. Two plants were grown in each pot. Fertilization was performed with a Hoagland solution that did not include B. Plants were harvested after 25 days (2 pots from each treatment) and after 45 days (5 pots from each treatment). Plants were analyzed as in the preliminary study. Soil was analyzed at the beginning of the experiment and 25 and 45 days thereafter.

RESULTS AND DISCUSSION

Adsorption isotherms for the soil mixtures with organic matter are presented in Figs. 1-5. Isotherms were determined at 3 pH values. The points shown are experimental whereas the lines are calculated according to Yermiahu et al. (1987). The model lines fit well the measured data. Adsorption increases with pH at any given B concentration in the solution.

A comparison of the adsorption levels shows that they increase with increasing organic matter. This comparison should be done at the same pH level. The adsorption coefficients are listed in Table 2. Fig. 6 shows calculated adsorption vs. pH at a constant B level. Maximum adsorption can be derived from this figure and the comparison between the various levels of organic matter is presented. Since the pH values of the soil paste were 7.7 ± 0.2 , this pH was used to calculate B adsorption at the 3 B concentrations that were used in this experiment (Fig. 7). Adsorbed B levels increase with organic matter contents. The increase is more prominent at high B levels. Boron concentration vs. organic matter levels are presented in Fig. 8. The concentrations were measured in the saturated paste of pots at the end of the experiment. The results are corrected to field capacity conditions. The broken curves describe measured data whereas the continuous ones present lines calculated by the adsorption model for pH 7. The soils to which no B was added exhibited very low B concentrations ranging from $0.2 - 0.5 \text{ mg L}^{-1}$ and are therefore not included in the figure. At low B application (0.1 mmol kg^{-1}) the concentrations of B in the solution are relatively low ranging from $0.5 - 0.7 \text{ mg L}^{-1}$. Application of $0.5 \text{ mmol B kg}^{-1}$ soil increased the soluble B levels to a range of $2 - 3.5 \text{ mg L}^{-1}$ whereas the highest application level of B (1.0 mmol kg^{-1}) resulted in concentrations of $4 - 8.5 \text{ mg L}^{-1}$ soluble B.

In general, B concentration decreased significantly with increasing

levels of organic matter. This phenomenon is prominent at the high B application levels and only slightly detectable at the lower ones. The calculated lines show higher concentrations of B than the measured ones, but the correlation between calculated and measured values as presented in Fig. 9 is very high ($r=0.96$). The slope of the curve is 0.74 indicating lower measured values than calculated ones. This correlation provides a predictive correction value for the calculations.

The dry yields of the plants were measured even though it was secondary in importance to B concentrations in the leaves. OM levels of 3, 6 and 10% resulted in a similar effect on the yield of leaves that ranged from 3.4 to 3.8 g/plant. At lower OM levels the yields were lower ranging from 2.1 to 2.5 and 0.3 to 0.5 g/plant for 1% and 0% OM, respectively. Boron application levels did not have any effect on the yield in the presence of added OM. However, at 0% OM an optimum in growth was obtained at 10^{-3} mol B kg^{-1} soil and the yield decreased from this point by about 45% at B levels of 10^{-2} mol kg^{-1} .

Photographs of the plant growth experiment are presented in Figs. 10, 11 and 12. Fig. 10 presents the plants in the preliminary experiment. Growth was significantly inhibited in the absence of OM. Whether this effect is due to aeration problems or to toxic levels of B will be discussed later along with the discussion on B concentration in the leaves. In soil systems mixed with OM (1, 3, 6 and 10%) B applications did not affect growth to any extent. The toxicity effect of B at 10^{-2} mol kg^{-1} B that almost killed the plants at 0% OM disappeared completely at higher levels of OM in the soil. Plant growth improved, in general, in the presence of OM as compared to 0% OM treatments. It seems that improved soil aeration was the reason for this effect.

The major parameters of concern for the assessment of B effects on the plants is B concentration in the leaves. In Figs. 13, 14 and 15 B

concentration in the leaves are presented. According to Chapman (1966) B concentration in pepper leaves in the range of 34 - 118 mg kg⁻¹ are considered as intermediate range whereas at concentration higher than 330 mg kg⁻¹ toxicity effects are likely. In the preliminary experiment plants grown at 0% OM exceeded the critical level in the second and third harvest (Fig. 14) whereas 5 and 10% prevented B uptake to toxic levels. In general, B concentration in the leaves increased from the first to the third harvest. The differences between treatments increased with time. Similar results were obtained in the major experiment in which 5 OM levels and 4 B application levels were tested. In the first harvest B concentrations did not exceed 330 mg kg⁻¹, but the effect of OM in reducing B concentration in the leaves is prominent at the higher B application rates of 5 and 10 x 10⁻³ mol kg⁻¹ soil (Fig. 14). In the second harvest the trends are the same but the concentration of B in the leaves is higher, especially at 0 and 1% OM. Boron concentration in the leaves exceeds 330 mg kg⁻¹ in the high B application rate and 0% OM only. It seems that the pepper plants grown at B levels of 5 x 10⁻³ mol kg⁻¹ and 0% OM were significantly retarded by high levels of B (Fig. 12) even though toxicity symptoms on the leaves were not detected in any of the treatments. The correlation between B concentration in the second harvest vs. the first one is excellent (r=0.98; Fig. 16) exhibiting a slope of 1.33 indicative of the higher concentrations in the second harvest. Fig. 16 shows that B concentration measured during the growth period can assist in predicting the concentration at a later growth stage but the rate of increase must be a known factor.

The hypothesis that B concentrations in the soil solution will be of predictive value to B concentration in the leaves was one of the scopes of this investigation. Linear correlations between B concentration in the leaves vs. B concentrations in the soil solution are presented in Figs. 17

and 18. Fig. 17 presents a correlation based on measured B concentration in the soil solution whereas Fig. 18 is based on calculated data for the soil solution based on the model described earlier (Yermiahu et al., 1987). It seems that B concentrations in the soil solution are well correlated with B in leaves. The correlation coefficients are 0.89 and 0.99 for measured soil B and calculated soil B, respectively. In both cases B concentration in the leaves at zero B concentration in the soil solution is about 33 mg kg^{-1} . According to Fig. 18 and 19, an increase in B concentration in the soil solution of 1 mg L^{-1} will result in an increase of about 30 mg B kg^{-1} dry matter of leaves. As long as B concentrations in the soil solution do not exceed 1 mg L^{-1} concentration in leaves is practically the same. From this concentration and up any increase has a large potential effect on the leaves. Organic matter was shown to have a prominent effect on the reduction of B uptake to the leaves. Concentrations in the leaves were independent of growth but strongly dependent on B concentrations in the soil solution. The model proposed seems to predict well B adsorption on soil mixtures with OM and predicted values can be correlated with B uptake to plants. Similar tests are required for other plants to obtain the correlation between soil and leaves concentrations.

ACKNOWLEDGMENT

The authors wish to thank BARD for the kind financial support.

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Table 1. Properties of the Alumim loessial soil.

Clay	Silt	Sand	pH	Saturated paste		
----- % -----				EC	B	
				%H ₂ O (d	Sim m ⁻¹)	(mg L ⁻¹)
24	14	62	7.8	54	0.51	0.3

Organic	CaCO ₃	CEC	Exchangeable cations			
Matter	(%)	(meq/100g)	Na	K	Ca+Mg	
(%)			-----meq/100 g-----			
1.1	4.5	16.8	0.44	0.72	15.64	

Table 2. Adsorption coefficients used in computations.

Organic	T	KB	KOB	KOH
Matter				
(%)	(mmol/g ⁻¹)	(M ⁻¹)	(M ⁻¹)	(M ⁻¹)
0	0.0075	54	1034	5973
1	0.0100	43	1938	25913
3	0.0120	88	1488	43938
6	0.0143	94	1609	266124
10	0.0250	37	4169	1545958

Fig. 1. Adsorption isotherms for B on Alumim soil at 0% organic matter.

Fig. 2. Adsorption isotherms for B on Alumim soil at 1% organic matter.

Fig. 3. Adsorption isotherms for B on Alumim soil at 3% organic matter.

Fig. 4. Adsorption isotherms for B on Alumim soil at 6% organic matter.

Fig. 5. Adsorption isotherms for B on Alumim soil at 10% organic matter.

Fig. 6. Calculated functions for B adsorption vs. pH at 5 levels of organic matter (B level $10^{-3} \text{ mol kg}^{-1} \text{ soils}$).

Fig. 7. Calculated functions for B adsorption vs. organic matter contents at 3 levels of B.

Fig. 8. Calculated and measured values for B concentration in soil solutions vs. organic matter level at 3 levels of B:

$Q_2 = 10^{-3} \text{ mol Kg}^{-1}$; $Q_3 = 5 \cdot 10^{-3} \text{ mol Kg}^{-1}$; $Q_4 = 10 \cdot 10^{-3} \text{ mol Kg}^{-1}$

Fig. 9. Measured vs. calculated values of B in the soil solution.

Fig. 10. Plants in the preliminary experiment. Organic matter levels are indicated. Applied B level was $0.5 \times 10^{-3} \text{ mol kg}^{-1}$.

Fig. 11. Plants grown at 0 and 1% organic matter and increasing levels of applied B.

Fig. 12. Plants grown at 3, 6 and 10% organic matter and increasing levels of applied B.

Fig. 13. Boron concentration in the leaves vs. organic matter contents in the preliminary experiment. One concentration of B and 3 harvests.

Fig. 14. Boron concentration in the leaves vs. organic matter at four levels of applied B - first harvest.

Fig 15. Boron concentration in the leaves vs. organic matter levels at four levels of applied B - second harvest.

Fig. 16. Boron in the leaves of the second harvest vs. the first harvest.

Fig. 17. Boron concentration in the leaves of the second harvest vs. measured B concentration in the soil solution.

Fig. 18. Boron concentration in the leaves of the second harvest vs. calculated B concentration in the soil solution.