THE INTERACTION OF ANIONS WITH HUMIC SUBSTANCES

Thesis submitted for the degree
"Doctor of Philosophy"

by

PHILLIP BARAK (BURKE)

Submitted to the Senate of the Hebrew University of Jerusalem

August 1987

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This work was carried out under the
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My thanks to Prof. Yona Chen who supplied patience, support and encouragement beyond that expected of an academic advisor. Above all, recognition is due to my wife Sara and sons Ariel and Yishai, who by their steadfastness made their own immeasurable contribution.

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ABSTRACT

Very little is known of the interactions of humic substances with the various anions in the soil environment, other than several reports of adsorption of phosphate, silica, borate, and fluoride and exclusion (negative adsorption) of chloride. The object of this research was to expand the knowledge of the interactions of humic substances with the anions nitrate, ethylenediamine di(o-hydroxyphenylacetic acid) [EDDHA] and borate. In the course of the research, analytical techniques employing ion chromatography for nitrate and EDDHA have been developed and published.

Nitrate was found to be excluded by sodium humate solutions under all conditions tested--pH 3.5 to 6 and ionic strength from 1 mM to 100 mM. At pH 6 and 1 mM electrolyte, exclusion was as much as 400 mL g^{-1} humic acid.

The adsorption of FeEDDHA on humic acid was dependent upon pH and ionic strength. At pH 3.5 and 25 μM, adsorption was 30 μmol g^{-1} at 180 mM NaCl and 12 μmol g^{-1} in 0.8 mM NaCl. Some adsorption is still present at pH 4.5, but at pH values from 6.8 to 11 the net interaction is exclusion; exclusion of FeEDDHA may be as much as 350 mL g^{-1} humic acid. The adsorption mechanism is proposed to be one of hydrogen bonding with carboxylic groups of the humic acid.

Boric acid was found to be unreactive with humic acid at pH 5, to be adsorbed at pH 7.5 to 8.5, and excluded at pH 10.5. Adsorption at 1 mM boric acid/borate did not exceed 0.05 mmol g^{-1} humic acid. The adsorption mechanism is probably due to condensation between $B(OH)_4^-$ and either cis-diol or catechol ligands present in small quantities in the humic acid.

Exclusion phenomena due to electrostatic repulsion between the solute anion and the humic macromolecule were measured for all three anions considered, at least under conditions of neutral or basic pH. Nitrate exclusion data were combined with acid-base titration data using double electric layer theory with cylindrical geometry to model the electrostatic behavior of extended linear polyelectrolytes; the model yields an apparent radius of 0.35 to 0.85 nm, depending upon degree of ionization, for the humic macromolecule. These dimensions are similar to those of other biomolecules such as DNA and α-helix and are in accordance with well-known models of chemical structure of humic substances.

INTRODUCTION

LITERATURE REVIEW

Of all of the organic carbon at the earth's surface, thereby excluding coals and sediments, some $30*10^{14}$ kg C are present as soil organic matter compared to $5*10^{14}$ kg C present in the biomass (Bohn, 1976). Considering some 60-70% of soil organic matter to be composed of humic substances (Schnitzer, 1978), the quantity of humic substances on earth exceeds that of living organisms at least threefold. Humic substances are however unlike the organic compounds of living organisms which are manufactured according to genetic instructions and therefore may be regarded as having well-defined, even if complex, composition and structure. In marked contrast, humic substances are heterogenous with respect to size and composition by nature, not by artifact, so many of the standard procedures for extraction and isolation of individual organic compounds are not applicable here, as they are in organic chemistry and biochemistry. Instead, the best that can be hoped for is standardized extraction, fractionation, and purification without major alteration of the natural product, including its heterogeneity. It would be unreasonable to neglect for reasons of convenience the research of humic substances, heterogenous as they may be, since they not only exceed on a weight basis any organic homogenous compound, or all of them together, but also play a major role in shaping soil structure and chemistry, an essential element for all terrestrial life, as well as serving as the ultimate fate of much of the biomass.

Humic substances are by nature dark-colored substances originating from microbial decomposition of lignin and/or cellulose, and with a mean residence time in the soil of several hundred to several thousand years. Typically, humic substances are fractionated on the basis of solubility and include humic acid (soluble in alkali, insoluble in acid), fulvic acid (soluble in alkali and acid) and humin (insoluble in either alkali or acid). Humic substances contain significant quantities of aromatic carbon; known functional groups include carboxyl, phenolic, quinone, alcoholic, ketonic, and methoxy groups. The material is macromolecular with a molecular weights typically ranging from 500-2000 for fulvic acids to 50,000-100,000 dalton for humic acids. In the 200 years of humus chemistry, every attempt to fractionate humic acids into a finite number of fractions based on properties of size or charge have failed to produce truly homogenous preparations.

Intensive research has elucidated many aspects of humic chemistry, particularly that of humic acids. Investigation of the reactions of the acidic functional groups has lead to measurements of the acid strength of numerous preparations of humic acid, as well as the stability constants for numerous cations of the alkali earth and transition metal groups; this aspect of humus chemistry has had direct implications on the understanding of cation exchange phenomena, as well as mobility of trace elements in the soil. Research has also been conducted on the reaction of humates with many nonpolar, hydrophobic substances of types which comprise many pesticides, with direct application to pesticide chemistry in the soil. Various aspects of the colloidal chemistry of humic substances in solution, particularly sodium humates, have measured particle sizes, diffusion coefficients, and viscosity. Understanding of the humic substances has benefited from the application of physical methods of chemical analysis such as infrared spectroscopy, electron spin resonance spectrometry and nuclear magnetic resonance spectrometry, both liquid- and solid-state.

One aspect of humate chemistry which has not been intensively studied is the reaction of humic substances with anionic molecules of various types or their acids. Book-length reviews on soil organic matter and humic substances and their chemistry by M. Kononova (1966), Schnitzer (1978), F. J. Stevenson (1982), G. C. Choudry (1984) and G. R. Aiken et al. (1985) do not mention this subject at all. The several reports of anion interactions with humic substances scattered through the literature include adsorption through condensation (borate and possibly silica), adsorption through a partially coordinated metal ion (phosphate and fluoride) and anion exclusion (chloride).

Considerable evidence collected for whole soils points to the role of soil organic matter in boron solubility, sorption and availability in the soil. Berger and Truog (1945) found that boron availability in 34 virgin and 48 cultivated soils was most significantly correlated with organic matter content (r = 0.83). Baser and Saxena (1967) found that soluble boron in 30 Indian soils was correlated with organic matter content. Elrashidi and O'Connor (1982) introduced an organic carbon term into a multiple linear regression model which explained 98% of the variation of boron adsorption in a group of soils. Evans (1987) found that for nine Canadian soils the boron adsorption maxima of nine Canadian soils with pH > 6.8 were correlated with both organic carbon (r = 0.87). Russell (1973) speculated that the reaction between boron and soil organic matter was due to boric acid condensing with diol groups associated with carboxylic groups of humic acid. In what appears to be the only research on boron retention of pure humic acid systems, Parks and White (1952) found that calcium humate extracted with bicarbonate retained 0.6 mg B g^{-1} HA at 9 mg L^{-1} soluble B, bicarbonate-extracted humic acid retained 1.4 mg B g^{-1} HA at 7.8 mg L^{-1}, and a pyrophosphate-extracted humic acid retained 18.2 mg B g^{-1} HA at 44 mg L^{-1} B; no pH control was reported and other than the three measurements reported here, no adsorption isotherms were measured. Results were attributed by Parks and White to be due to cis- diols present in the humic acid.

Another ubiquitous component of soil chemistry, silica, also appears to react with humic substances. Silica is a significant component of the ash impurities of crude humic acid preparations (Stevenson, 1982), which is in itself not surprising considering that amorphous silica is also extractable from soils with NaOH. On the other hand, the drastic means required for the removal of silica from the crude humate preparation may indicate some type of reaction; use of 0.3 N HF is not unusual. Griffith and Schnitzer (1975) found that even 36 hr in 0.5% HCl+HF left between 9-13% silica in humic acids. Schnitzer and Desjardins (1969) reported that half of the water-soluble humic compounds that had leached through soil, when concentrated a hundredfold, precipitated to form a sediment containing 52% ash, which was 98% silica. The nature of the $Si(OH)_4$-humate interaction has yet to be elucidated, but x-ray diffraction rules out the possibility of crystalline silicaceous minerals which have accidentally escaped the purification steps.

A different type of interaction between humic substances and anions is due to the formation of mixed ligand complexes with metal ions at the center of a complex with two types of coordinating groups, one humic and the other an inorganic anion. Levesque and Schnitzer (1967) prepared fulvic-metallo-phosphate complexes with aluminum and iron(III) and differentiated complexes from mixtures by dialysis; the molar ratio of both Fe:P and Al:P in dialyzable complexes was as low as 4:3. Phosphate added in excess led to the removal of iron and aluminum and precipitation as metal phosphates. A mixed ligand complex of a similar, but less defined nature was reported by Farrah et al. (1985) who studied the sorption of fluoride on commercial humic acids. A maximum of 0.2 mmol F^- g^{-1} humic acid was adsorbed at $6*10^{-4}$ M F^- at pH 3.5-4.5, declining to zero at pH 6.5. The fluoride was observed not only to be sorbed to the humic acid but to also release cations, primarily aluminum, from the humic acid and form soluble complexes with those cations; presumably fluoride was distributed between F^-, AlF_x3^{-x} and a humic-alumino-fluoride complex.

A different type of reaction of anions with humic substances is electrostatic repulsion leading to anion exclusion. The anionic nature of humic substances when acidic functional groups are ionized 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, anions are expected, in the absence of various adsorptive reactions, to be repelled from the negatively charged humic surface. Exclusion of chloride 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 coal-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 probable geometry or dimensions of humic substances.

A detailed literature review specific to each anion considered in this work is included in the relevent chapter.

OBJECTIVES

The purpose of this work was to elucidate the presence and nature of interactions of humic substances with three anions of agricultural importance--nitrate, borate and ethylenediamine di(o-hydroxyphenylacetic acid) [EDDHA]. The chemistry of borate in soils, important because of phenomena of toxicity and deficiency, is unclear, as is the fate of the majority of EDDHA added to soil as an iron chelate to remedy iron deficiencies in plants grown in calcareous soils; the interest in nitrate stems from the fact that nitrate is a major nutrient whose chemistry is otherwise relatively well-known. Furthermore, these three substances provide a wide range of properties, ranging for example in acid strength from the salt of strong acid (nitrate) to very weak acids (borate and EDDHA), and in size from ~0.3 nm diameter (nitrate and borate) to ~1.0 nm (EDDHA). It may be hoped that by studying humic-anion interactions, including adsorption and exclusion, both the chemistry of humic substances and of the anions in question will be elucidated.

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 (Small, 1983; Fritz, 1987). 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 concommitant 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. The results were published as

"Three-minute analysis of chloride, nitrate, and sulfate by single column anion chromatography" by Phillip Barak and Yona Chen in Soil Science Society of America Journal 51:257-258, as follows:

Three-Minute Analysis of Chloride, Nitrate, and Sulfate by Single Column Anion Chromatography[1]

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 μL sample loop, detection limits were 0.05 mmol$_c$ 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.

Additional Index Words: anion chromatography, chloride, nitrate, sulfate

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.

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[1] Contribution of the Seagram Center for Soil and Water Sciences, Faculty of Agric., Hebrew Univ. of Jerusalem, P. O. Box 12, Rehovot, Israel 76-100. This research was supported by the U.S.-Israel (Binational) Agric. Res. and Dev. Fund (BARD). Received 23 Apr.1986.

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 were refiltered immediately before injection with a Millipore membrane filter (0.45 µm). Standards were prepared from reagent grade sodium salts of Cl^-, NO_3^-, and SO_4^{2-}; 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 the 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 SO_4^{2-} (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^-, NO_3^-, and SO_4^{2-} 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 curvers 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 $mmol_c$ L^{-1} when based on peak height, and 0.03 to 0.16 $mmol_c$ 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 $\text{mmol}_c\ L^{-1}$ 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 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 pH values bor eluents, i.e., pH 4.5, the organic compontents 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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Table 1: Precision of analysis of Cl^-, NO_3^-, and SO_4^{2-} based on 12 injections of 50 µL of combined standard.

		Peak height		Peak area	
Anion	Concentration Injected	Standard Deviation	Coefficient of variation	Standard Deviation	Coefficient of variation
	——— $mmol_c\ L^{-1}$	———	%	$mmol_c\ L^{-1}$	%
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

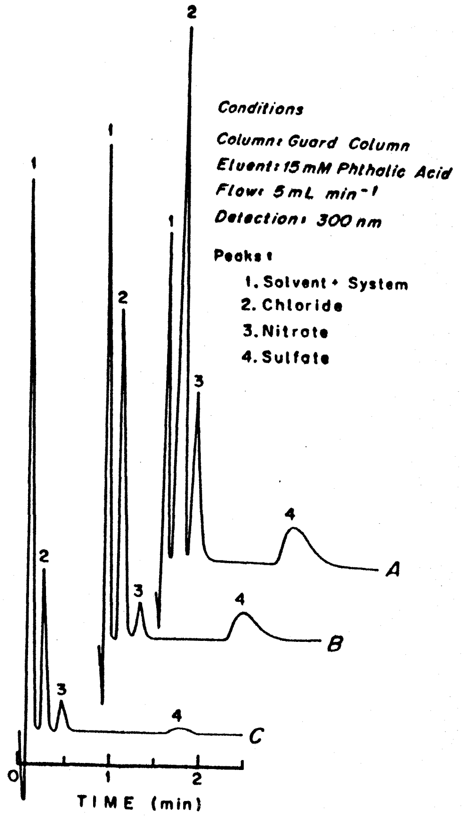

Fig. 1: Chromatograms of: a) combined standard containing 8, 4, and 4 mmol$_c$ L^{-1} Cl$^-$, NO$_3^-$, and SO$_4^{2-}$, respectively, b) a saturated extract of a clay loam soil (Mitzpeh Massuah, xerorthent, 4.41, 0.78, and 2.82 mmol$_c$ L^{-1} Cl$^-$, NO$_3^-$, and SO$_4^{2-}$, respectively), and c) groundwater from the coastal aquifer of Israel (Rehovot, 1.99, 0.81, and 0.37 mmol$_c$ L^{-1} Cl$^-$, NO$_3^-$, and SO$_4^{2-}$, respectively).

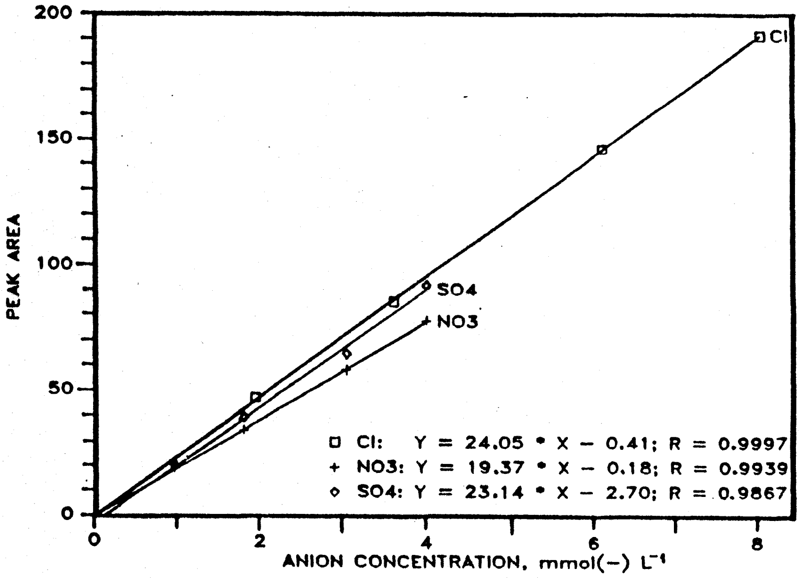

Fig. 2: Calibration plots for Cl$^-$, NO$_3^-$, and SO$_4^{2-}$ as measured by indirect UV detection. Each point is the average of three injections; regression equations and correlation coefficients were calculated with all 12 injections.

EDDHA and FeEDDHA — Published analytical techniques included spectroscopic measurement of the colored chelate (Hill-Cottingham, 1962), solvent extraction followed by spectroscopic measurement (Batra and Maier, 1964), and gel chromatographic separation followed by spectroscopic measurement (Boxema, 1979). 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, as follows:

Determination of FeEDDHA in Soils and Fertilizers by Anion Exchange Chromatography[1]

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 $Fe_2(SO_4)_3$ 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 $Fe_2(SO_4)_3$, 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 µmol L^{-1} using a 50 µL sample; the detection limit was 1.2 µmol 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.

Additional Key Words: Fe(ethylenediamine di-o-hydroxyphenyl-acetic acid), chelates, high performance liquid chromatography, iron nutrition.

Barak, P., and Y. Chen. 1987. Determination of FeEDDHA in soils and fertilizers by anion exchange chromatography. Soil Sci. Soc. Am. J. 51:000-000.

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[1] Contribution of the Seagram Center for Soil and Water Sciences, Faculty of Agriculture, Hebrew Univ. of Jerusalem, P.O. Box 12, Rehovot, Israel 76-100. This research was supported by the U.S.-Israel (Binational) Agricultural Research and Development Fund (BARD). Received 29 July 1986.

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-o̲-hydroxyphenylacetic acid) [NaFaEDDHA], also known as NaFe(N,N'-ethylene-bis (o̲-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 resulting bands 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 $Mg(FeEDDHA)_2*9H2O$ 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 $Fe_2(SO_4)_3$ as the eluent.

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

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, the 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 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 $Fe_2(SO_4)_3$, eluting at 4 and 6 min at a flow rate of 3 cm min^{-1}. The dl-racemic isomer prepared from Mg(FeEDDHA)$_2$*9H2O 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 purple (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 $Fe_2(SO_4)_3$ 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, declines 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 is similar to that found by Follett 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 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

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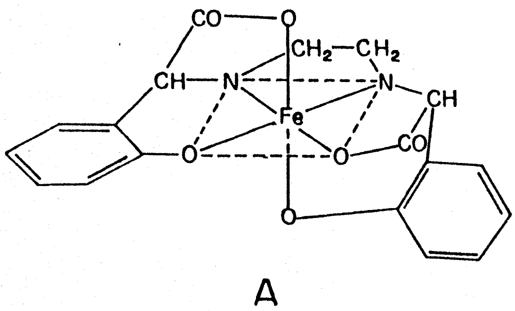
Table 1. Selected properties of soils used in this study.

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



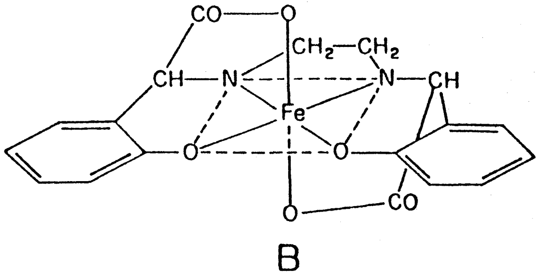


Fig. 1. Schematic diagrams of FeEDDHA: (A) meso isomer, R_f = 0.65 (paper chromatography); and (B) dl-racemic isomer, R_f = 0.44.

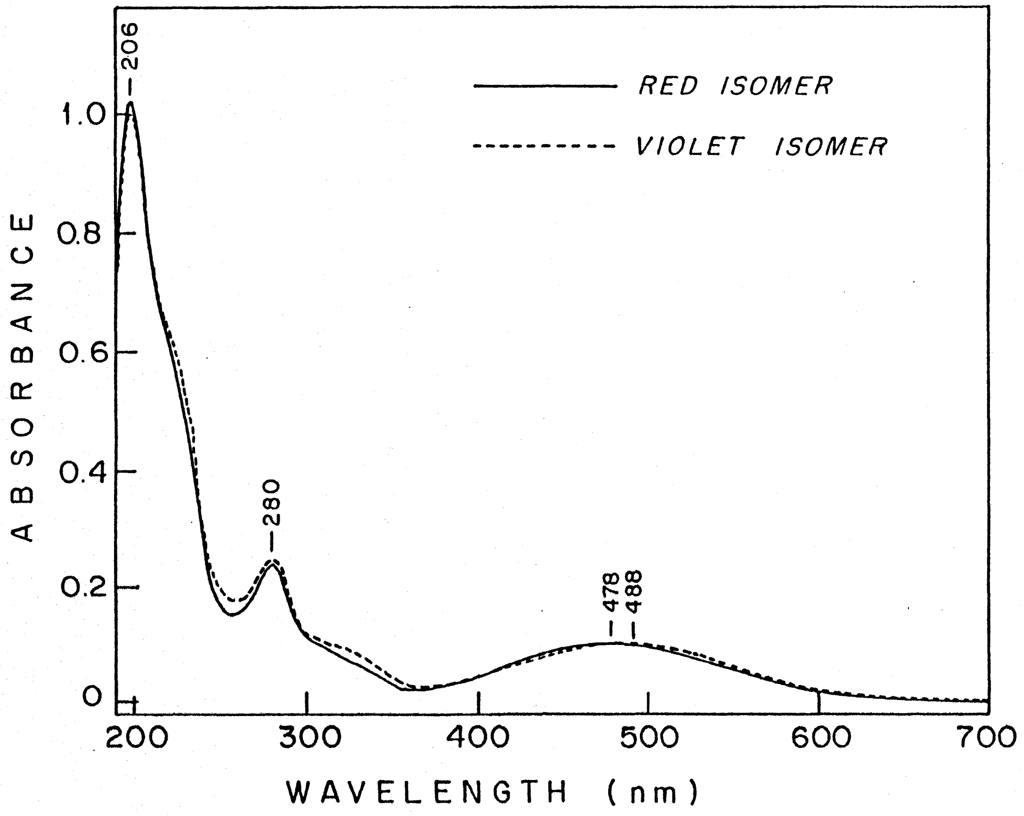


Fig. 2. Ultraviolet and visible spectra of red and violet isomers of FeEDDHA at a concentration of 0.022 mM.

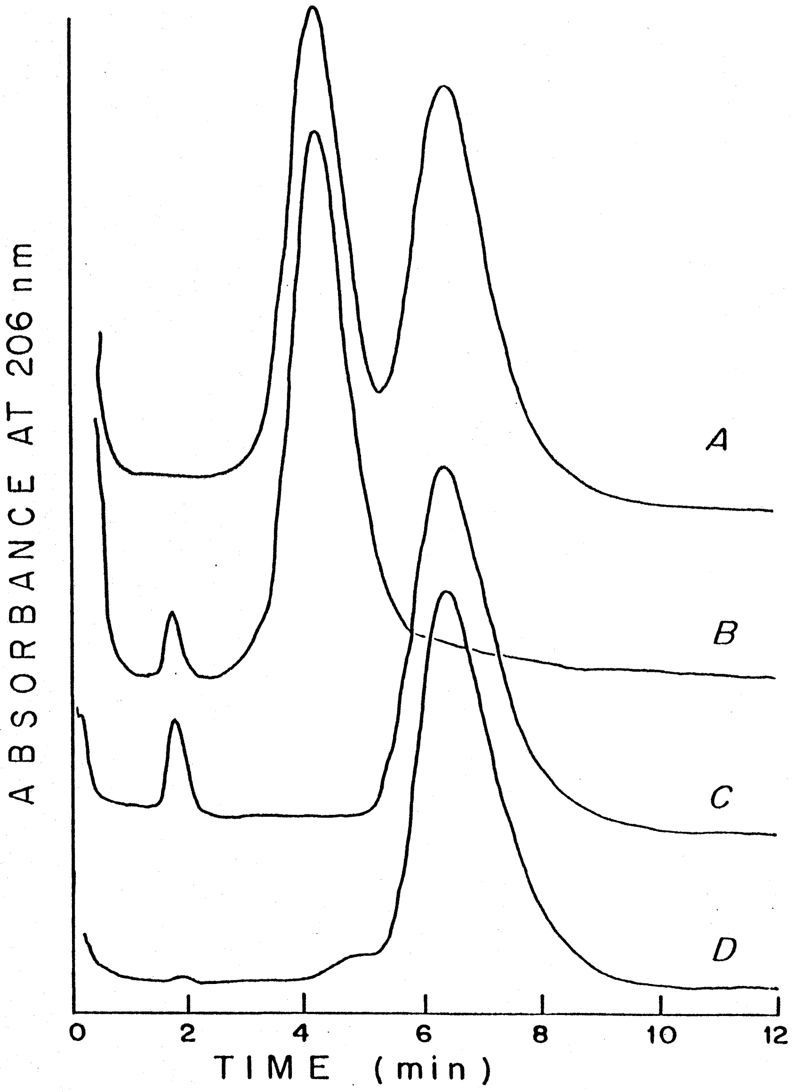


Fig. 3. Chromatograms of (A) original NaFeEDDHA solution, (B) violet, (C) red isomers separated from A by paper chromatography, and (D) solution of $Mg[Fe(\underline{rac}\text{-}EDDHA)]_2$.

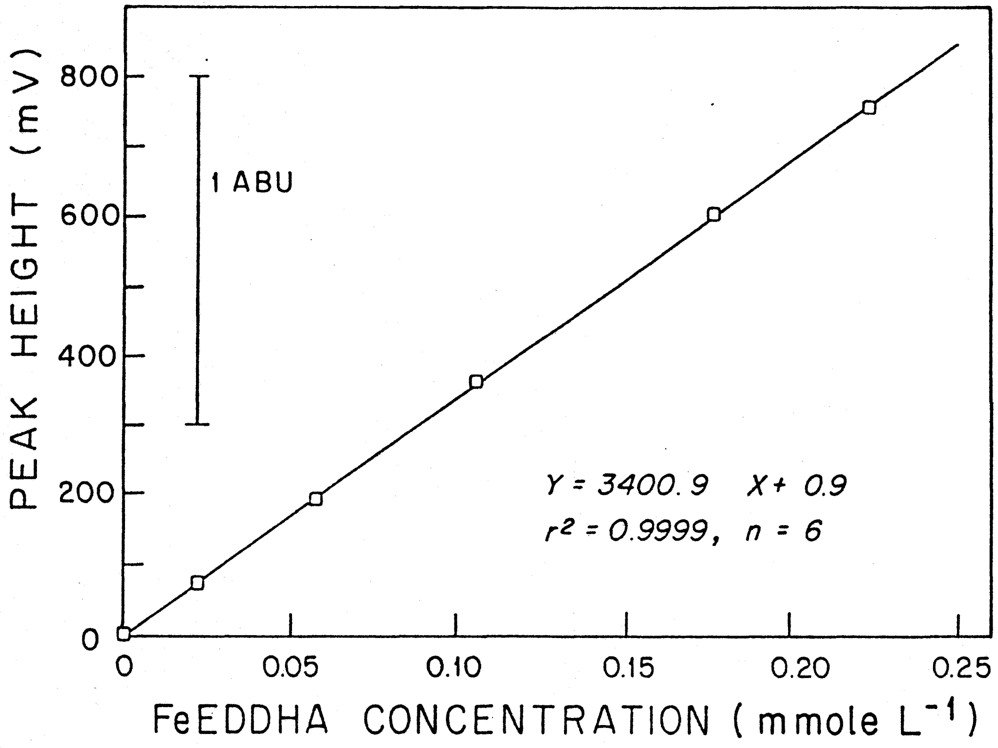


Fig. 4. Calibration curves for FeEDDHA isomers based on peak height of absorption at 206 nm (ABU = absorbance unit).

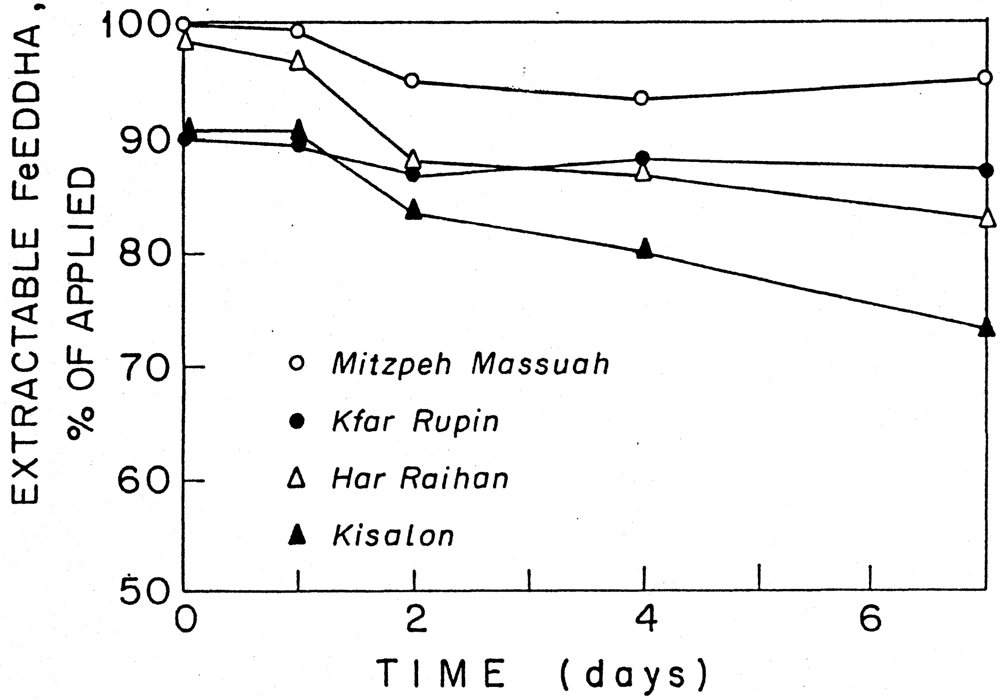


Fig. 5. Recovery of FeEDDHA applied to soil as measured by chromatography.

NITRATE EXCLUSION BY HUMIC ACID

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 unmeasurable 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 coal-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 either $\kappa a \gg 10$ or when $\kappa a \gg 1$ 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.

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 and 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 capacitites 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) and have been modified so as to produce a chromatographic separation of nitrate from other inorganic anions and measurement against a standard background in the course of a short and accurate procedure (Barak and Chen, 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 were both employed in this research aiming to measure interactions of nitrate with humic substances.

MATERIALS AND METHODS

Titrations with Nitrate Specific Electrodes--Titrations of humic acid solutions were conducted using two manufactures of nitrate specific electrodes to measure nitrate activities. First, an Orion nitrate specific electrode, Model 92-07, which employs a water-immiscible liquid ion exchanger, with a K_2SO_4 reference electrode, was used to perform titrations of two types--titration with base at a constant nitrate level and titration with nitrate at a constant pH, using a Beckman 4500 millivolt (pH) meter to record electrode potentials.

1. A 30-mg sample of humic acid was dissolved in 40 mL H_2O (control) or 10^{-4} M KNO_3, adjusted to pH 6 with 0.1 M KOH, and made to 50 mL. Nitrate activity was recorded by the Orion ISE as the pH was raised stepwise from 6 to 10 by the addition of 0.1 M KOH. The solution was then back-titrated to pH 2.2 with 0.1 H_2SO_4 and the nitrate activity recorded.
2. A 30-mg sample of humic acid was dissolved in 50 mL of 5×10^{-6} M KNO_3 solution. The sample was then titrated with 1.0 mM KNO_3 and the nitrate activity recorded. The pH was maintained at the desired value (3, 4, 5, or 7) by the addition of 0.01 M H_2SO_4 or KOH. At the end of the titration, the pH was adjusted to ~10 and the nitrate activity recorded.

Two types of titrations were conducted with a Radiometer nitrate specific electrode (F2412NO_3), which employs a nitrate-specific ion exchanger impregnated in a polyvinyl chloride (PVC) membrane, and a calomel reference electrode (Radiometer K401), using a Metrohm 686 Titroprocessor to measure electrode potential.

3. Dialyzed Hula humic acid and HA-A at pH 3.5 were titrated with 0.1 M NaOH, with monitoring of the potential on the Radiometer electrode, without the addition of nitrate.
4. Aliquots of stock solution of Hula humic acid were titrated to pH values of 2.5, 4, 6, and 9, and brought to a concentration of 0.25% w/w. Fifteen-mL aliquots were titrated with increments of 0.1 M $NaNO_3$ delivered by a Metrohn Dosimat 665. A Radiometer nitrate specific electrode with a calomel reference electrode was used to monitor nitrate activity in the humic acid solution. The electrode was calibrated by titrating 15 mL of water with 0.1 M $NaNO_3$.

Dialysis and Ion Chromatography--Stock solutions of Hula humic acid extracted and purified as described before were prepared at a concentration of 0.25% and adjusted to pH values of approximately 3.5, 5, and 7. Four-mL aliquots of the humic acid solution, containing 10 mg humic acid per aliquot, were placed in dialysis cells constructed of dialysis tubing (Medicell International Ltd, London, wet diameter ~6.5 mm) and extensively dialyzed against distilled water.

Each dialysis cell was placed in a test tube and weighed. Weighed quantities of $NaNO_3$ solution and water were added to bring the total volume to approximately 10 mL with a total nitrate concentration range of 0 to 1.0 mM. The test tubes were shaken at 25°C overnight. Independent experiments had shown that electrolyte diffusion through the dialysis tubing was 90% complete in 60 minutes. The external solution was then analyzed for nitrate, pH, and sodium. Nitrate concentrations were measured using ion chromatography techniques described in a previous chapter (Barak and Chen, 1987) using a Perkin-Elmer Series 10

Liquid Chromatograph pump, a Wescan 269-003 Ion-Guard Anion Cartridge as an ultra-high speed anion column, Jasco Uvidec-100-V UV spectrophotometer with a 1 cm optical path flow-through cell for indirect UV detection at 300 nm, and a LDC/Milton Roy CI-10 integrator; the eluent was 25 mM phthalic acid at a flow rate of 5 mL min^{-1}. Nitrate standards were prepared gravimetrically from the sodium nitrate stock solution. Measurements of pH were made with a combined glass-calomel electrode and a Radiometer pHM 84 pH meter calibrated with phthalate and phosphate buffers. Sodium was analyzed with a Corning 400 flame photometer. Blanks were set up containing dialysis cells without humic acid. At the end of the experiment, the dialysis cells were extracted and dried at 60°C in order to permit calculation of total weight of liquid by difference.

Measured exclusion volume was calculated as follows:

$$W_O C_O = W_{incl} C_s$$

$$W_{ex} = W_s - W_{incl}$$

yielding the formula:

$$V_{ex} = \frac{W_s - W_o (C_o/C_s)}{\rho_w W_{HA}}$$

where: V_{ex} = volume from which nitrate is excluded ($cm^3 g^{-1}$)

W_s = total weight of liquid in sample

W_o = weight of nitrate stock solution added to sample

W_{incl} = weight of liquid in sample into which nitrate mixed

C_s = nitrate concentration in sample

C_o = nitrate concentration of nitrate stock solution

ρ_w = density of water

W_{HA} = weight of humic acid in dialysis cell

For the same dialysis cells, measurements were made with a nitrate specific electrode (Radiometer F2412NO3 with a Radiometer K401 calomel reference) in both the external and internal dialysis cells, using a Radiometer PHM84 pH-meter to measure potential. Calibration curves for the nitrate electrode were constructed using the readings of the external cell (without humic acid) and nitrate measurements by ion chromatography.

RESULTS AND DISCUSSION

Titrations with Nitrate Specific Electrodes -- The results of acid-base titration with nitrate activity measured by the Orion ISE (Fig. 1) show that in the absence of nitrate added to the humic acid solution, the apparent nitrate activity is on the order of 10^{-6} M, which is the equivalent of 0.03-0.07 meq NO_3 $g^{-1}HA$, and is relatively independent of pH and hysteretic effects. When the humic acid solution was prepared in 10^{-4} M NO_3^-, the measured nitrate activity was lower than the calculated nitrate activity, suggesting nitrate adsorption on the humic acids. This apparent adsorption declined sharply with increasing pH until at pH 10, the nitrate electrode reading was approximately equal to that expected assuming no nitrate sorption or repulsion. Upon back-titration, the apparent adsorption was reversible,

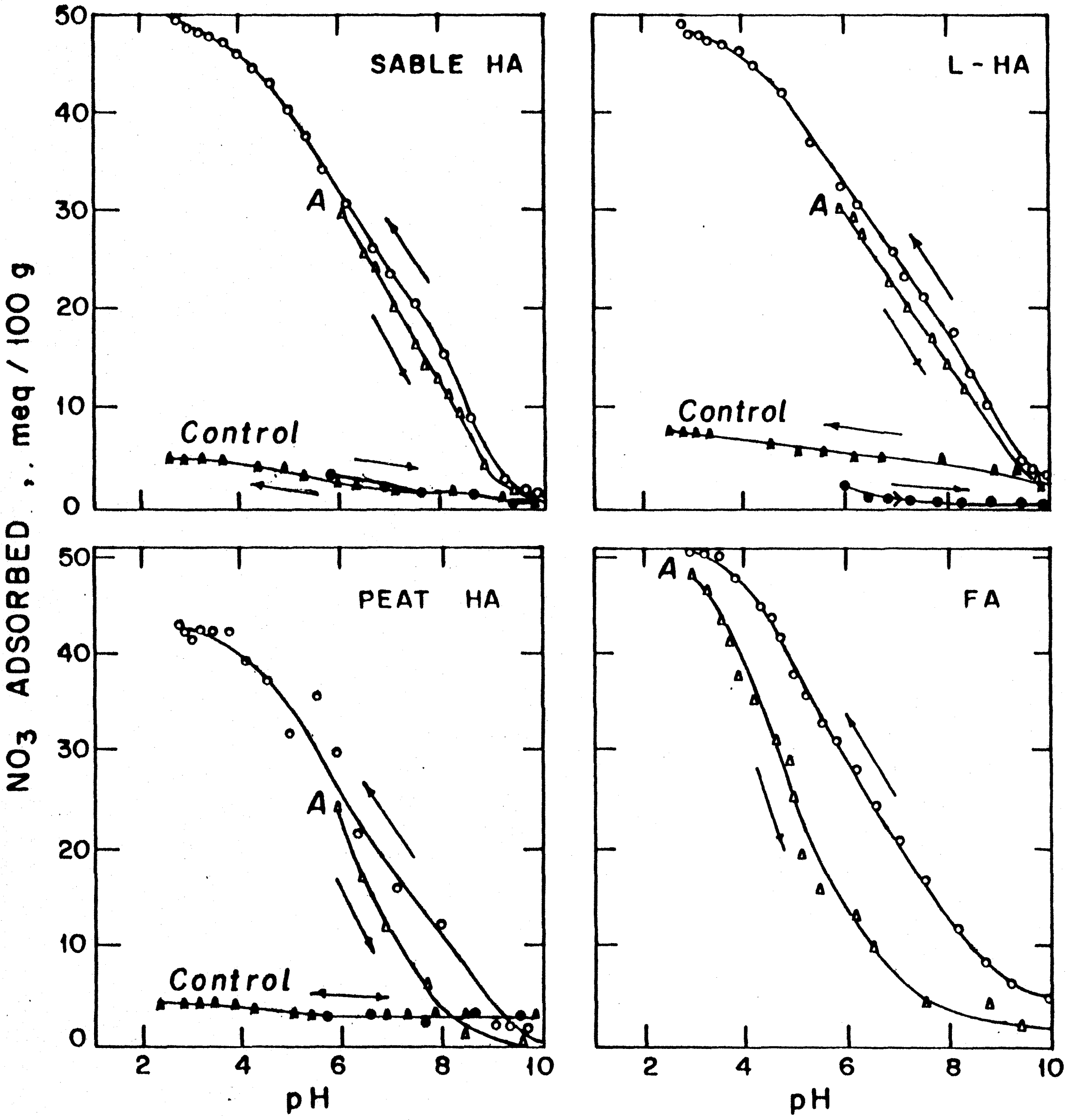


Fig. 1. Apparent nitrate activity of four humic substances as measured by Orion nitrate specific electrode, calculated as apparent nitrate adsorption. Titration with base in the presence of 10^{-4} M KNO_3 (Δ), titration with acid in the presence of 10^{-4} M KNO_3 (○), titration with base - KNO_3 absent (▲), and titration with acid - KNO_3 absent (●).

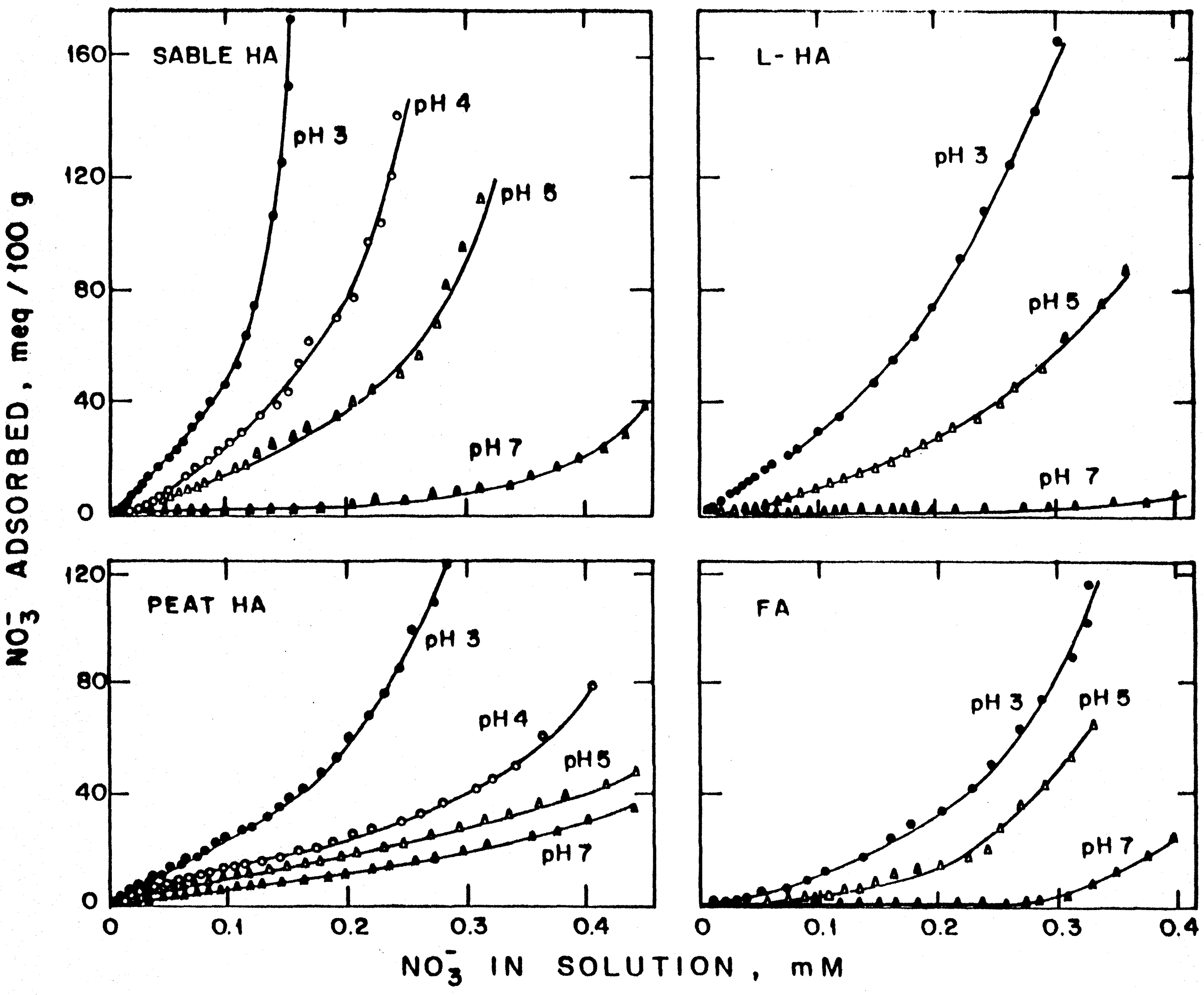


Fig. 2. Apparent nitrate activity of four humic substances as measured by Orion nitrate specific electrode at constant pH, calculated as apparent nitrate adsorption.

although some hysteresis occurs for two of the samples, and reached a maximum of about 0.5 meq NO_3^- g^{-1}HA at pH 2.2.

When titrations were conducted at constant pH and with increments of KNO_3 and nitrate activity measurements using the Orion ISE, results consistent with strong nitrate adsorption at acidic pH were noted (Fig. 2). All humic substances show decreasing slope of adsorption isotherms with increasing pH; at pH 7, adsorption was negligible or nonexistant at the lower concentration range. The largest value of apparent adsorption measured was 1.65 meq NO_3^- g^{-1} HA at 0.14 mM NO_3^- at pH 3, but none of the adsorption isotherms showed signs of approaching saturation of presumed adsorption sites; in fact, the electrode data would seem to indicate an increasing affinity for nitrate adsorption as the amount of adsorption increased. All humic substances show decreasing slope of the adsorption isotherms with increasing pH; at pH 7, adsorption was negligible or nonexistant for three of the four humic substances tested at the lower concentrtion range.

Measurements with the Radiometer nitrate specific electrode differed significantly from those of the Orion electrode. The magnitude of the apparent nitrate activity measured by the Radiometer ISE upon immersion in a humic acid solution is shown by Fig. 3 to be both greater in magnitude and strongly dependent on pH. The apparent nitrate activity ranged from 1 to 17 mM nitrate, equivalent to 0.4 to 6.8 meq NO_3^- g^{-1} in the purified humic acids. This inital apparent nitrate concentration seems to be due exclusively to humic acid interference since no nitrate was otherwise detectable in the humic acids, which had undergone careful extraction and purification using only analytical reagents, followed by extensive dialysis. The apparent presence of

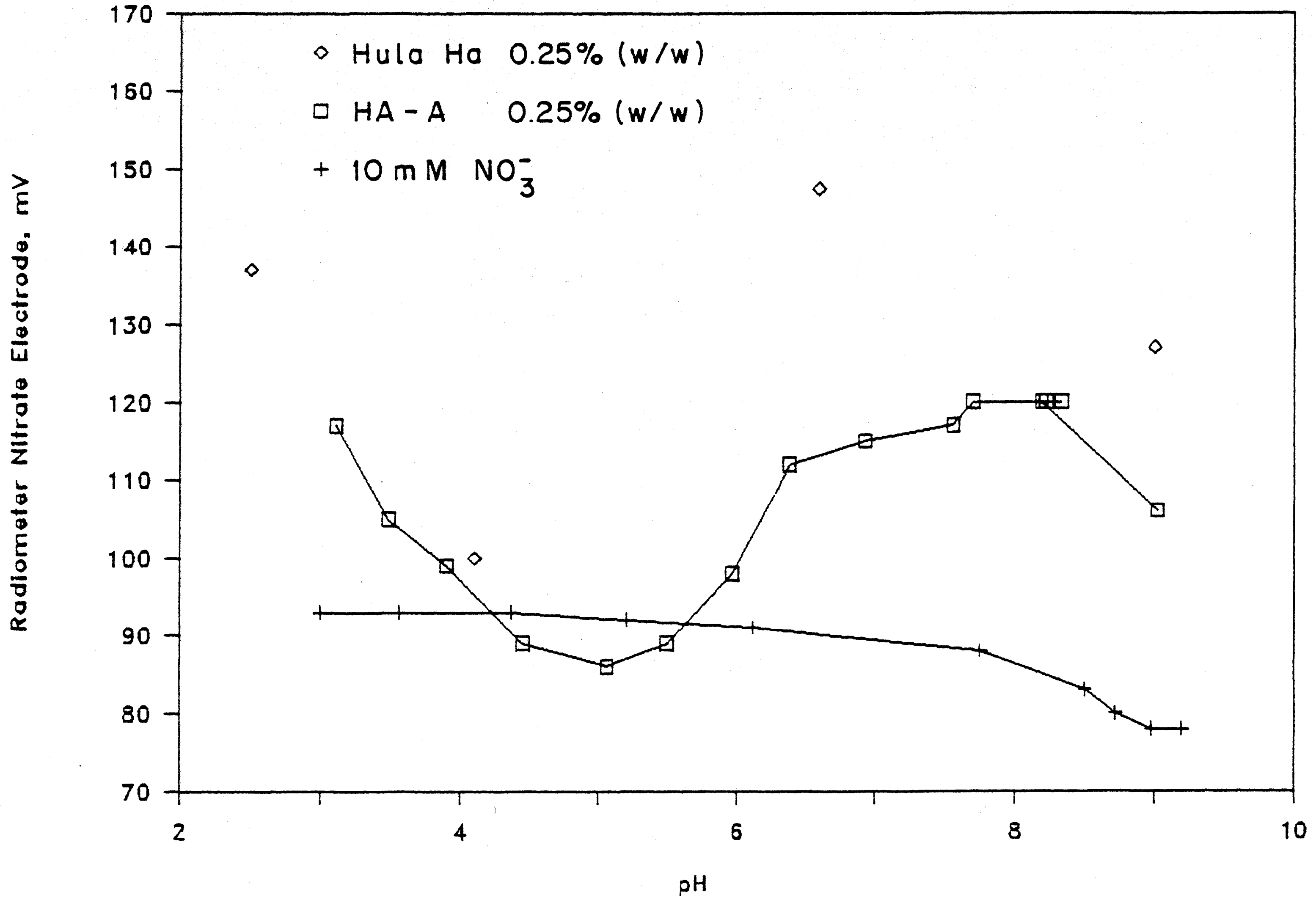


Fig. 3. Apparent nitrate activity of a 0.25% w/w Hula and HA-A humic acid solutions (no nitrate added) as measured by a Radiometer nitrate specific electrode.

nitrate upon immersion of the Radiometer electrode was also noted in humic substances prepared in the laboratories of Prof. M. Schnitzer (Ottawa, Ontario, Canada) and Prof. F.J. Stevenson (Urbana, IL, USA).

For titrations of 0.25% (w/w) humic acid solutions with nitrate stock solutions at fixed pH values with the Radiometer ISE, the measured nitrate activity was always greater than the total concentration of added nitrate (Figs. 4 and 5). In an attempt to remove the effect of the apparent nitrate activity upon immersion, the initial nitrate activities were subtracted from the readings during the course of the titration (Figs. 6 and 7). The results of such calculations suggest that that the electrode is measuring the effects of nitrate exclusion by the humic macromolecule. Furthermore, the extent of appparent exclusion increases with pH from 2.5 to 4.1 to 6.1, which is in general accord with theoretical considerations. However, at pH 9, no exclusion is apparent, which casts doubt upon the validity of the nitrate electrode measurements since exclusion at pH 9 must be at least as large as at pH 6, and is probably even greater due to the ionization of very weakly acidic groups.

Overall, although both manufactures of nitrate specific electrode were able to produce excellent calibration curves in water, the presence of high concentrations of humic substance in solution caused marked anomalies, even though the readings were steady and reversible. Readings indicative of either nitrate adsorption or nitrate exclusion could be measured for the same humic substance, depending upon whether the results of the Orion or the Radiometer electrode were considered. Trial calculations were made to extract a selectivity ratio of the type:

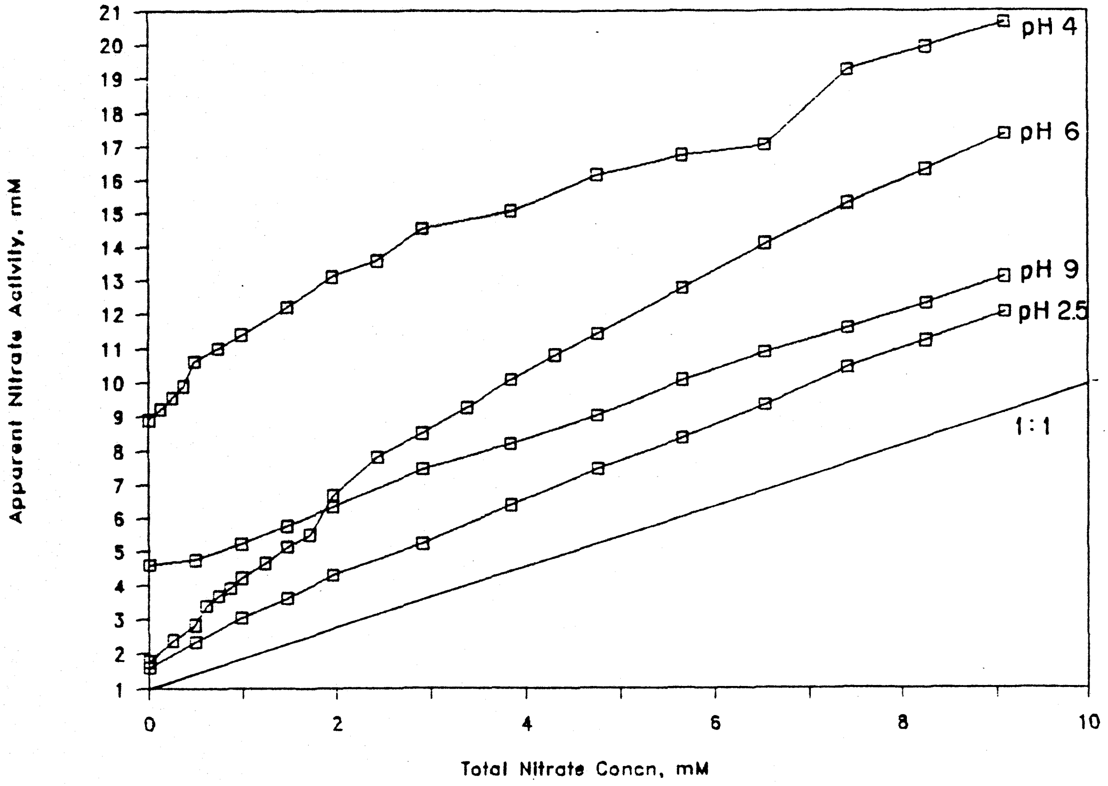


Fig. 4. Apparent nitrate activity measured by a Radiomseter nitrate specific electrode of a 0.25% w/w Hula humic acid solution titrated with nitrate.

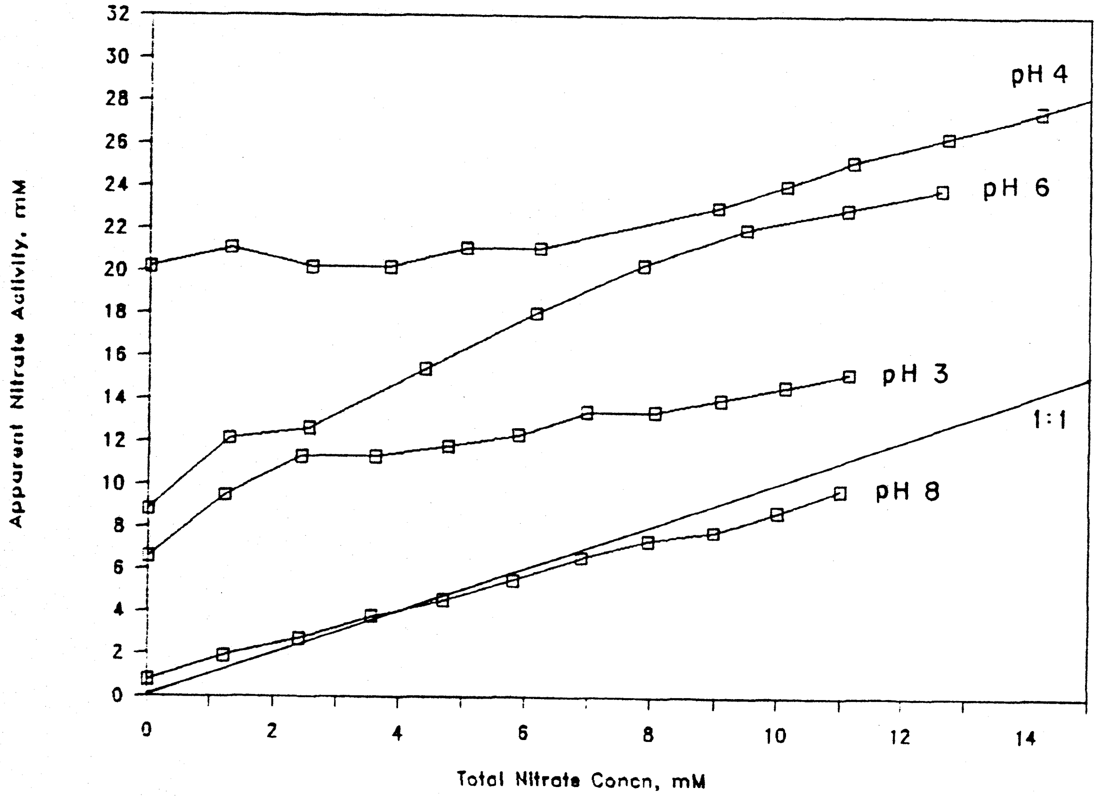


Fig. 5. Apparent nitrate activity measured by a Radiometer nitrate specific electrode of a 0.25% w/w HA-A solution titrated with nitrate.

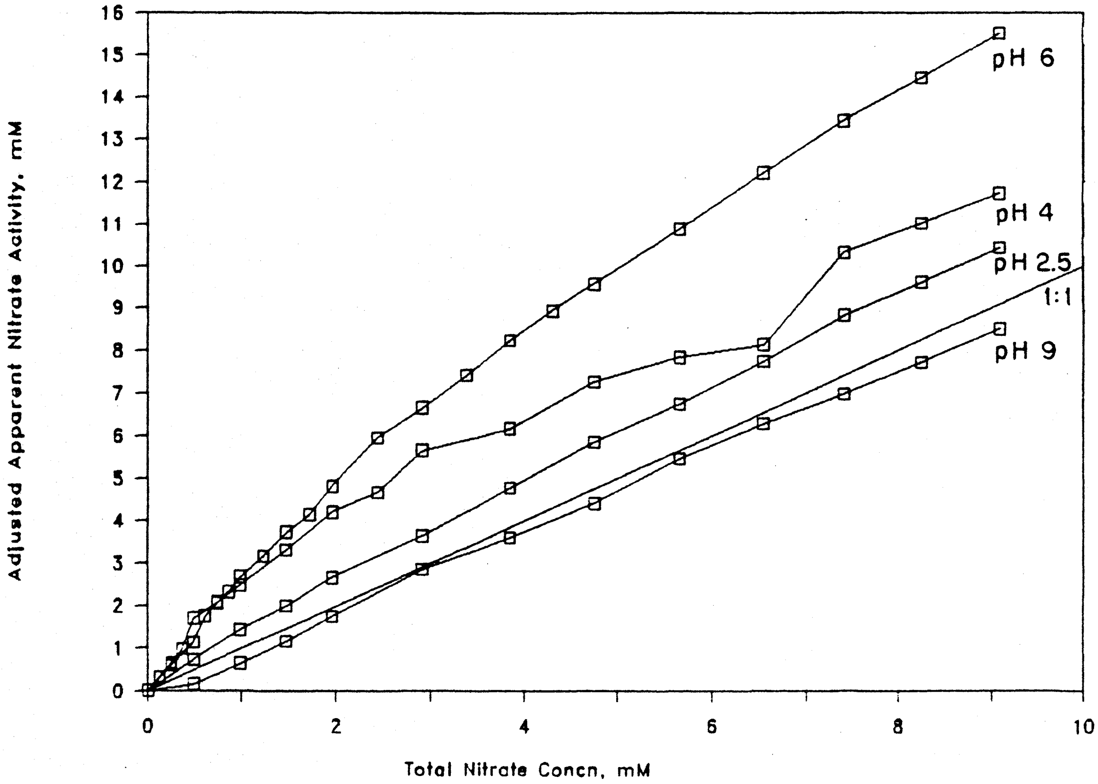


Fig. 6. Apparent nitrate activity measured by a Radiometer nitrate specific electrode of a 0.25% w/w Hula humic acid solution titrated with nitrate, after deduction of apparent initial nitrate activity.

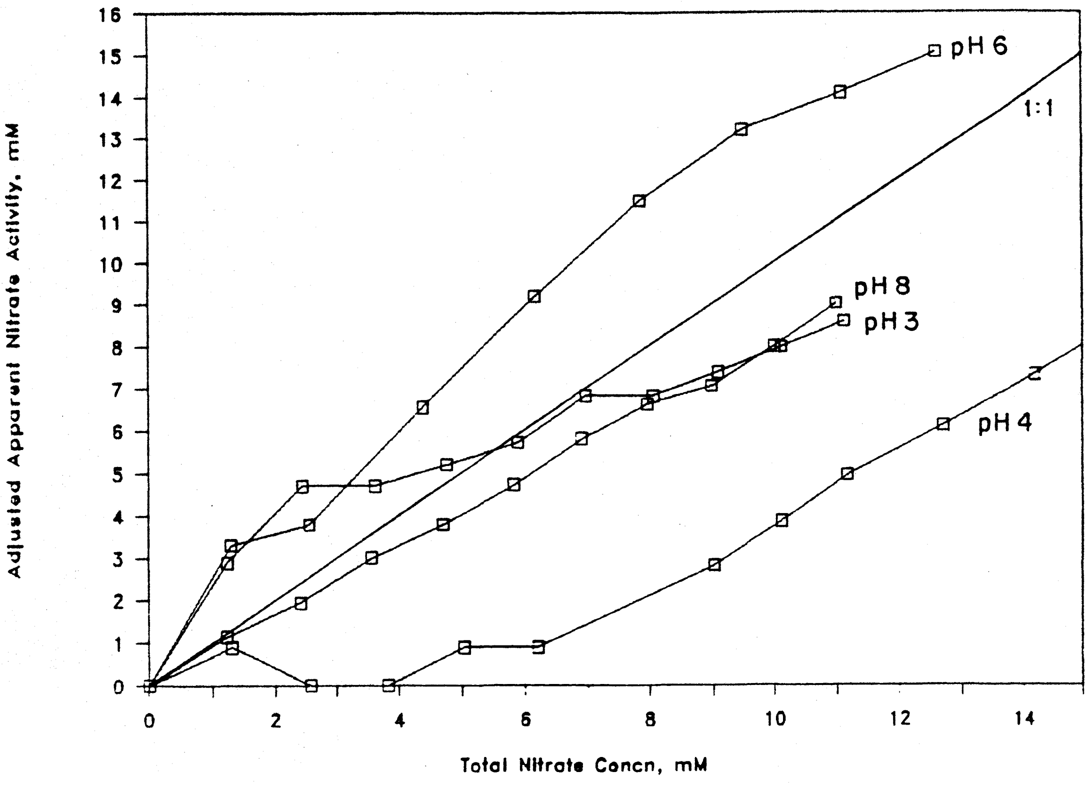


Fig. 7. Apparent nitrate activity of a 0.25% w/w HA-A solution titrated with nitrate, after deduction of apparent initial nitrate activity as measured by a Radiometer nitrate specific electrode.

$$E = E_0 - 2.303\ RT/F\ \log(a_1 + Ka_2)$$

where E and E_0 are measured and reference potentials, R is the gas constant, T is absolute temperature, F is the Faraday constant, a_1 is the activity of the primary univalent anion, a_2 is the activity of a second univalent anion, and K is the selectivity ratio of anion 2 for the given electrode, following Srinivasan and Rechnitz (1969). This approach is inapplicable for the Orion ISE since K would have to be negative to fit the data, but the Radiometer electrode data failed to yield a single-valued selectivity ratio, even at a constant pH. Calculations attempting to extract a junction potential created by the humic acid also failed to produce a single-valued result. The possibility of some type of reversible chemisorption of the humic macromolecule on the electrode membrane cannot be ruled out as the source of interference. In any case, the use of nitrate electrodes immersed in solutions of humic substances during the course of titration with nitrate does not produce unequivocable data, and batch experiments to produce high-quality data seem to be required. However, these difficulties do not rule out the use of nitrate specific electrodes in solutions not containing dissolved humic substances and probably also in natural waters containing typically low levels of dissolved humic substances, which was not relevent here.

Dialysis and Ion Chromatography __ Measurement of nitrate exclusion phenomena using dialysis cells and ion chromatography is the most conservative system since the dialysis cell separates the humic acid solution from its equilibrium solution and ion chromatography separates nitrate from all other anions which may be present in the equilibrium solution before the measurement is made against a standard background. Results of such analyses are presented in Table 1. The measured exclusion volumes ranged from 60-80 $cm^3\ g^{-1}$ for those humic acid treatments at 100 mM by the addition of Na_2SO_4 to 300-400 $cm^3\ g^{-1}$ for those humic acids at 1 mM or less. The exclusion volume increases with pH over the range of 3 to 7.5.

The Radiometer nitrate specific electrode measurements correlated well with ion chromatographic measurements of nitrate in the external solutions without humic acid and permitted drawing of calibration curves (Fig. 8). However, the nitrate activities measured upon immersion in the internal solutions containing humic acid were consistently higher than the activities measured in the external solutions in equibilibrium across the dialysis membrane (Fig. 9). This discrepancy was on the order of 1-2 mM NO_3^- and is contrary to both theoretical anion exclusion considerations and material balance equations using ion chromatography which show exclusion of nitrate from the humic acid solution and therefore a lower nitrate concentration in the internal solution. Clearly, the nitrate electrode is responding properly to nitrate in the absence of humic acid but is reading high in its presence, indicating some type of reversible chemical interference with the nitrate-specific ion exchanger.

Table 1. Nitrate exclusion data measured by ion chromatography. Net exclusion of nitrate by Hula humic acid is given in mL/g after correction for dialysis cell blank.

#	10 mM NO$_3$ Added, -- mL --	Total Volume -- mL --	Nitrate Conc'n		Nitrate Volume		Net Excln, mL/g HA	Ionic Str, mM	pH
			Ideal	Meas'd	Included	Excluded			
			---- mM -----		------ mL -----				
NITRATE1:									
1	0.117	11.492	0.102	0.148	7.93	3.56	298	1.67	7.23
2	0.195	9.951	0.196	0.282	6.91	3.04	244	1.62	7.15
3	0.428	9.947	0.430	0.596	7.18	2.77	227	2.08	7.47
4	0.628	10.113	0.621	0.870	7.22	2.89	240	2.13	7.20
5	0.795	9.745	0.816	1.137	6.99	2.75	231	2.51	7.16
6	1.003	9.952	1.008	1.281	7.83	2.12	172	2.76	7.05
7	0.136	11.692	0.116	0.090	15.08	-3.39	25	44.40	6.77
9	0.391	9.993	0.391	0.301	13.00	-3.01	62	41.10	6.82
10	0.594	10.149	0.585	0.477	12.45	-2.30	133	42.00	6.67
11	0.774	10.018	0.773	0.604	12.80	-2.79	85	40.50	6.67
12	0.990	10.038	0.986	0.760	13.02	-2.98	65	42.00	6.54
NITRATE2:									
1	0.103	10.013	0.103	0.144	7.13	2.88	213	0.43	3.51
2	0.211	10.029	0.210	0.282	7.47	2.56	139	0.23	3.57
3	0.398	9.992	0.398	0.513	7.76	2.23	150	0.45	3.56
4	0.608	9.992	0.608	0.755	8.05	1.94	134	0.56	3.53
5	0.790	10.001	0.790	0.930	8.49	1.51	103	0.72	3.54
6	1.015	10.001	1.015	1.170	8.68	1.32	99	1.04	3.55
NITRATE3:									
3	0.414	10.854	0.381	0.468	8.85	2.01	139	0.36	3.67
4	0.618	10.471	0.590	0.707	8.74	1.73	119	0.49	3.56
5	0.788	10.692	0.737	0.856	9.20	1.49	101	0.58	3.58
6	0.972	10.358	0.938	1.069	9.09	1.27	84	0.77	3.51
15	0.386	10.467	0.369	0.390	9.90	0.57	57	14.10	3.95
16	0.705	10.533	0.669	0.713	9.89	0.65	65	15.20	3.92
18	1.091	10.035	1.087	1.157	9.43	0.61	61	16.55	3.85
NITRATE4:									
1	0.093	11.456	0.081	0.127	7.34	4.12	401	0.47	5.61
2	0.223	11.021	0.202	0.280	7.97	3.05	294	0.51	5.50
3	0.412	10.655	0.387	0.521	7.90	2.75	264	0.73	5.35
4	0.584	11.742	0.497	0.643	9.08	2.66	255	0.82	5.31
5	0.799	10.873	0.735	0.983	8.13	2.74	264	1.11	5.17
6	0.990	10.699	0.925	1.211	8.18	2.52	241	1.27	5.18
7	1.193	10.967	1.088	1.411	8.46	2.51	240	1.44	5.10
8	1.403	11.073	1.267	1.620	8.66	2.41	230	1.58	5.14
9	1.583	11.463	1.381	1.977	8.01	3.46	335	1.73	5.08

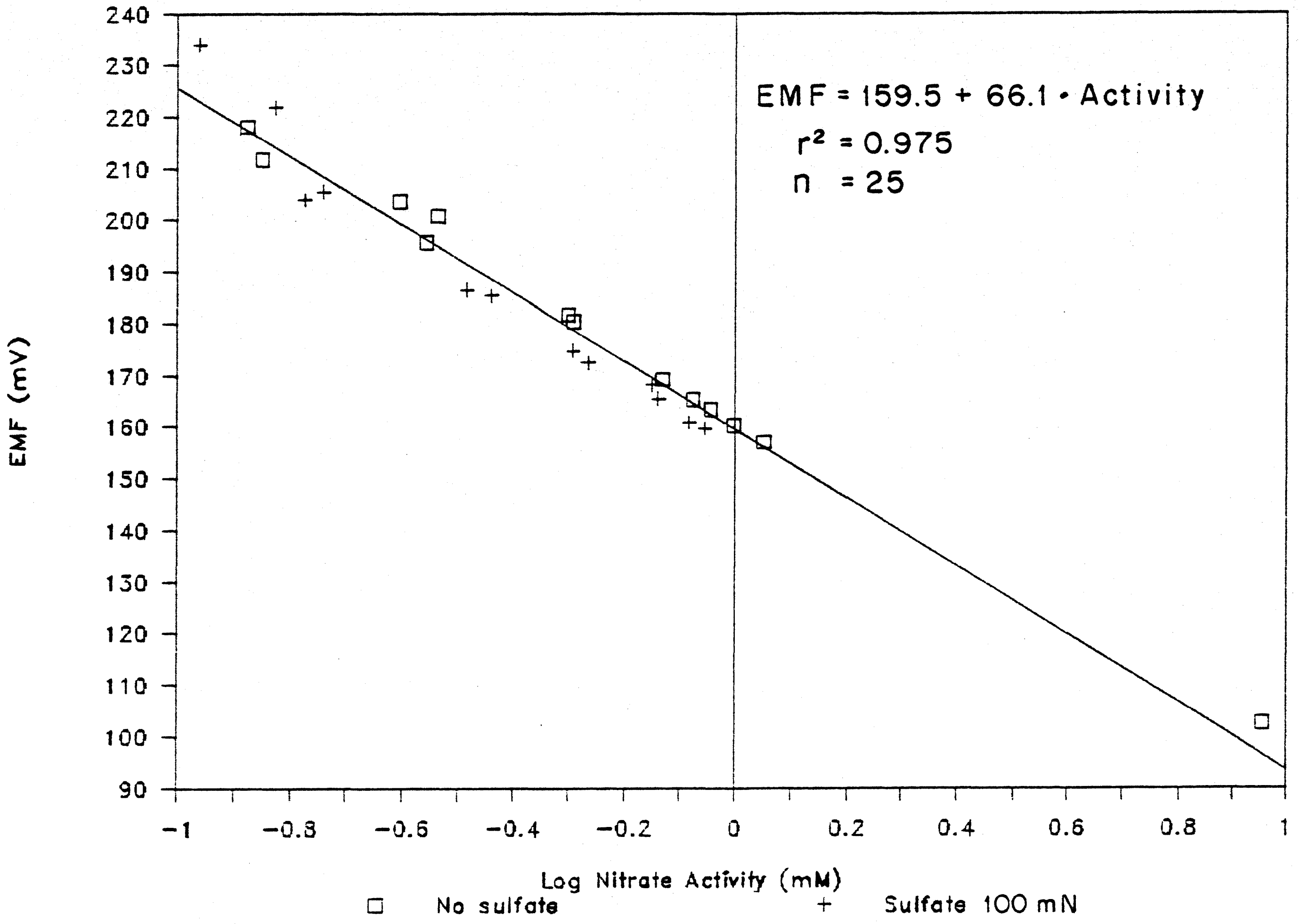


Fig. 8. Calibration of a Radiometer nitrate specific electrode against results of ion chromatography for external dialysis cells (no humic acid) in batch experiments. Nitrate activities were calculated from nitrate concentrations and activity coefficients based on ionic strengths using the Debye-Huckel equations.

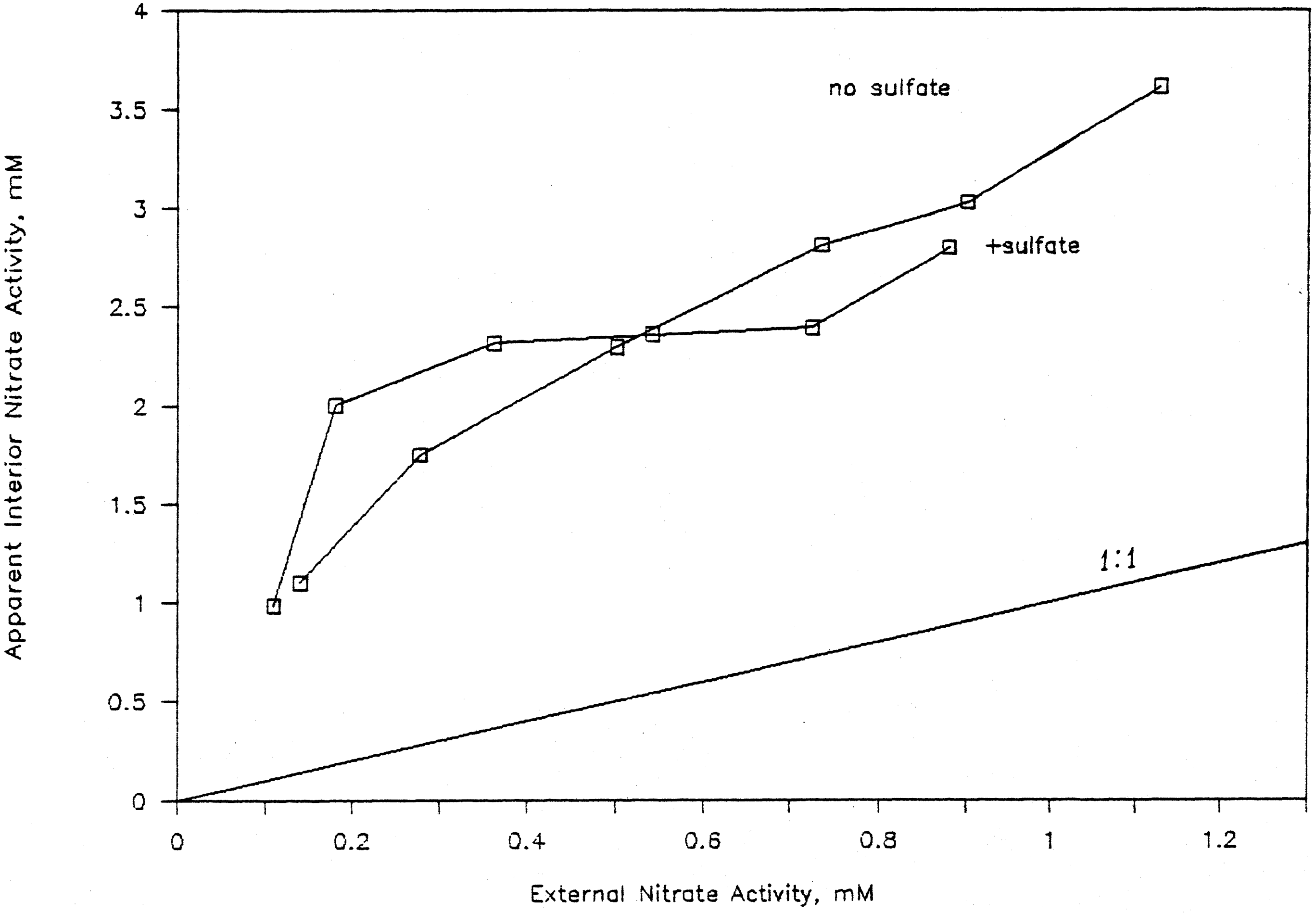


Fig. 9. Apparent nitrate activity measured by a Radiometer nitrate specific electrode in internal cells (0.25% HA) compared with measured nitrate concentration in external cells (no humic acid).

CONCLUSIONS

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. The possibility of some degree of nitrate adsorption by humic acid cannot be ruled out but the magnitude of nitrate exclusion measured masks whatever small amount of adsorption may exist.

In the next chapter, data gathered here by dialysis and ion chromatography will be combined with additional information on the nature of the acidic groups to formulate and define a quantitative model of the humic macromolecule and its double electric layer.

APPARENT RADIUS OF HUMIC MOLECULES FROM ACID-BASE TITRATION AND NITRATE EXCLUSION

Humic substances are natural, organic substances ubiquitous in soil and sediments. Humic substances have average 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:

$$RCOOH \Longleftrightarrow RCOO^- + H^+ \qquad (1)$$

where: (2)

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

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

$$K = (H^+)_s(RCOO^-)/(RCOOH) \quad \text{and} \quad pK = pH_s - \log[(RCOO^-)/(RCOOH)] \tag{3}$$

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

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

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

$$pK = pH + 0.434\,\phi_s - \log[(RCOO^-)/(RCOOH)] \tag{5}$$

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:

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

with the boundary conditions:

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

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 $\kappa = (8\pi e^2 IN/1000\epsilon kT)^{1/2}$ 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:

$$\sinh \phi = \phi + \phi^3/3! + \phi^5/5! + \ldots;$$

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 $\phi(x) \alpha \exp(-\kappa x)$ in planar systems for $\phi << 1$, (van Olphen, 1977) especially considering that the polynomial expansion for the modified Bessel function is:

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

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. Titrations were begun below pH 3 in order to titrate the most acidic groups of the humic acid (Takatsu and Yoshida, 1978); 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:

$$(V_0 + V)[H^+] \approx m(V_s - V) \qquad (8)$$

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 $(Vo + V)[10^{-pH}]$ against V.

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

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

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:

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

$$pH = pK + n \log [m(V_w - V) - ([H^+] - [OH^-])(V_0 + V)) \; / \; m(V - V_s) + ([H^+] - [OH^-])(V_0 + V)] \qquad (10)$$

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:

$$\phi(\kappa x) = \Sigma f_{2n-1} (C * K_0(\kappa x))^{2n-1} \qquad (11)$$

where $n = 0,1,2,..9$, f_{2n-1} is a numerically integrated constant, $K_0(\kappa x)$ 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:

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

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

where $I = 0,1,2...$, 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:

$$A_{ex} = 2\pi \int_a^\infty (1-\exp(\phi(x)))\ x\ dx$$

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

$$V_{ex} = A_{ex}\, L = \frac{2}{a^2 p} \int_a^\infty (1-\exp(\phi(x))\; x\, dx$$

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 ($cm^3\ g^{-1}$)

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

Integration of $(1-\exp(\phi(x))x$ from the cylinder radius a to a distance of $12/\kappa$ 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.

$I = 1$:

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

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

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

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

$I = 2$:

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

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

$I = 3$:

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

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

$I = 4$:

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

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

RESULTS AND DISCUSSION

Characterization of Acidic Groups:

The strong acid equivalence point V_s, at which the titration of humic acid commences, was determined by Gran plot calculations. The end of the titration of the weakly acidic groups (and the beginning of the titration of the very weakly acidic groups) V_w 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 unambigous extrapolation of the strong (free) acid and total acid equivalent points (Fig. 2). Strong (free) acid equivalence points were all between pH 2.8 and 3.1, confirming the recommendation of Takamatsu and Yoshida (1978) to add strong acid before titration. 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; the contents of acidic groups is therefore fairly typical of soil-derived humic acids.

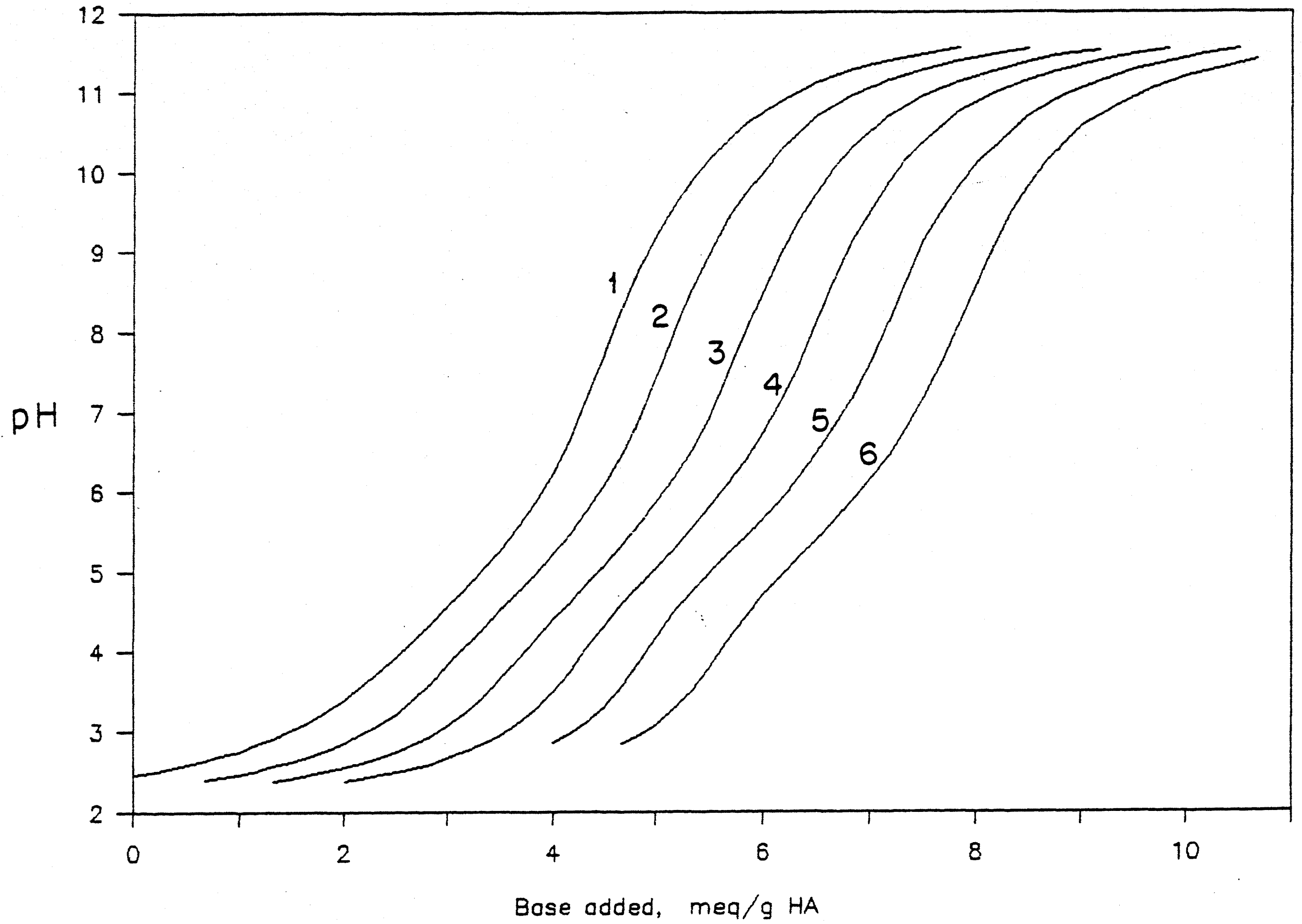


Fig. 1. Titration of Hula humic acid at several ionic strengths, 0.0927 M (1), 0.0266 M (2), 0.0135 M (3), 0.0046 M (4), 0.0018 M (5), and 0.0017 M (6). Curves have been displaced for purposes of display.

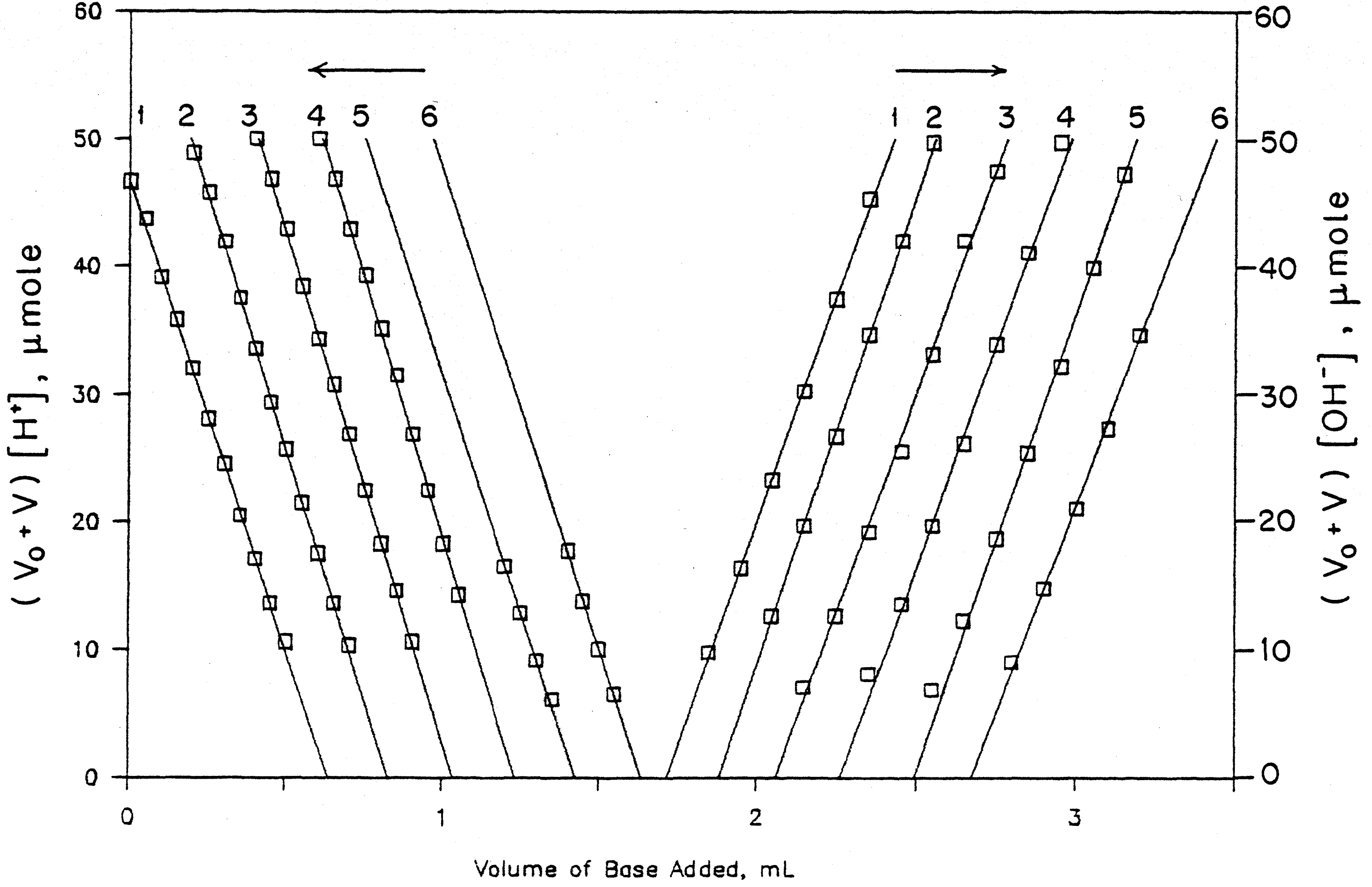


Fig. 2. Gran plot calculations for Hula humic acid. Sample labels as in Fig. 1. Curves have been displaced for purposes of display.

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 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).The shape of the Henderson-Hasselbach plot, although curvilinear, was not appreciably affected by ionic strength. The pK_a values at 0.100 M ionic strength and $\underline{n}$ 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 $\underline{n}$ = 2.07 (as the first derivative at 50% ionization), 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

Table 2. Characteristics of Hula Valley humic acid.

Ionic Strength mol L^{-1}	Weakly acidic group meq g^{-1}	pK_a	n	Very weakly acidic group meq g^{-1}	Total Acidity meq g^{-1}
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

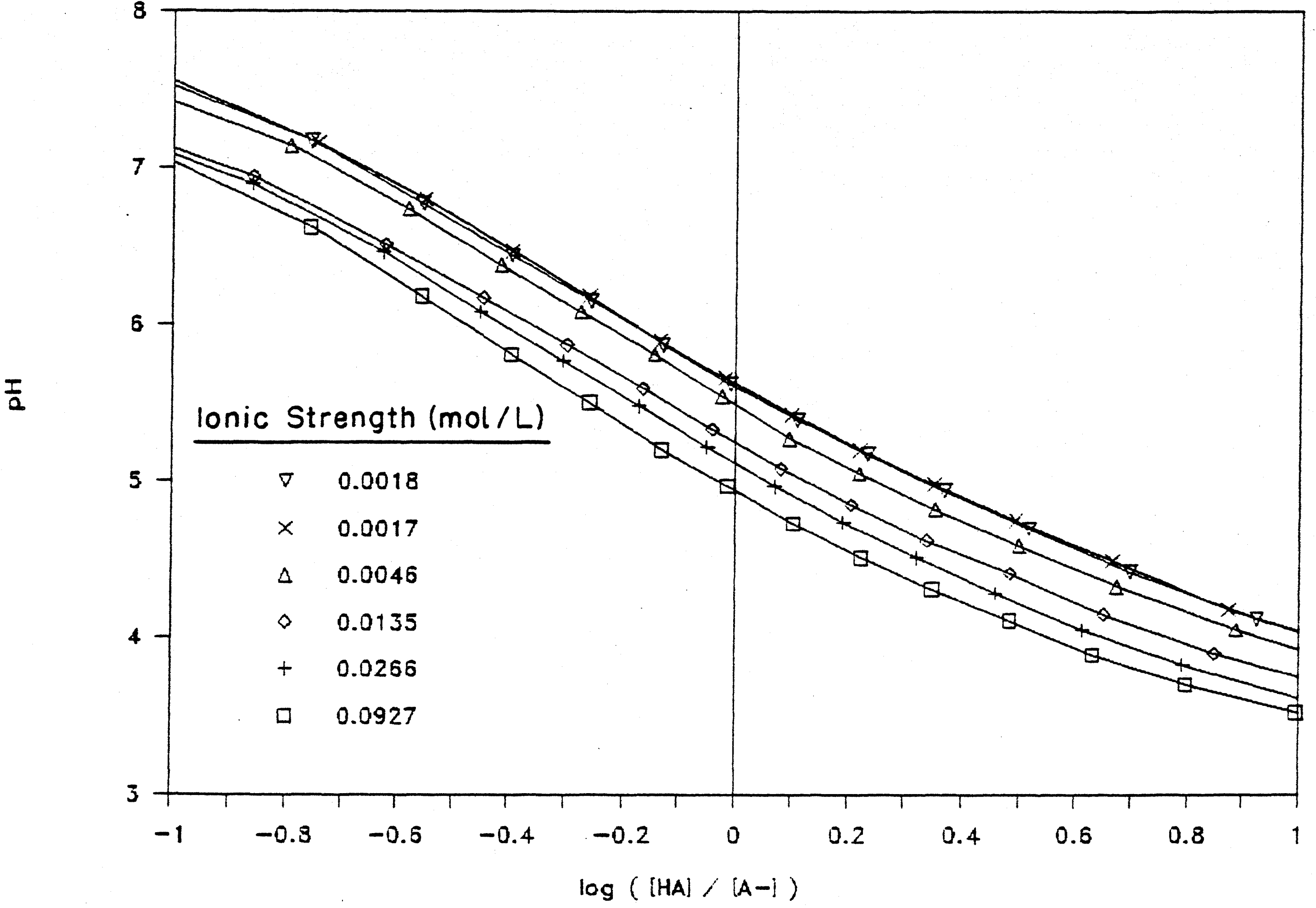


Fig. 3. Henderson-Hasselbach plots as a function of ionic strength for six titrations of Hula humic acid.

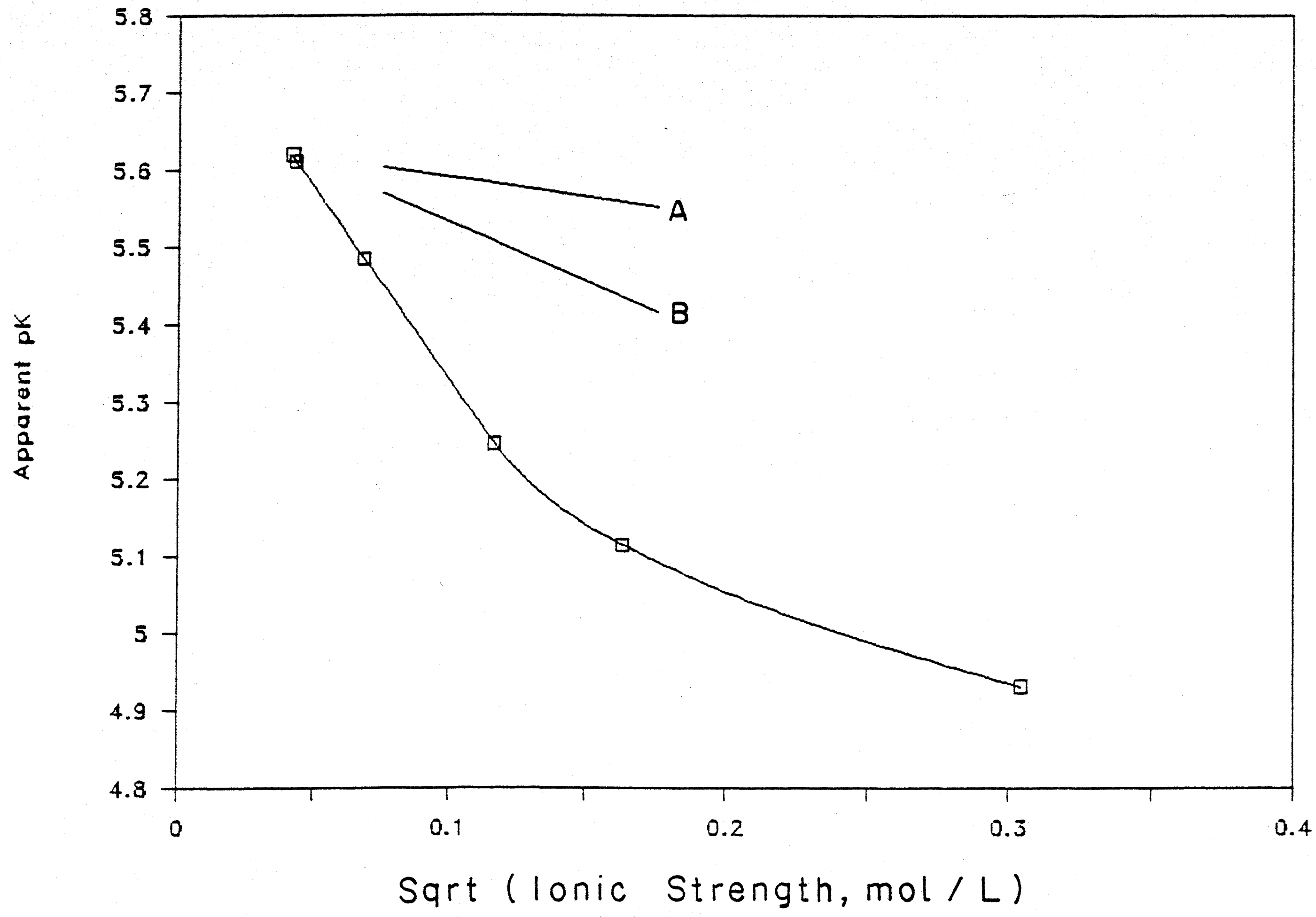


Fig. 4. Apparent pK at 50% ionization as a function of square root of ionic strength and radius. Lines A and B show slopes predicted by Debye-Huckel activity equations for a monocarboxylic and dicarboxylic acid, respectively.

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 discrepency 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 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 pH + 0.434 ϕ_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 pH + 0.434 ϕ_s about a mean for all six ionic strengths measured (Fig. 6).

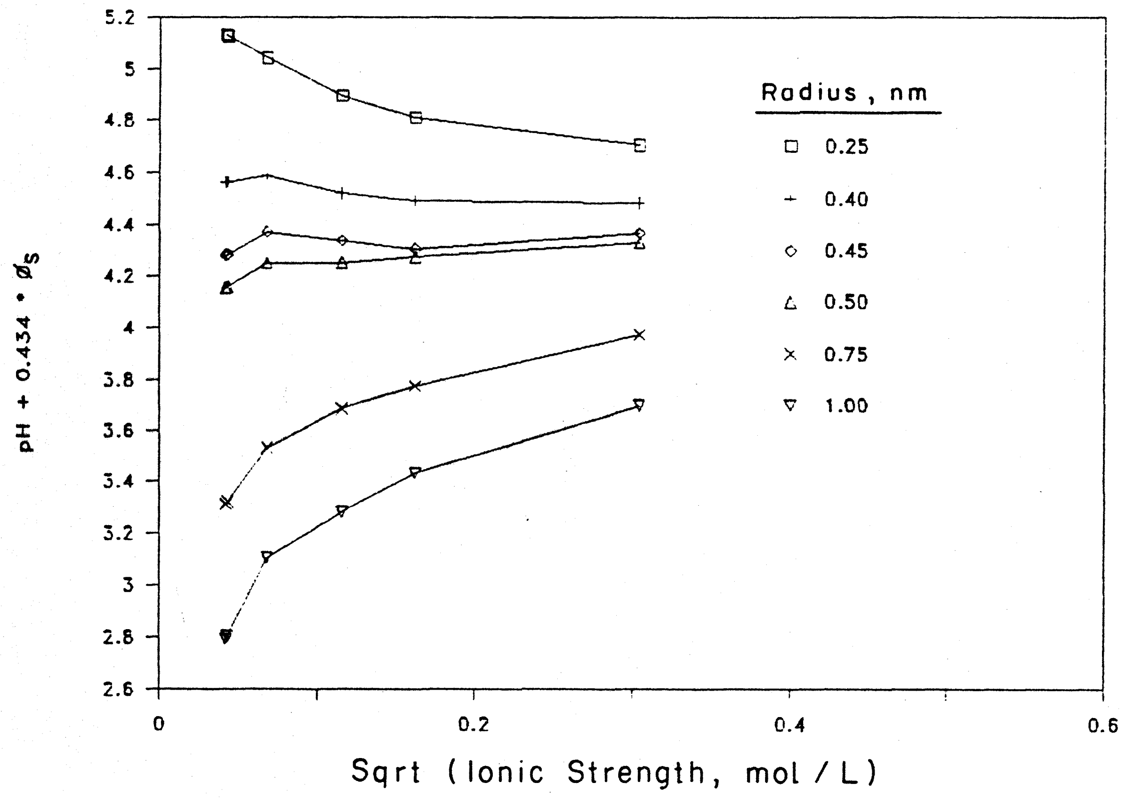


Fig. 5. Effect of assumed apparent radius on pH + 0.434 ϕ_S at 50% ionization for the range of ionic strengths measured.

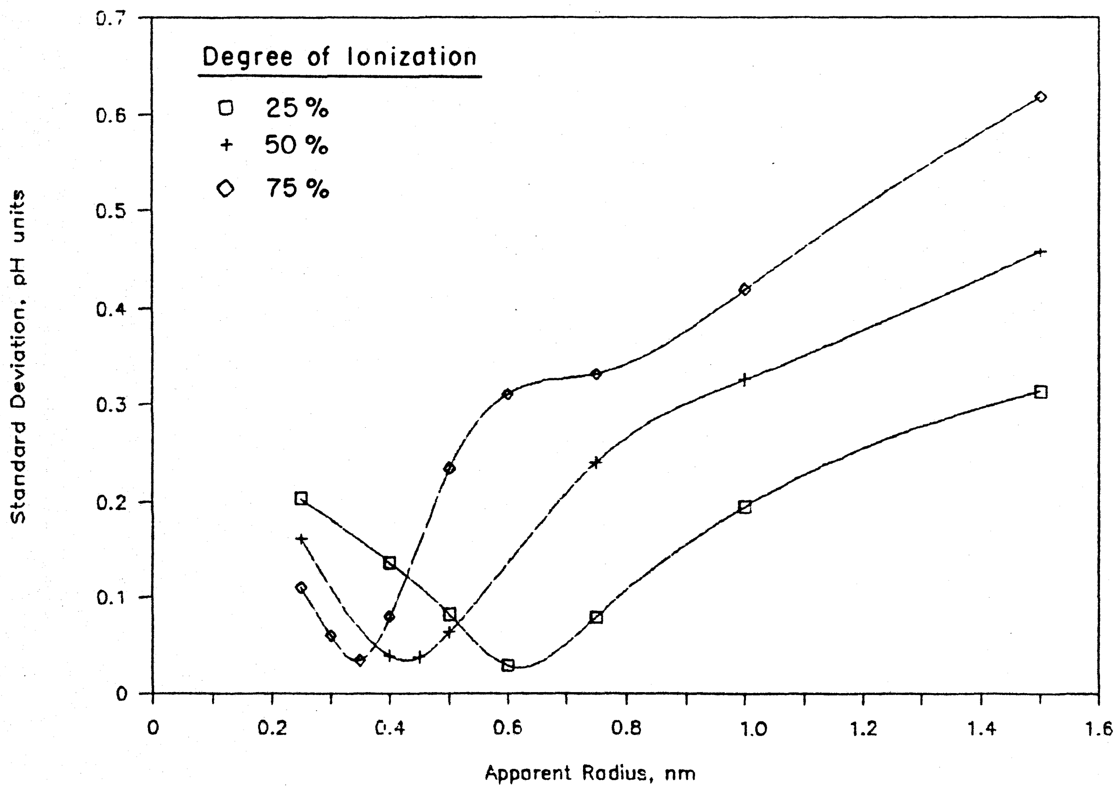


Fig. 6. Effect of choice of radius on variance of pH + 0.434 ϕ_S for 25, 50, and 75% ionization.

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 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 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 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 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 quantitity of negative charge and the extension and subsequent thinning of the humic macromolecule resulting from the electrostatic repulsion between ionized acidic groups.

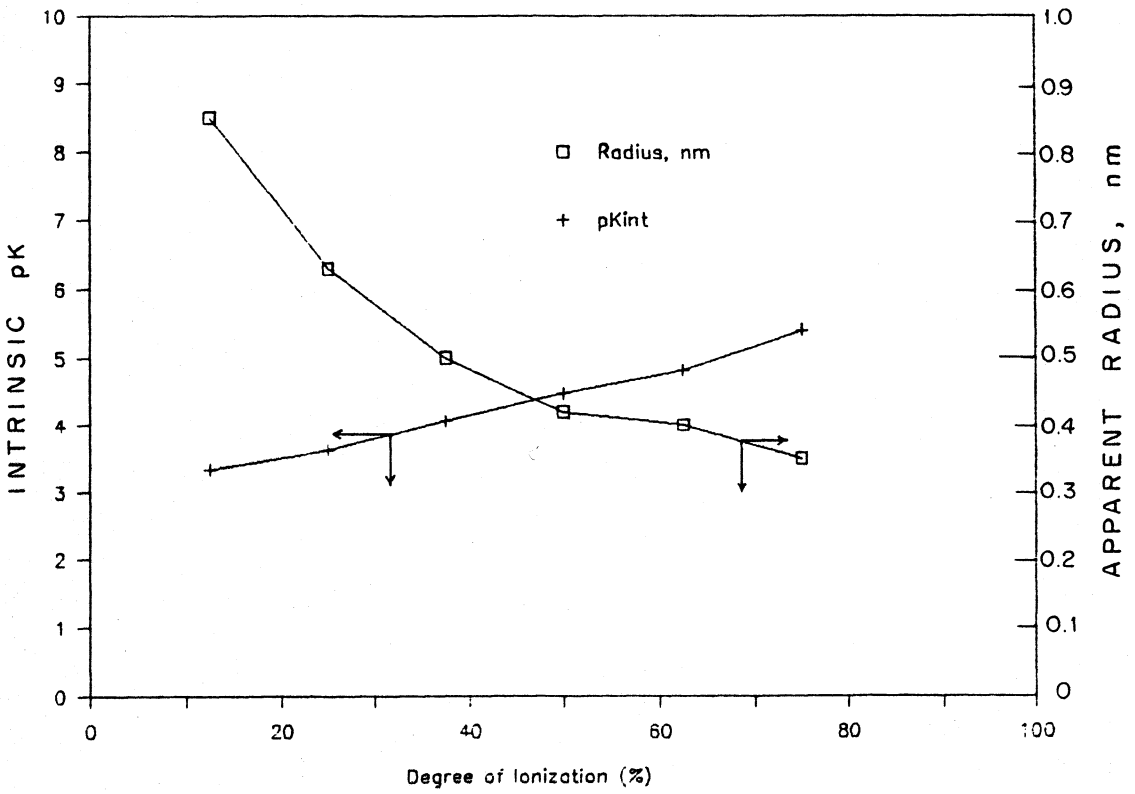


Fig. 7. Apparent radius and pH + $0.434\phi_s$ as a function of degree of ionization.

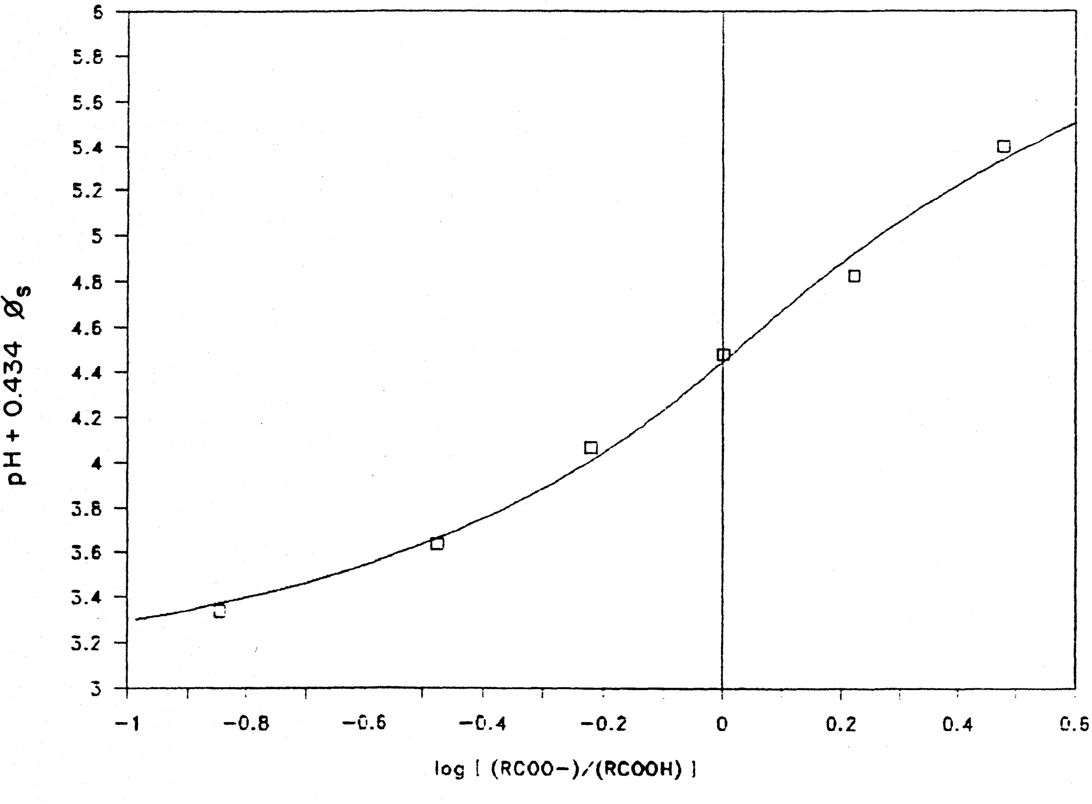


Fig. 8. Intrinsic Henderson-Hasselbach plot (symbols) and resolution by a mixture of two weak acids, 1.22 meq g^{-1} and pK = 3.59 and 1.17 meq g^{-1} and pK = 5.32 (solid line).

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

Degree of Ionization	Apparent Radius (nm)	$pH + 0.434\phi_s$ average ± st.dev.	Specific Surface Area ($m^2\ g^{-1}$)	Area per Charge (nm^2)
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

When the intrinsic $pH + 0.434\ \phi_s$ is plotted against $\log[(RCOOH/RCOO^-)]$ (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:

$$H^+ - OH^- = \frac{a_1K_1}{K_1 + H^+} + \frac{a_2K_2}{K_2 + H^+} - b_s \qquad (14)$$

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^+:

$$H^4 + H^3 [K_1+K_2+b_s] + H^2 [K_1K_2-W+(K_1+K_2)b_s-a_1K_1-a_2K_2] \qquad (15)$$
$$-H [(K_1K_2(a_1+a_2-b_s)+W(K_1+K_2)] - K_1K_2W = 0$$

where W is the dissociation constant of water. The equation was solved for $H^+(b_s)$ 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

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

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 distance^2), 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

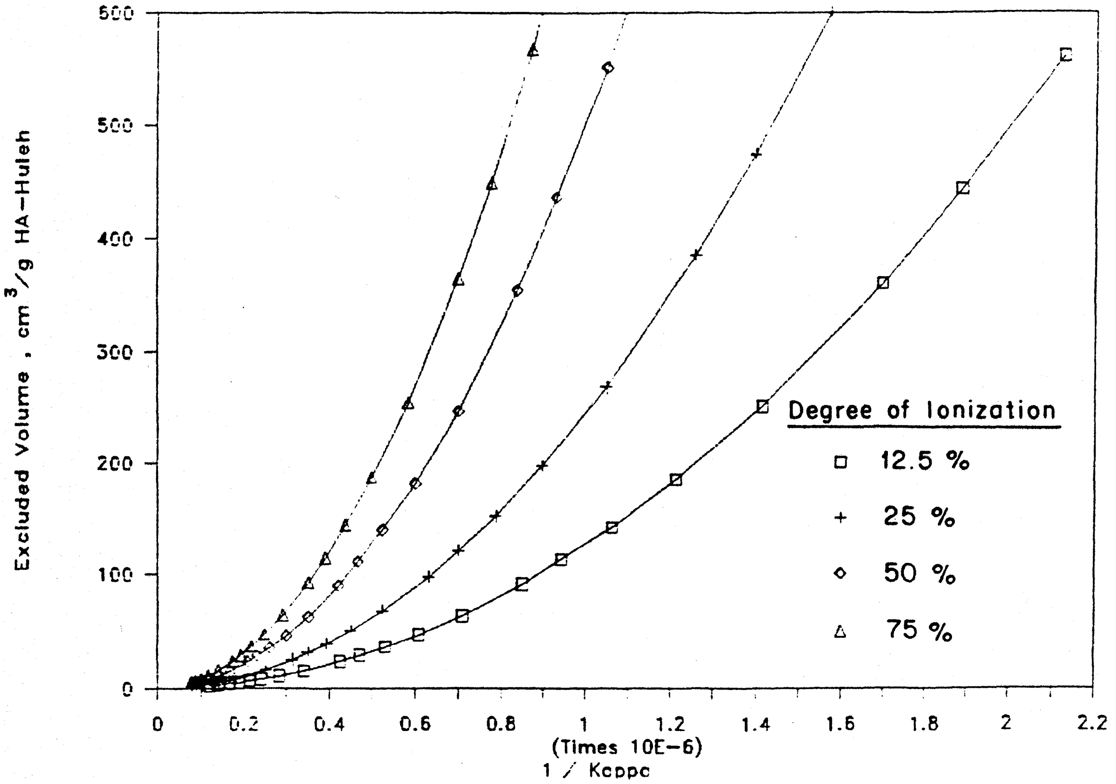


Fig. 9. Predicted anion exclusion volume (cm^3 g^{-1}) plotted against Debye-Huckel distance $1/K$, based on acid-base behavior.

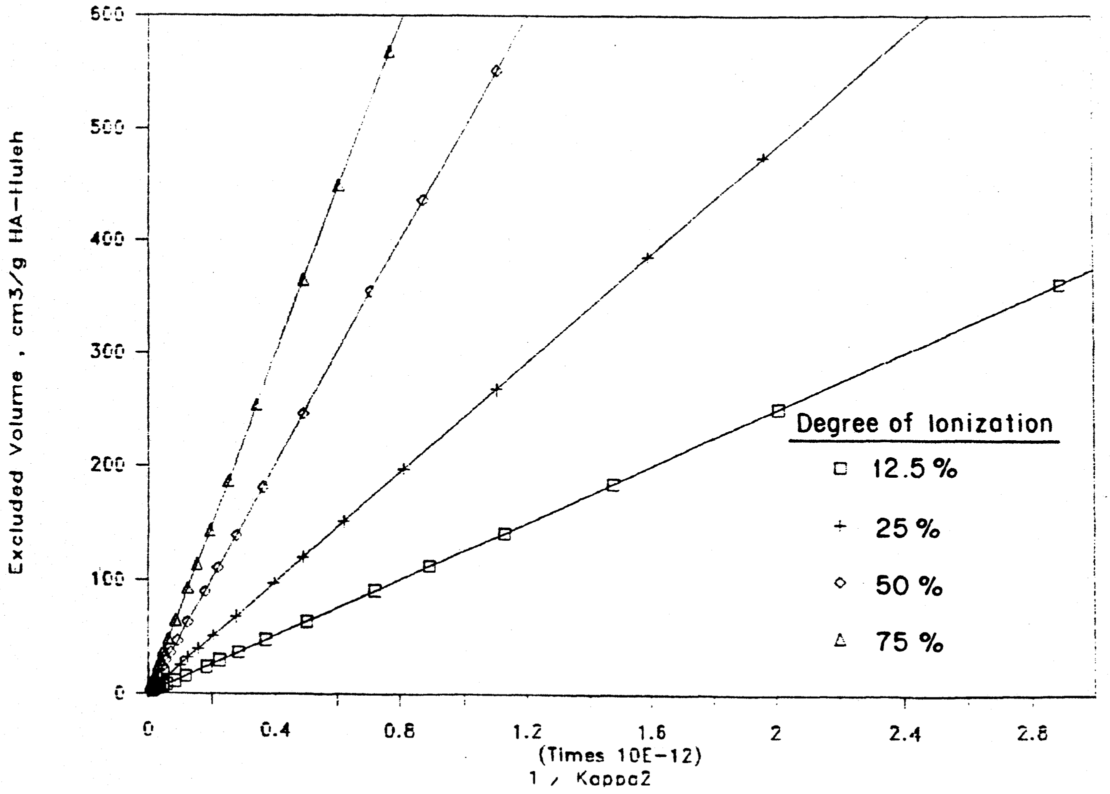


Fig. 10. Predicted anion exclusion volume (cm^3 g^{-1}) plotted against $1/K^2$

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 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 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).

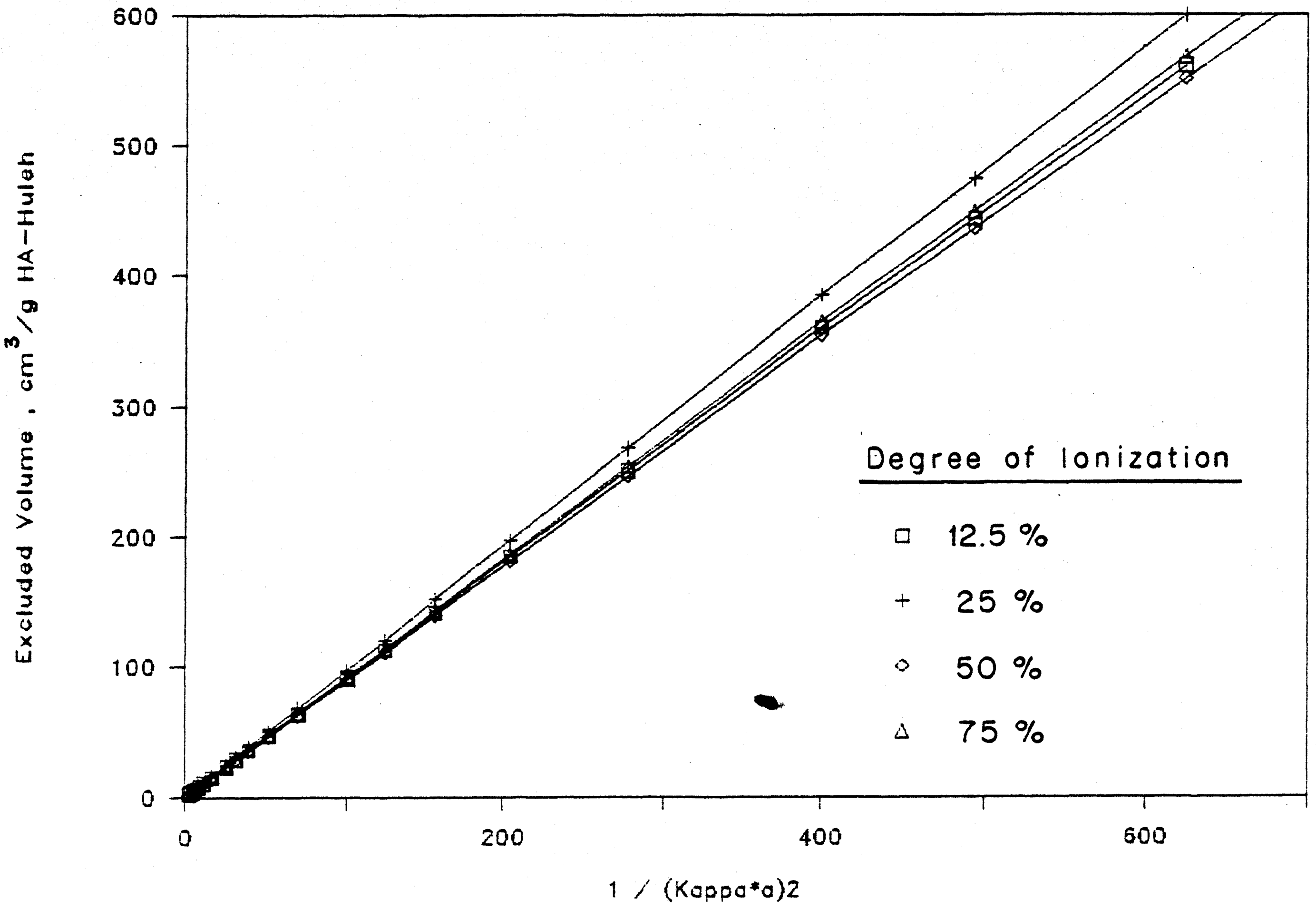


Fig. 11. Predicted anion exclusion volume (cm^3 g^{-1}) plotted against $1/(Ka)^2$.

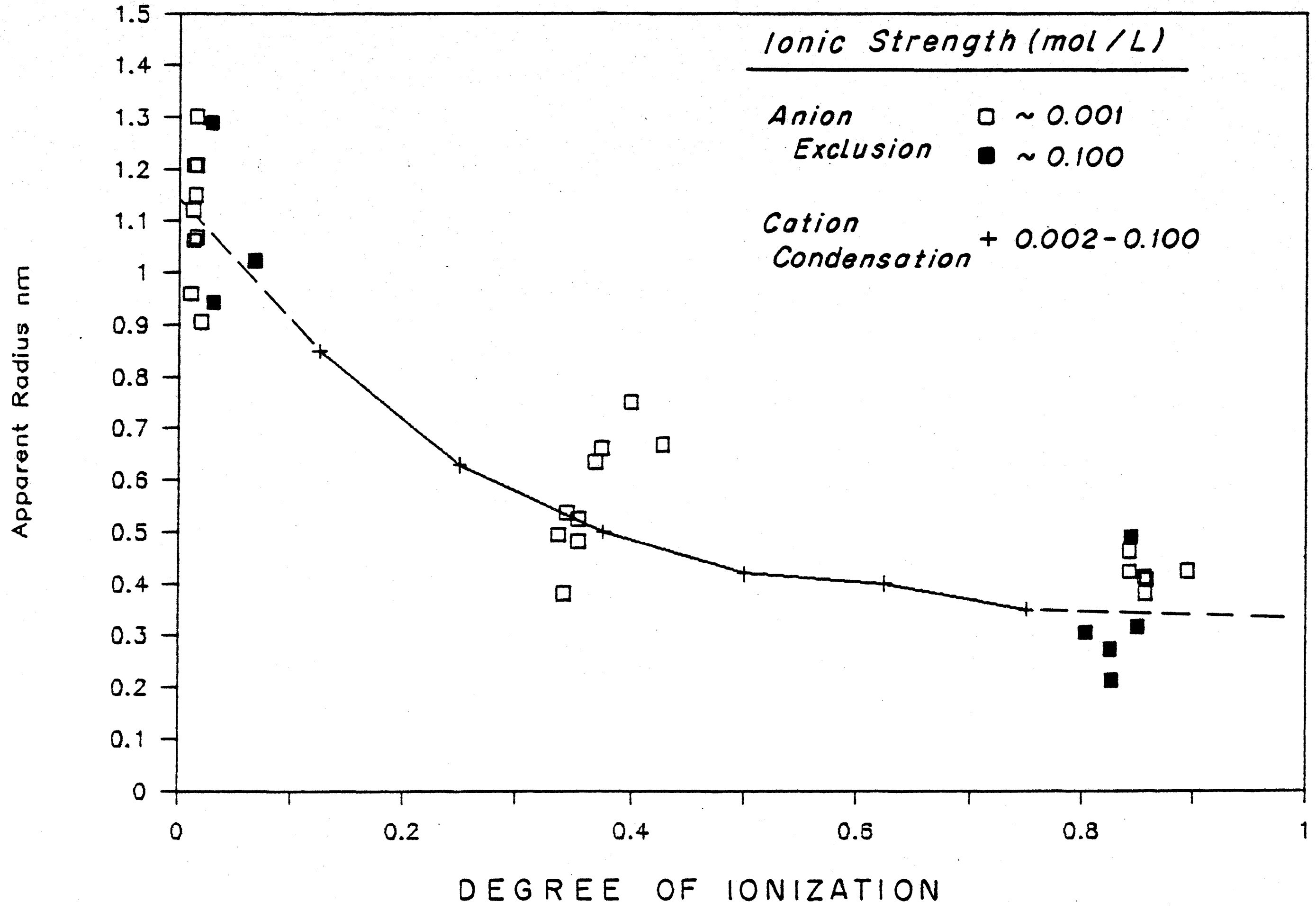


Fig. 12. Apparent radius of humic molecules based on nitrate exclusion (crosses and squares) compared with radii measured by acid-base behavior. Cation condensation results extrapolated (---) for illustrative purposes.

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 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 ionic 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. These 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 relevent 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 of 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 neglible 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 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.

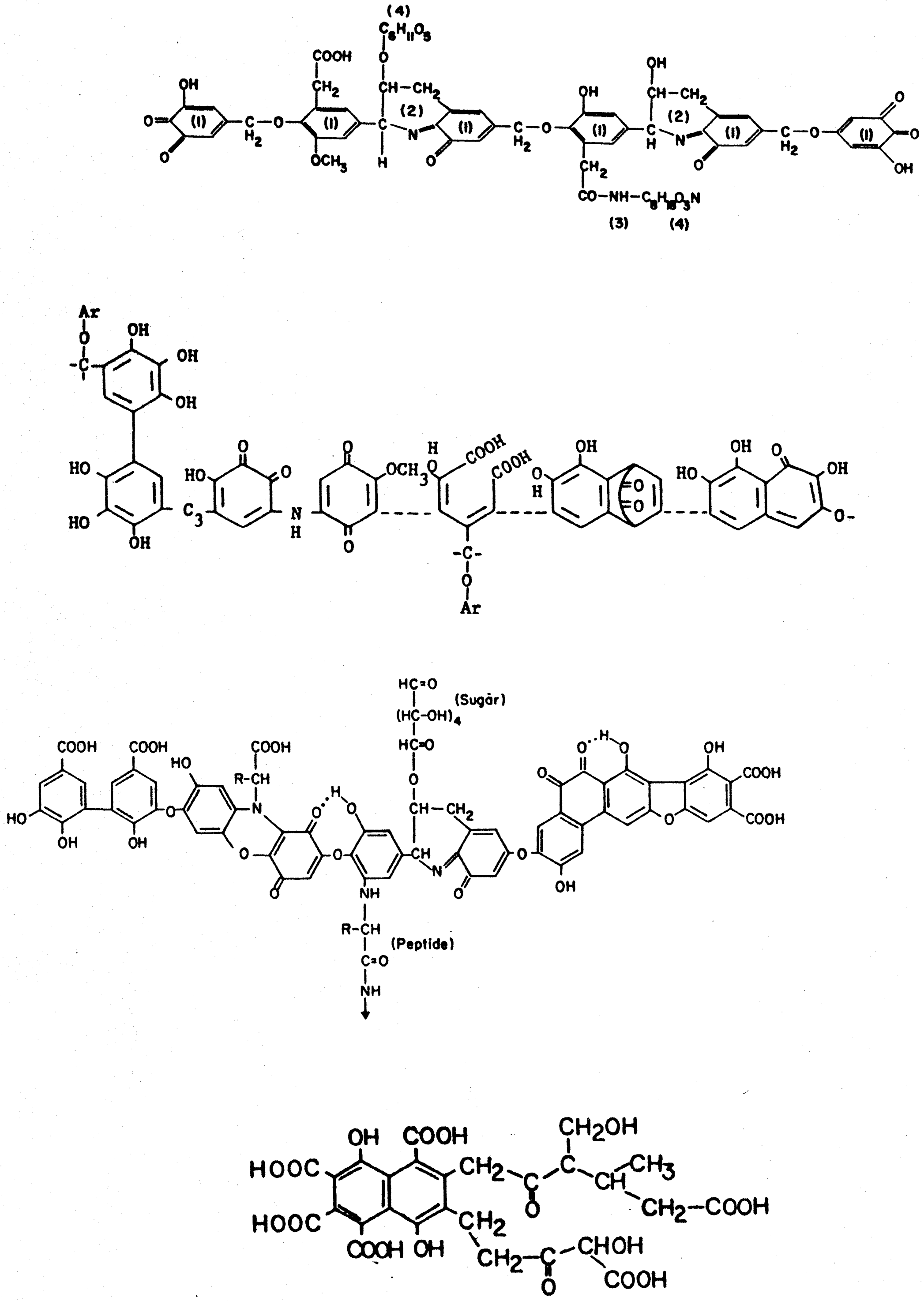


Fig. 13. Structural models of humic substances proposed by Dragunov (Kononova, 1966), Flaig (1960), Buffle (1977), and Stevenson (1982).

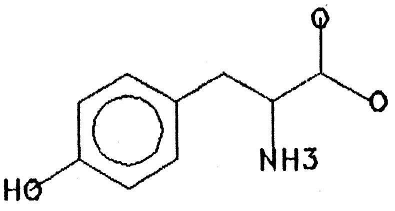
INTERACTIONS OF EDDHA AND FeEDDHA WITH HUMIC SUBSTANCES

Abnormalities of iron nutrition are common for many sensitive crops grown in calcareous soils (Chen and Barak, 1982). These abnormalities, which are expressed in a visual symptom termed lime-induced chlorosis, are due to the low solubility of iron from soil minerals in soil solution. The remedy of choice, but for its cost, is the synthetic chelate ethylenediamine di(o-hydroxyphenylacetic acid) [EDDHA] and also known as N,N'-ethylene bis-2-(o-hydroxyphenyl)glycine [EHPG] and as N,N'-1,2-ethanediylbis[N-(2-hydroxyphenyl)-glycine] [CAS Registry No. 10328-28-6] by the American Chemical Society. This substance, Fig. 1, is a synthetic amino acid which is chemically similar to both the synthetic chelate ethylenediamine tetraacetic acid and the natural aromatic amino acids tyrosine and dihydroxyphenylalanine (DOPA). By virtue of the highly specific chelation of iron by EDDHA, plant availability of iron may be raised to normal levels.

Although EDDHA may be second only to nitrogen in fertilizer input costs in cases where needed, the level of fertilizer utilization is often on the order of one percent, much lower than any of the macroelement fertilizers, in spite of the fact that the FeEDDHA complex is anionic and extremely soluble. Results of reported FeEDDHA-soil incubation experiments summarized in Table 1 show that a significant portion of the FeEDDHA gradually disappears although up to 13% becomes fixed immediately upon contact with the soil; the fate of

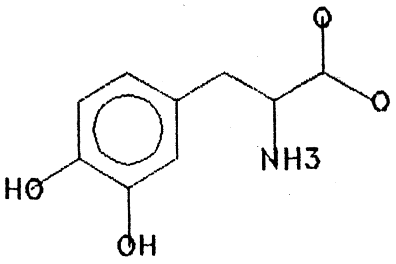
Tyrosine

A



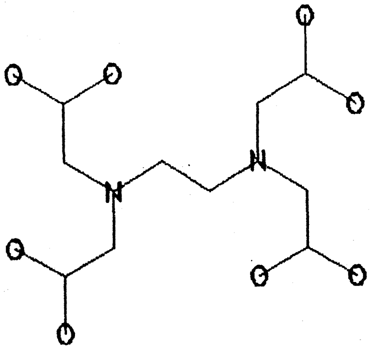
Dihydroxyphenylalanine

B



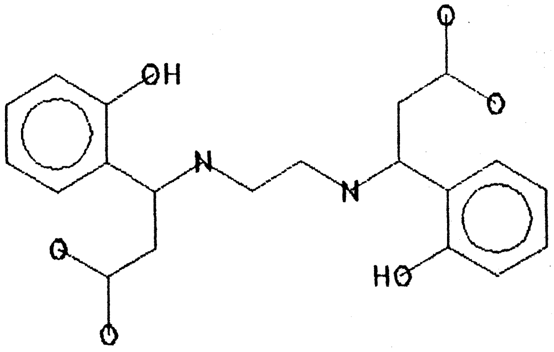
Ethylenediamine tetraacetic acid

C



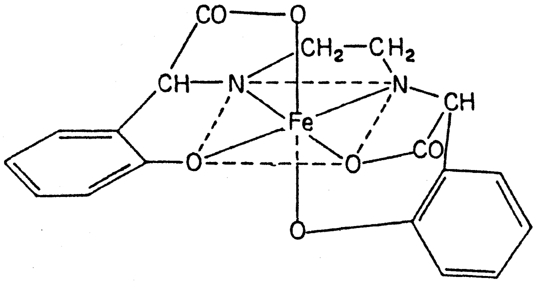
Ethylenediamine di(o-hydroxyphenylacetic acid)

D



meso FeEDDHA

E



d, l- racemic Fe EDDHA

F

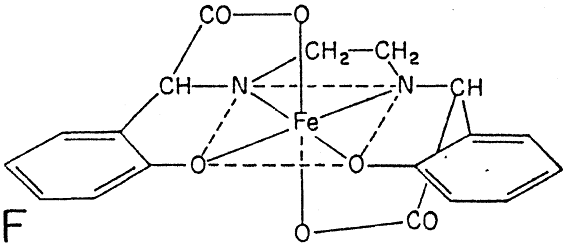


Fig. 1. Chemical structures of tyrosine (A), dihydroxyphenylalanine (B), EDTA (C) and EDDHA (D). Three-dimensional structure of $FeEDDHA^-$: meso (E) and d,l-racemic (F) isomers.

Table 1. Fixation of iron applied as FeEDDHA to soil, as summarized from the literature.

Soil	Fe added (mg/kg) as EDDHA	Time Elapsed	Fe Fixed (mg/kg)	% Fe Fixed	Extractant	Reference
Hacienda loam (calcareous)	200	4 d	18	9	water	Wallace et al. (1955)
		30 d	44	22		
Hacienda loam (calcareous)	200	30 min	36	18	water	Wallace and Lunt (1956)
		4 d	40	20		
Everton pH 7.8 35% clay, 28% $CaCO_3$	100	2 d	5	5	water	Hill-Cottingham (1957)
		15 d	9	9		
11 soils	5	"imm"	0.65	13	DTPA	Follett and Lindsay (1971)
		7 d	0.95	19		
		49 d	1.65	33		
		98 d	3.70	74		
Ulysses silt loam pH 6.6, 0.2% $CaCO_3$	20	7 d	4.2	21	DTPA	Salardini and Murphy (1977)
		14 d	9.2	46		
		28 d	8.4	42		
		56 d	10.8	54		
	40	7 d	10.8	27		
		14 d	24.0	60		
		28 d	22.4	56		
		56 d	26.4	66		
	80	7 d	46.4	58		
		14 d	54.4	68		
		28 d	52.0	65		
		56 d	63.2	79		

Ulysses silt loam (subsoil), pH 7.8 9.8% $CaCO_3$	20	7 d	9.6	48		
		14 d	10.4	52		
		28 d	12.2	61		
		56 d	13.6	68		
	40	7 d	22.0	55		
		14 d	24.0	60		
		28 d	26.4	66		
		56 d	28.4	71		
	20	7 d	48.8	61		
		14 d	56.6	77		
		28 d	60.0	78		
		56 d	64.8	81		
Pima clay loam	200	"imm"	8	4	water	Elgala et al. (1971)
		5 d	26	13		
		10 d	38	19		
		15 d	36	18		
Laveen loam	200	"imm"	10	5		
		1 d	10	5		
		5 d	14	7		
		10 d	18	9		
		15 d	22	11		
Laveen loam + 3.8% plant material	200	"imm"	16	8		
		1 d	24	12		
		5 d	58	29		
		10 d	90	45		
		15 d	86	43		
Cypriot soil pH > 7.3	24	4 d	3	12.5	DTPA	Orphanos and Hadjiloucas (1984)
		11 d	4	16.7		
		28 d	6	25		

Mitzpe Massua	2	1	h	0.02	0.9	water	Barak and Chen (1987)
light clay		1	d	0.02	0.9		
63.0% $CaCO_3$		2	d	0.10	5.2		
pH 8.0		4	d	0.14	7.0		
		7	d	0.10	5.2		
Har Raihan	2	1	h	0.03	1.7		
heavy clay		1	d	0.07	3.5		
0.7% $CaCO_3$		2	d	0.24	12.2		
pH 7.3		4	d	0.26	13.0		
		7	d	0.35	17.4		
Kfar Rupin	2	1	h	0.19	9.6		
heavy clay		1	d	0.21	10.4		
58.2% $CaCO_3$		2	d	0.26	13.0		
pH 7.8		4	d	0.24	12.2		
		7	d	0.26	13.0		
Kisalon	2	1	h	0.19	9.6		
heavy clay		1	d	0.19	9.6		
2.8% $CaCO_3$		2	d	0.33	16.5		
pH 8.1		4	d	0.40	20.0		
		7	d	0.54	27.0		

the added FeEDDHA is uncertain--although leaching, fixation on soil components and microbial decomposition have all been suggested as possible routes for removal of EDDHA from the soil system. Fixation on soil clays has been considered by Wallace and coworkers (1955, 1956) to be insignificant.

Methods of analysis of FeEDDHA in soil extracts are particularly crucial to the results. FeEDDHA is both red due to an absorption peak at 480 nm and iron-bearing (~6.5% by weight). However, methods based on spectrophotometric analysis of the soil extracts confront interference from soluble humic substances which absorb at the same wavelength (Hill-Cottingham, 1957). On the other hand, methods based on analysis of iron contents of soil solutions must also contend with the high background due to soluble iron in soil extracts (Bradford et al., 1971), presumably chelated by soluble humic substances. It has been proposed to isolate FeEDDHA from soil extracts by extraction into n-amyl alcohol after addition of either ammonium sulfate (Batra and Maier, 1964) or tetra-n-heptylammonium iodide (Elgala et al., 1971) to the aqueous phase. These extractions are particularly noxious because of the amyl alcohol and also tedious because of the need to extract the aqueous phase twice, combining the extracts and making to volume, followed by spectrophotometric measurement at 480 nm. Boxema (1979) developed a gel chromatographic method using size exclusion on Sephadex G-10 for analysis of FeEDDDHA in fertilizer mixes. This method employs column beds containing 180 mL of gel, elution volumes of over 300 mL, and sample sizes on the order of 8-25 mg FeEDDHA per injection, and is clearly not suitable for microanalytical work. Based on the work of Silkey (1983), the Association of Official Analytical Chemists has adopted a procedure for measuring chelated iron in fertilizers, but the method, based upon the ability to chelate iron at pH 8.5, is not specific for EDDHA. More recently, a method was developed specifically for this research which provides a quick method of separating FeEDDHA from other components of samples, including dissolved humic substances, by anion chromatography and determination of FeEDDHA by ultraviolet spectroscopy (Barak and Chen, 1987a).

MATERIALS AND METHODS

Stock solutions of Hula humic acid extracted and purified as described before were prepared at a concentration of 0.25% by dissolving in NaOH and adjusted to pH values of approximately 3.5, 4.5, 7, and 11. Four mL aliquots were placed in dialysis cells contructed of dialysis tubing (Medicell International Ltd, London, 6.5 mm approximate wet diameter) in screw-top polycarbonate test tubes and extensively dialyzed against distilled water. For experiments using a Ca^{2+} ionic environment, the dialysis cells were extensively dialyzed against 10 mM $CaCl_2$ before dialysis with distilled water.

Each dialysis cell was placed in a test tube and weighed. Weighed quantities of stock solutions of either NaFeEDDHA, Na_2H_2FeEDDHA or Ca(FeEDDHA)$_2$, electrolyte solutions of either NaCl or $CaCl_2$ and water were added to bring the total volume to approximately 10 mL. The NaFeEDDHA was a commercial sample from Ciba-Geigy; the other salts were prepared from H_4EDDHA (Sigma Chem. Co., St. Louis, MO). The test tubes were shaken at 25°C overnight. The external solution was then sampled for EDDHA as FeEDDHA by anion chromatography with the technique of Barak and Chen (1987) using a Perkin-Elmer Series 10 Liquid Chromatograph pump, a Wescan 269-003 Ion-Guard Anion Cartridge as an ultra-high speed anion exchange column, a Jasco Uvidec-100-V UV spectrophotometer set at 209 nm with a 1 cm optical path flow-through cell, and a LDC/Milton Roy CI-10 integrator; the eluent was 3 mM H_2SO_4 + 50 mM Na_2SO_4 + 0.01 mM $Fe_2(SO_4)_3$; to measure the concentration of unferrated EDDHA as FeEDDHA, samples were spiked to a final concentration of 1 mM $FeCl_3$. The pH of the external solution was measured with a combined glass electrode and a Radiometer pHM 84 pH meter calibrated with phthalate and phosphate buffers. Sodium concentrations of the external solution were measured with a Corning 400 flame photometer. At the end of the experiment, dialysis cells were dried at 60°C in order to permit calculation of total weight of liquid by difference.

Where applicable, adsorbed EDDHA or FeEDDHA was calculated by material balance as follows:

$$Q_{ads} = (C_oW_o - C_sW_s)/p_w\ W_{HA}$$

where Q_{ads} = quantity of adsorbed EDDHA or FeEDDHA (mmole g^{-1} HA)

W_s = total weight of liquid in sample

W_o = weight of EDDHA or FeEDDHA stock solution added to sample

C_s = EDDHA or FeEDDHA concentration measured in sample

C_o = EDDHA or FeEDDHA concentration of stock solution

p_w = density of water

W_{HA} = weight of humic acid in dialysis cell

When the measured FeEDDHA concentration was less than that predicted by the uniform distribution of the added chelate in the total volume, measured exclusion volume was calculated as follows:

$$Vol_{ex} = \frac{W_s - W_o \ (C_o/C_s)}{p_w \ W_{HA}}$$

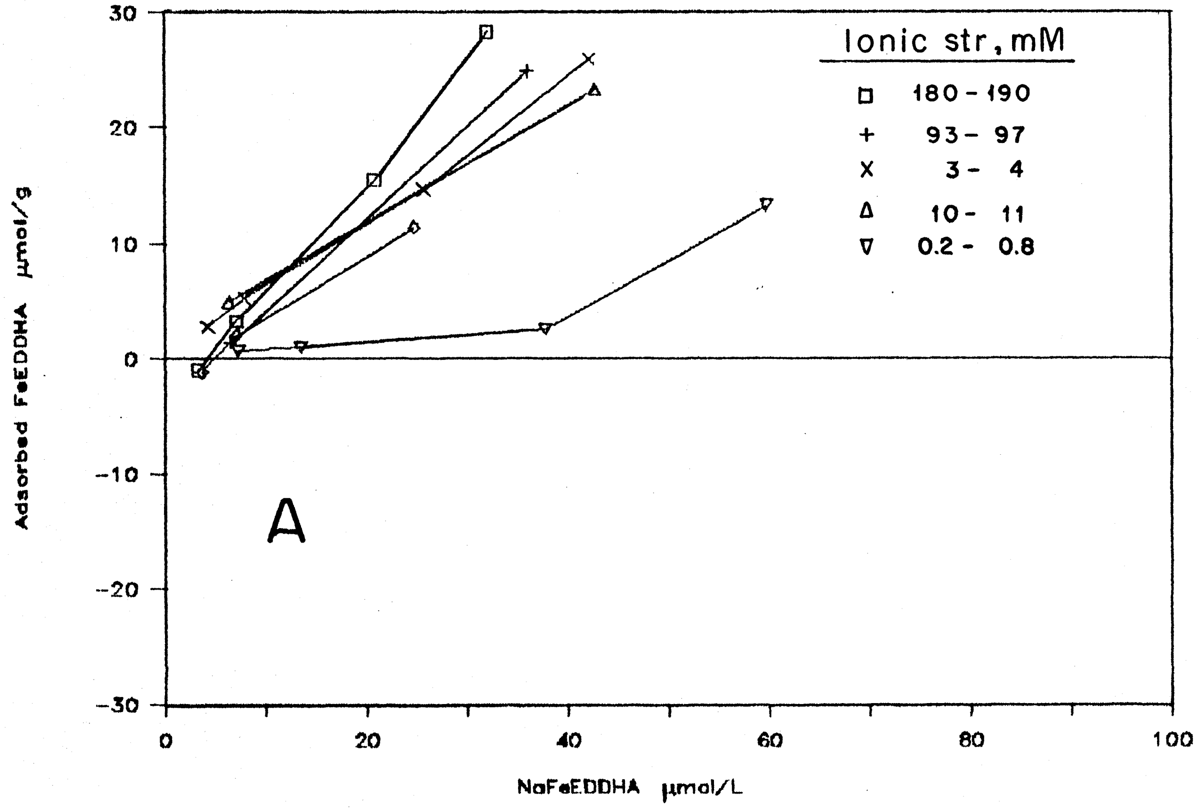
where Vol_{ex} is the volume from which EDDHA or FeEDDHA is excluded ($cm^3 \ g^{-1}$)

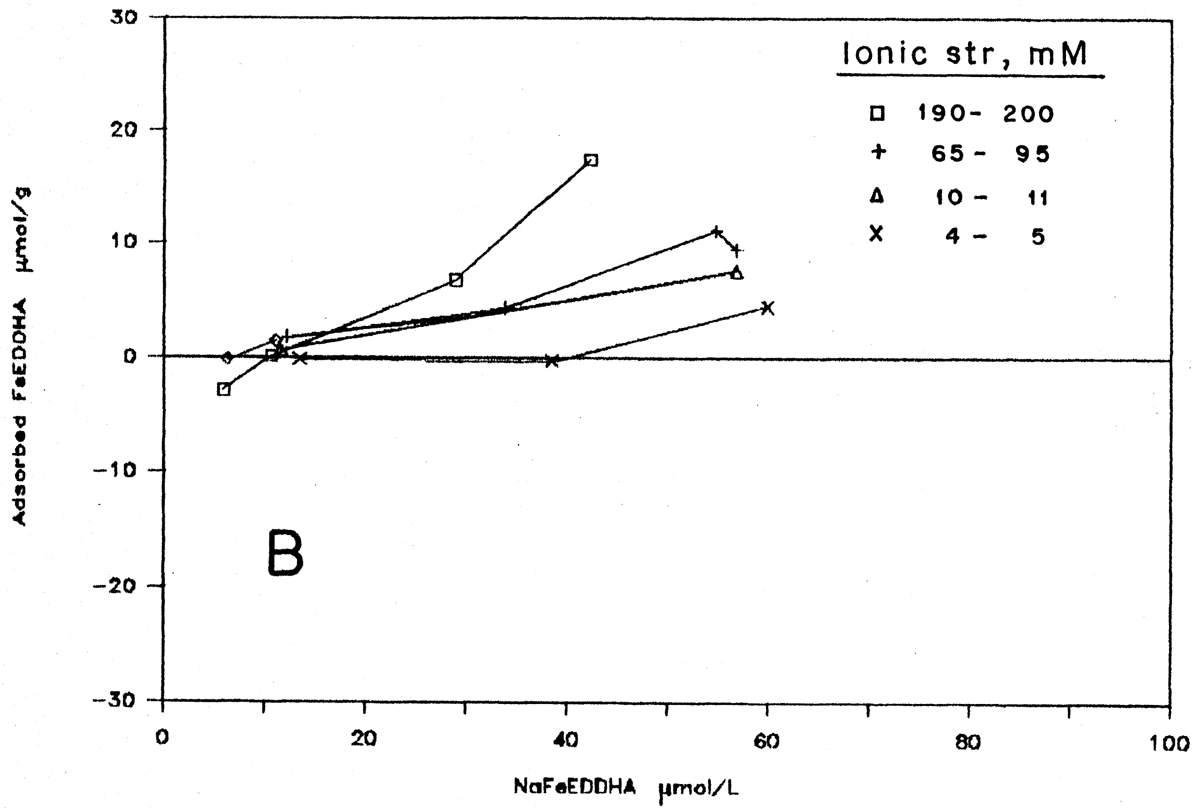
In a supplementary experiment, the interaction of montmorillonite and FeEDDHA was measured in a Ca^{2+} system. The <2 µm fraction of Wyoming montmorillonite (Fisher Scientific) was saturated with Ca^{2+} and excess electrolyte removed by repeated rinsing with distilled water and centrifugation; the monoionic clay was freeze-dried for storage. A 0.5 g sample of clay was weighed into a test tube with 5 mL of a 400 µM FeEDDHA solution as $Ca(FeEDDHA)_2$ and 100 mM $CaCl_2$ and water to produce a Ca^{2+} concentration range of 0.1 to 45 mM in approximately 9 mL. The test tubes were shaken overnight and the equilibrium solution was tested chromatographically after centrifugation at 10,000 rpm.

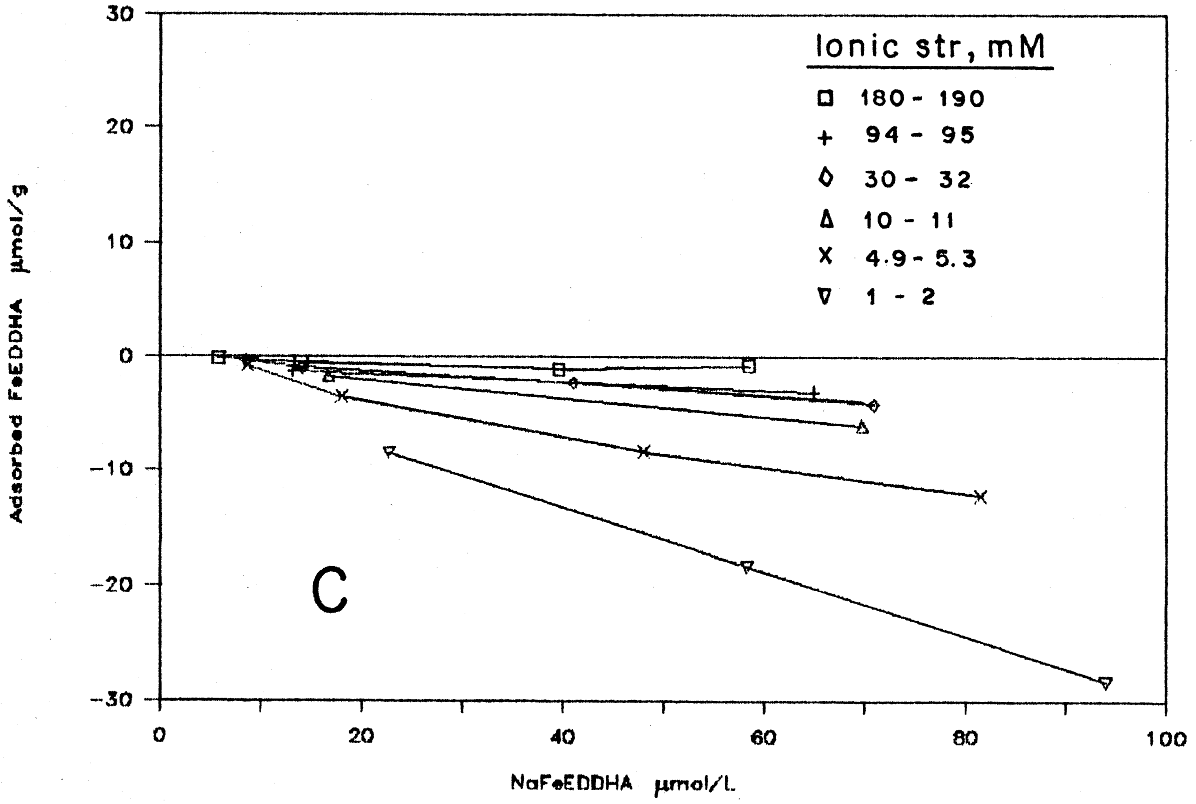
RESULTS AND DISCUSSION

NaFeEDDHA -- The results of reacting NaEDDHA with Hula humic acid at four pH values, 3.5, 4.5, 7, and 11, are presented as adsorption isotherms in Fig. 2. At pH 3.5 (Fig. 2a), all experimental data point to adsorption of FeEDDHA on the dissolved humic substance, with a strong dependence on the concentration of the equilibrium solution, which ranged up to 200 mmol L^{-1} by the addition of NaCl. Addition of electrolyte increased adsorption of FeEDDHA on the humic acid. At pH 4.5 (Fig. 2b), adsorption was markedly less than at pH 3.5; at low electrolyte concentrations of 4 mmol L^{-1}, adsorption was almost nonexistant at this pH. At pH 7, measurements show clearly that for all but the highest electrolyte concentrations, FeEDDHA concentrations were higher in the equilibrium solution than those expected if the added FeEDDHA had mixed into all of the solution volume equally, i.e., exclusion phenomena are evidenced at this pH. When presented in the form of adsorption isotherms, the data of pH 7 (Fig. 2c) shows negative values of adsorption, strongly dependent on electrolyte concentration. The effect of electrolyte concentration may, as with the case of nitrate interaction, be translated by use of the Debye-Huckel length $1/\kappa = (8\pi e^2/\epsilon kT)^{1/2} = 3.29*10^7 \sqrt{I}$, where I is the ionic strength of the equilibrium solution, to a scaled distance of the double electric layer about a charged cylinder using $1/\kappa^2$. When redrawn in terms of FeEDDHA exclusion volumes against $1/\kappa^2$ (Fig. 3c), the data show clearcut anion exclusion sensitivity to electrolyte

Fig. 2. Interaction of FeEDDHA⁻ with sodium humate in NaCl, with results presented as adsorption isotherms at (A) pH 3.5, (B) pH 4.5, (C) pH 6.8-7.5, and (D) pH 11.






Ionic str, mM
□ 180 - 190
+ 94 - 95
◊ 30 - 32
Δ 10 - 11
X 4.9 - 5.3
∇ 1 - 2
C
Adsorbed FeEDDHA µmol/g
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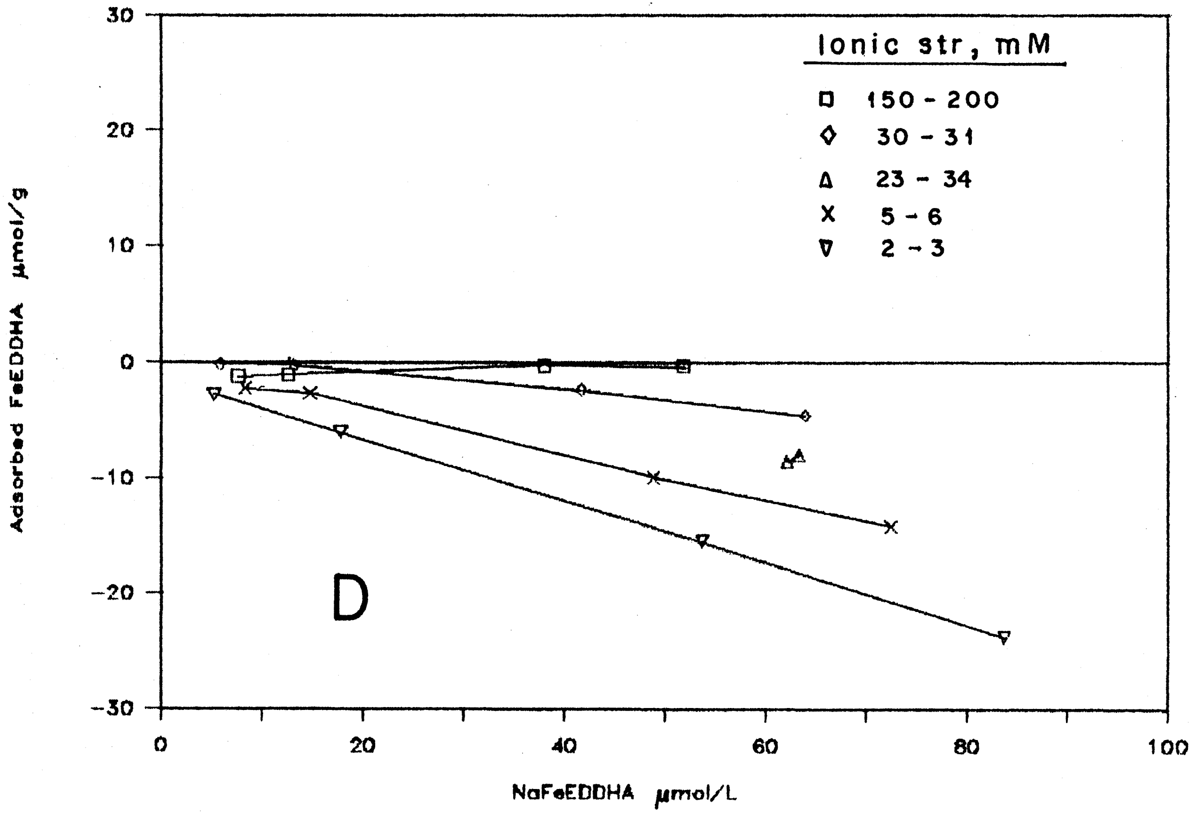
20
10
0
-10
-20
-30
0
20
40
60
80
100
NaFeEDDHA µmol/L


Ionic str, mM
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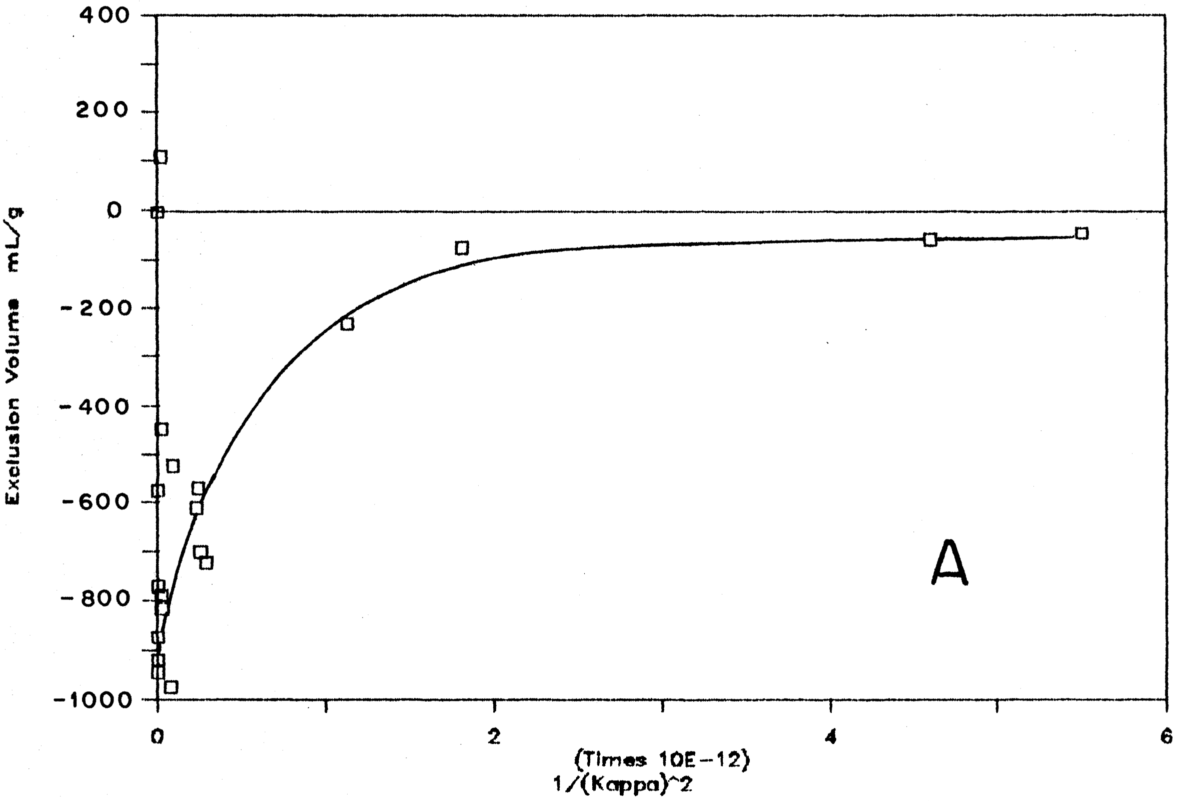
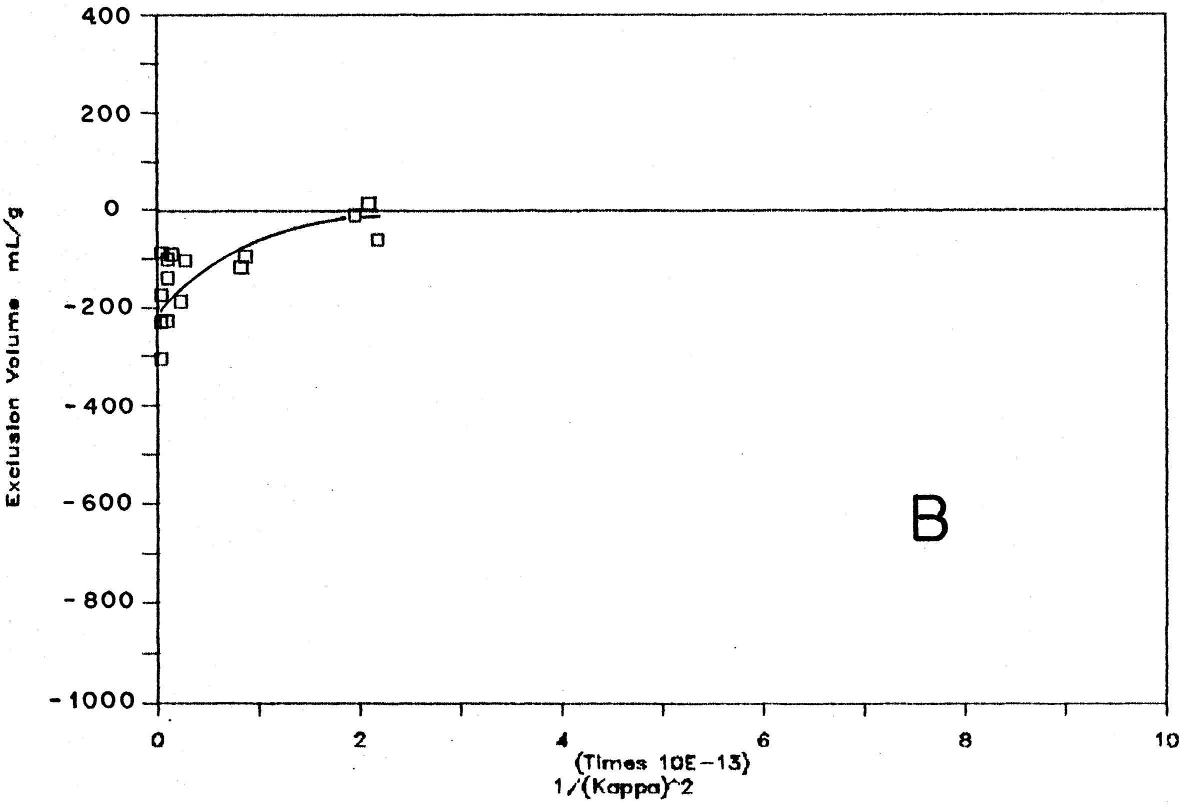
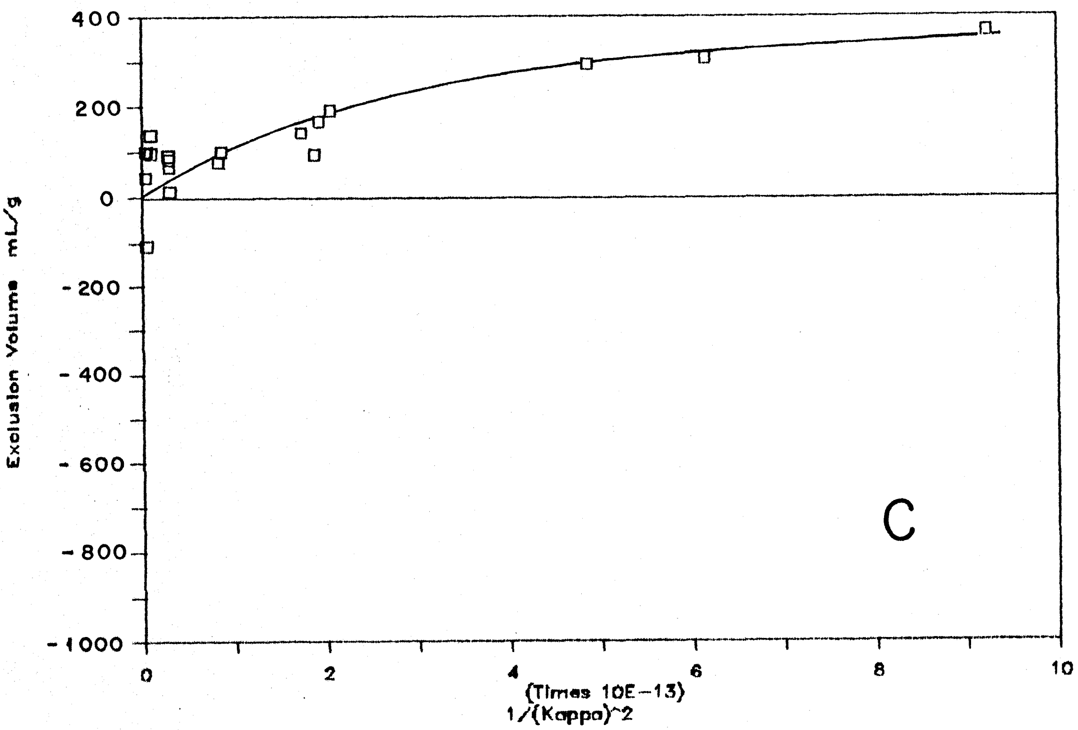
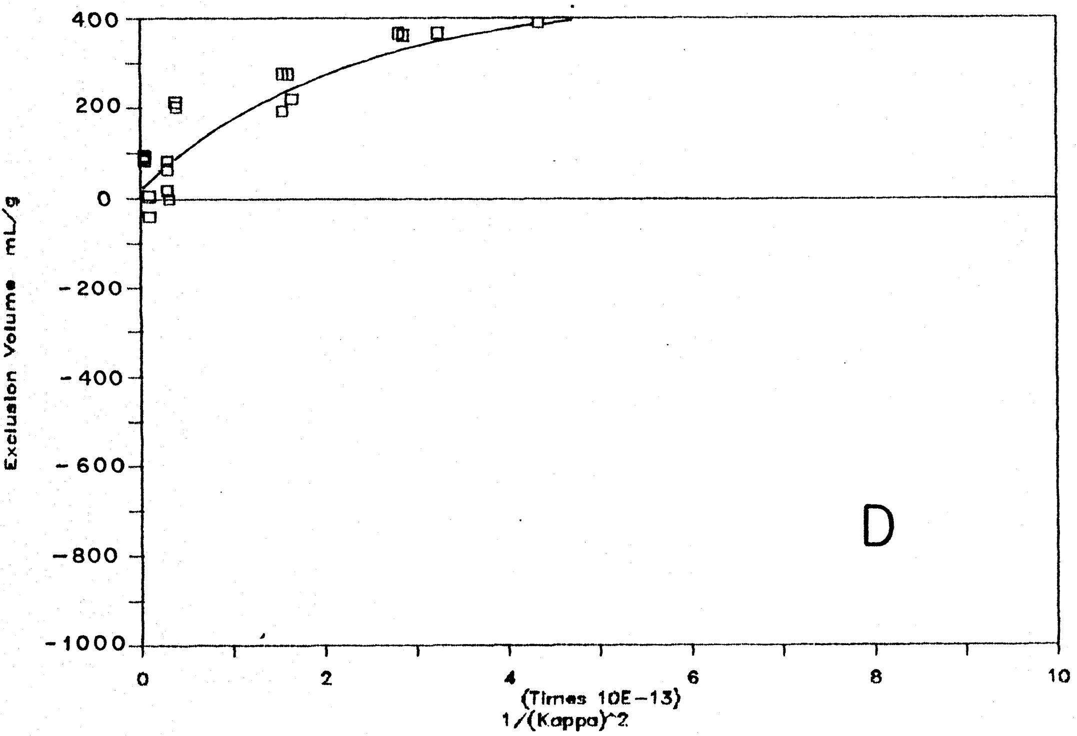
◊ 30 - 31
Δ 23 - 34
X 5 - 6
∇ 2 - 3
D
Adsorbed FeEDDHA µmol/g
30
20
10
0
-10
-20
-30
0
20
40
60
80
100
NaFeEDDHA µmol/L

Fig. 3. Interaction of FeEDDHA$^-$ with sodium humate in NaCl, with results presented as exclusion volumes at (A) pH 3.5, (B) pH 4.5, (C) pH 6.8-7.5, and (D) pH 11.






Exclusion Volume mL/g
400
200
0
-200
-400
-600
-800
-1000
0
2
4
6
8
10
(Times 10E-13)
1/(Kappa)^2
C


Exclusion Volume mL/g
400
200
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-200
-400
-600
-800
-1000
0
2
4
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8
10
(Times 10E-13)
1/(Kappa)^2
D

concentration. At pH 11, FeEDDHA exclusion volumes (Fig. 3d) were similar to those at pH 7.

$Ca(FeEDDHA)_2$ -- The second set of data measured the interaction of $Ca(FeEDDHA)_2$ with calcium humate with $CaCl_2$ as the supporting electrolyte. This system differs from the Na^+ system in that the humic acid was precipitated rather than dissolved. As seen in Fig. 4, at low pH values around 4, the humic acid adsorbed FeEDDHA although not to the same extent as in the sodic system (Figs. 2a and 2b). Like the sodic system, the extent of adsorption of FeEDDHA declined with increasing pH. However, at neutral pH (Fig. 4b and 4c) the exclusion volumes were all nearly zero, compared to a maximum of 400 mL/g for the sodic system. These results as a whole indicate positive adsorption of FeEDDHA to humic acid in a calic system under low pH conditions, but at neutral pH the adsorption is considerably reduced even though no significant exclusion of FeEDDHA is present.

Na_2H_2EDDHA -- The third set of data measured the interaction of the unferrated ligand EDDHA with sodium humate with NaCl as the supporting electrolyte. The results at acid pH values (Figs. 6a and 6b) show adsorption of $EDDHA^{2-}$ to an extent nearly identical to that of the sodic and calcic systems with $FeEDDHA^-$. Measurements at pH 6.1-6.4 (Fig. 6c and 7c) show clearly the development of net exclusion of EDDHA by the humic acid.

Fig. 4. Interaction of FeEDDHA⁻ with calcium humate in $CaCl_2$, with results presented as adsorption isotherms at (A) pH 3.9-5.0 and (B) pH 6.5-7.5.

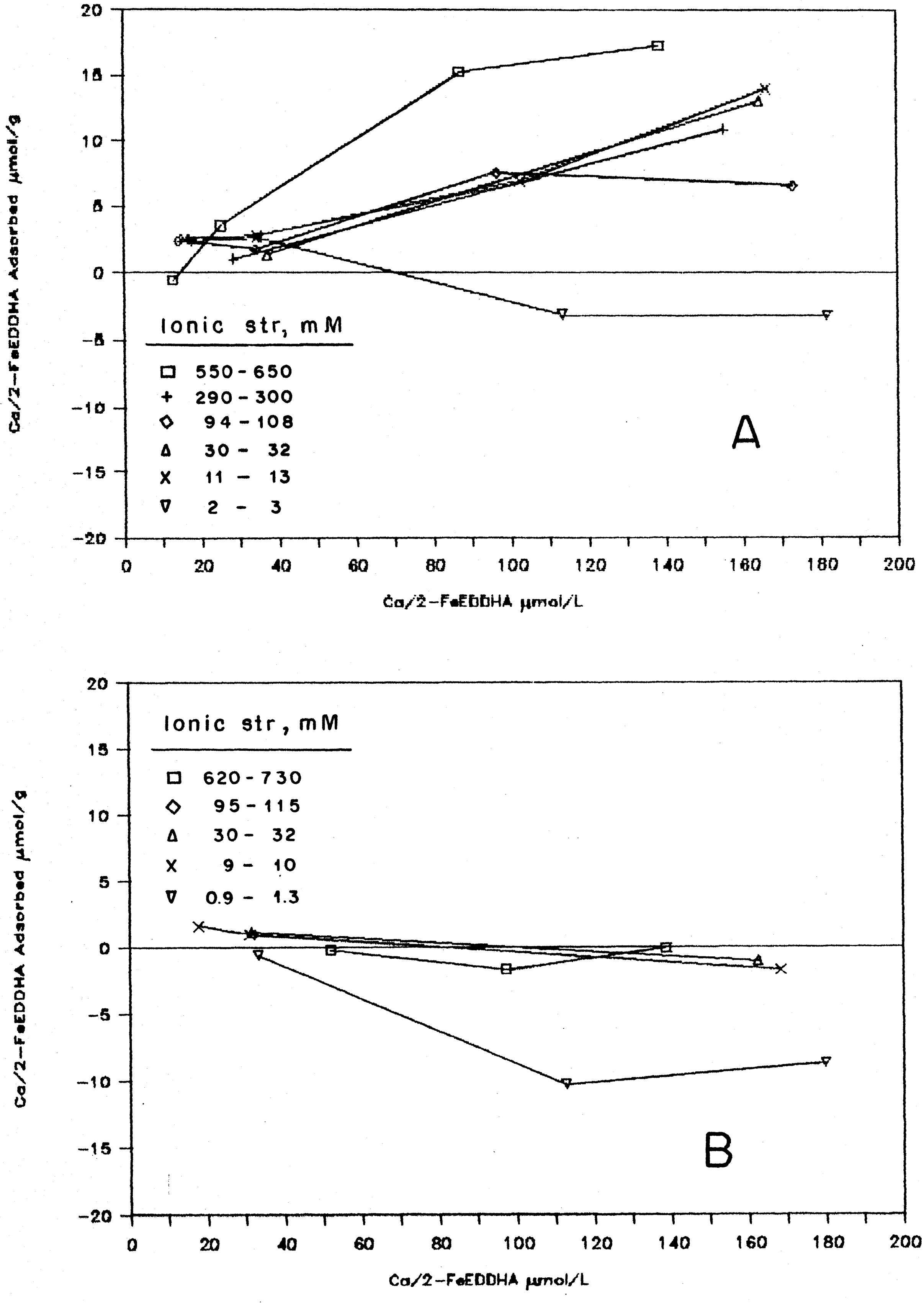


Fig. 5. Interaction of FeEDDHA$^-$ with calcium humate in $CaCl_2$, with results presented as exclusion volumes at (A) pH 3.9-5.0 and (B) pH 6.5-7.5.

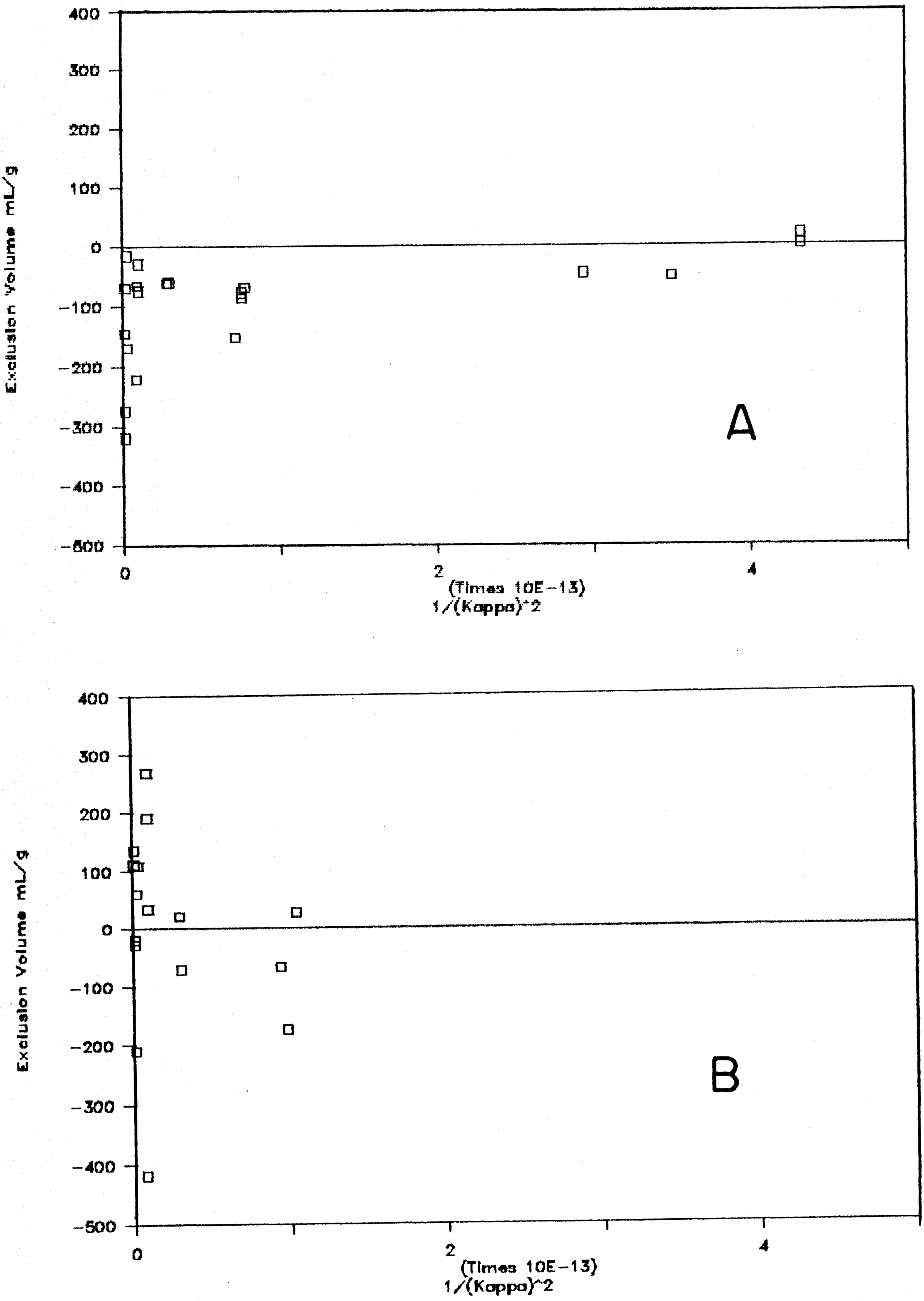


Fig. 6. Interaction of EDDHA^{2-} with sodium humate in NaCl, with results presented as adsorption isotherms at (A) pH 4.3-5.2 and (B) pH 5.2-6.0.

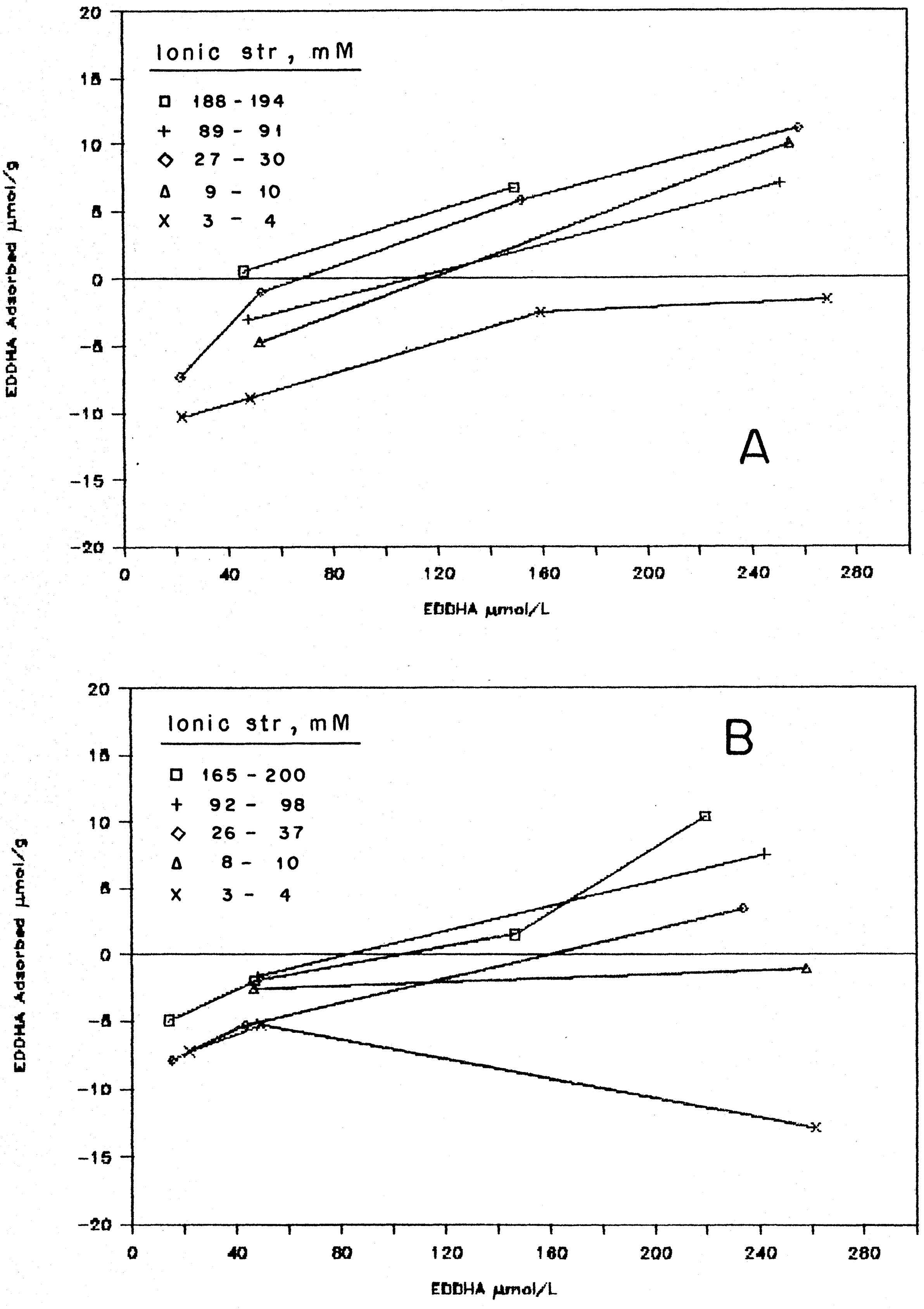
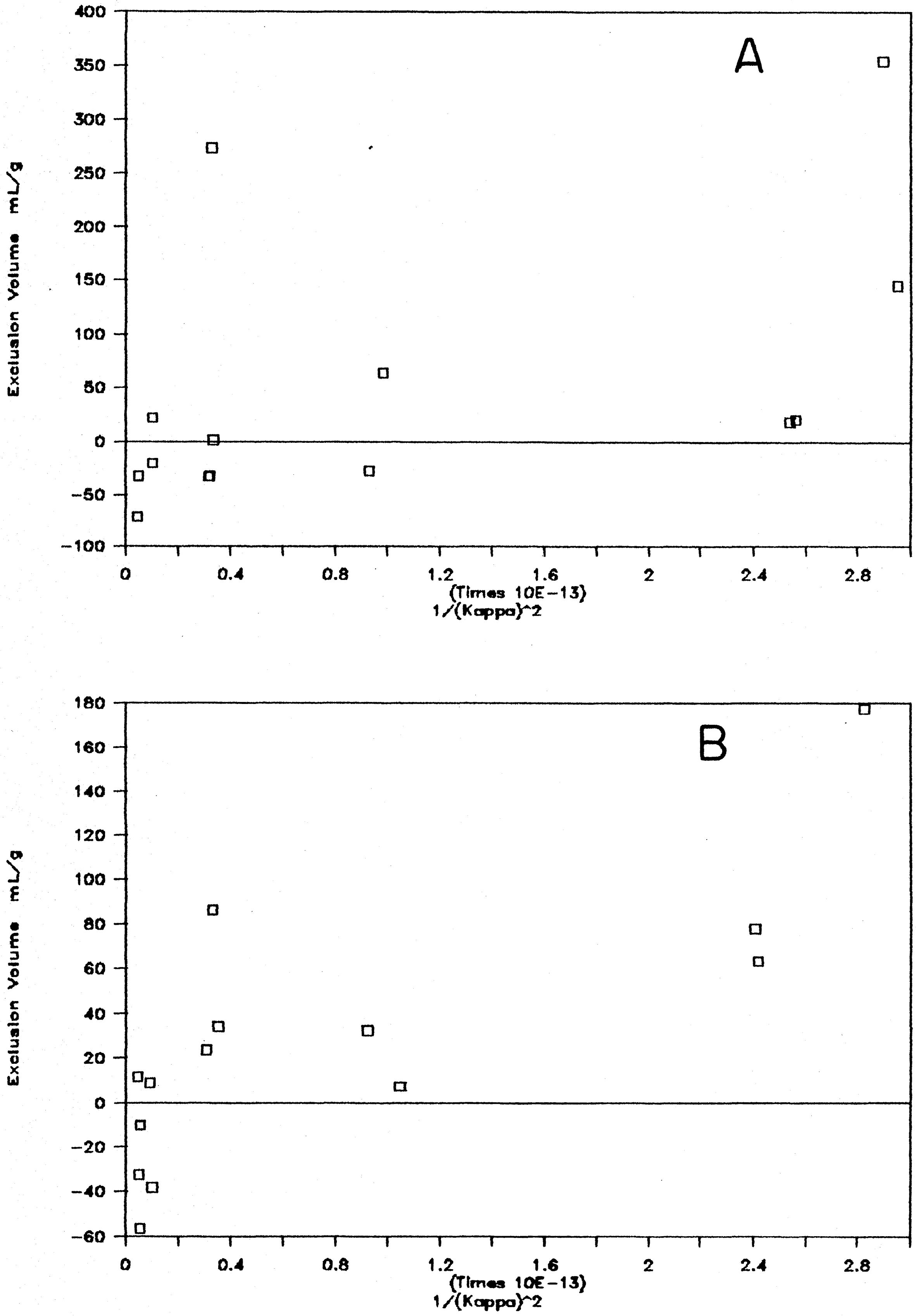


Fig. 7. Interaction of EDDHA^{2-} with sodium humate in NaCl, with results presented as exclusion volumes (A) pH 4.3-5.2 and (B) pH 5.2-6.0.



Within the pH range 2.5 to 7, as shown in a previous chapter, carboxylic groups of the humic substance undergo increasing dissociation and ionization, creating a double electric layer which simultaneously causes cation condensation (measured by acid-base titration) and anion exclusion (measured by nitrate exclusion). Within this pH range, FeEDDHA is a monovalent anion with iron fully complexed as evidenced by both the stability constants for H_4EDDHA°, H_3EDDHA^-, H_2EDDHA^{2-}, $HEDDHA^{3-}$, $EDDHA^{4-}$,and FeEDDHA (Lindsay, 1979) and by the fact that anion chromatography shows that FeEDDHA retains both ferration and anionic nature at pHs as low as 2.5 (Barak and Chen, 1987).

The evidence thus far is consistent with positive chemical adsorption of FeEDDHA to humic substances due to an unknown mechanism and with nonspecific anion exclusion of FeEDDHA due to the double electric layer. Anion exclusion, as described for nitrates, increases with increasing dissociation of the carboxylic group and decreases with increasing electrolyte concentration due to reduction of the width of the double electric layer. For the case of FeEDDHA and humic acid in the sodic system, both chemical adsorption and anion exclusion seem to be present in each of the pH values considered since the adsorption isotherms are positively correlated with electrolyte concentrations which, but for the reduction in the width of the double electric layer, represent in themselves increasing levels of a competing anion. In the Ca^{2+} system, FeEDDHA shows adsorption at acid pH but little exclusion at neutral pH. The sodic system with unferrated EDDHA shows adsorption at low pH and exclusion at near neutral pH.

The nature of the mechanism of chemical adsorption is not known but it would seem reasonable to consider the possible formation of a humic-Fe-EDDHA structure, a mixed ligand complex similar to the fulvic-Fe-phosphate complex prepared by Levesque and Schnitzer (1967) wherein the metal bridges two anions, as a remote possibilitity due to the crystal structure of FeEDDHA. The structure of hydrated FeEDDHA salts, and presumably FeEDDHA in solution as well, has been shown by Bailey et al. (1981) to coodinate iron sixfold with two nitrogen atoms and four oxygen atoms, which belong two each to the carboxyl groups and the phenolic groups. In this way, the EDDHA molecule is wrapped around the iron atom (see Fig. 1), coordinating the iron in near perfect octahedral geometry, and excluding water molecules from the immediate vicinity of the iron atom. It would seem improbable that this structure, which is remarkably tenacious in holding iron (log K $\neq$ 34), would dissociate in favor of a partially coordinated Fe-humate complex to which it would remain coordinated.

Another possible mechanism that may be considered as the explanation of bonding of FeEDDHA and EDDHA to humic acids is hydrophobic bonding, in which hydrophobic substances such as DDT and other organiochlorine insecticides are partitioned between water and hydrophobic portions of the humic acids. Humic acids have been shown by Khan and Schnitzer (1972) to adsorb as much as 2% by weight of hydrophobic organic compounds. Although EDDHA has a benzene ring which would contribute to hydrophobicity, the hydrophobic nature of

FeEDDHA is relatively small. The method of solvent extraction of Elgala et al. (1971) of FeEDDHA from aqueous solution into n-amyl alcohol succeeds only when the aqueous phase is nearly saturated with ammonium sulfate. Apparently the hydrophobic benzene ring of EDDHA does not overcome the hydrophilic nature due to the carboxylic and amine groups and the ionic charge resulting therefrom.

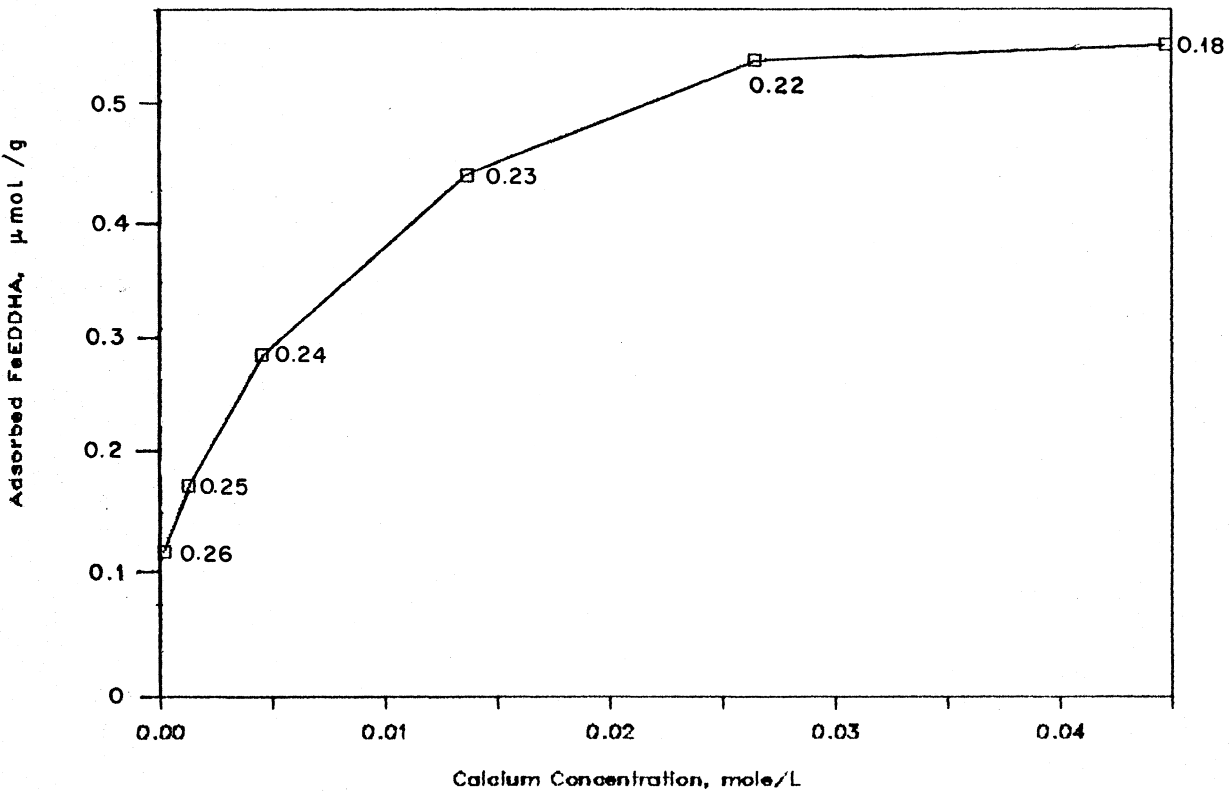
A third possible mechanism for FeEDDHA and EDDHA adsorption on humic acid is hydrogen bonding, which is a special kind of dipole-dipole interaction in which the hydrogen atom serves as a bridge between two electronegative atoms involving a partial charge transfer (Choudhry, 1984). In the case of humic acid and EDDHA, such a hydrogen bond could form between the protonated carboxyl group of the humic acid and one of the two NH groups of the EDDHA molecule. Such a mechanism would be limited in effect to acid conditions where carboxyl groups are unionized (Stevenson, 1972) and would be relatively insensitive to the difference between EDDHA and FeEDDHA, which would fit the empirical data well. Further, at higher pH upon deprotonation of the carboxyl groups and disruption of the hydrogen bonding, the reaction of humic acid with EDDHA and FeEDDHA would be expected to be purely one of exclusion if the carboxyl groups were ionized as in the sodic systems or to show little or no interaction at all if the carboxyl groups are neutralized with metal ions, as in the calcic system.

Although it is difficult to extrapolate from humic solutions to soil humates in situ, it would appear that under conditions prevailing in a calcareous soil - basic pH and low ionic strength - the data reported above would not suggest strong adsorption of FeEDDHA on humates; on the other hand, the information collected from the literature on FeEDDHA fixation in soils (Table 1) shows that some type of rapid reaction is present. A preliminary experiment with calcium montmorillonite, $Ca(FeEDDHA)_2$, and $CaCl_2$ at pH 7 showed adsorption of FeEDDHA on the clay, contrary to expectations of anion exclusion from the negatively charged planar surface of the clay. The adsorption was clearly affected however by electrolyte concentration (Fig. 8) indicating the presence of exclusion phenomenon as well as adsorption. Up to 0.7 µmol FeEDDHA per gram clay was adsorbed at 250 $\mu mol\ L^{-1}$ and 25 mM $CaCl_2$. Simple extrapolation to the plow layer of a valley rendzina soil with 30% clay of which 60% is montmorillonite and 0.01 M Ca^{2+} and 0.2 mM FeEDDHA in solution shows that ~150 kg FeEDDHA per hectare could be removed from solution by the clay upon contact. This figure would indicate FeEDDHA fixation greater than that actually encountered, indicating mitigating factors in the soil system. Resolution of the problem of fixation of FeEDDHA should be continued by further experiments with soil minerals, including treatments which alter the positive charge of edges.

CONCLUSIONS

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

Fig. 8. Adsorption of FeEDDHA on calcium montmorillonite in the presence of $CaCl_2$. Numbers to the right of symbols indicate the concentration of FeEDDHA (μmole L^{-1}) in the equilibrium solution.



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.

INTERACTION OF BORIC ACID AND BORATE WITH HUMIC SUBSTANCES

Boron is a light nonmetallic element which is found in natural environments in the +3 valency but is always coordinated with either three or four oxygen atoms, or more rarely with four fluoride ions, and is consequently never cationic; in many respects, boron resembles its metallic neighbor aluminum in the next row of the periodic table. The chemistry in solution is either that of boric acid $B(OH)_3$, a trigonal (planar) molecule, or that of the borate ion $B(OH)_4^-$, with regular tetrahedral configuration; the acid is very weakly acidic, with a pK of 9.18. The ionization of boric acid is unusual since its involves a change in coordination number of the central atom and consumes two water molecules, one to provide the additional hydroxyl group and another to form the resulting hydronium ion (Bell et al., 1967).

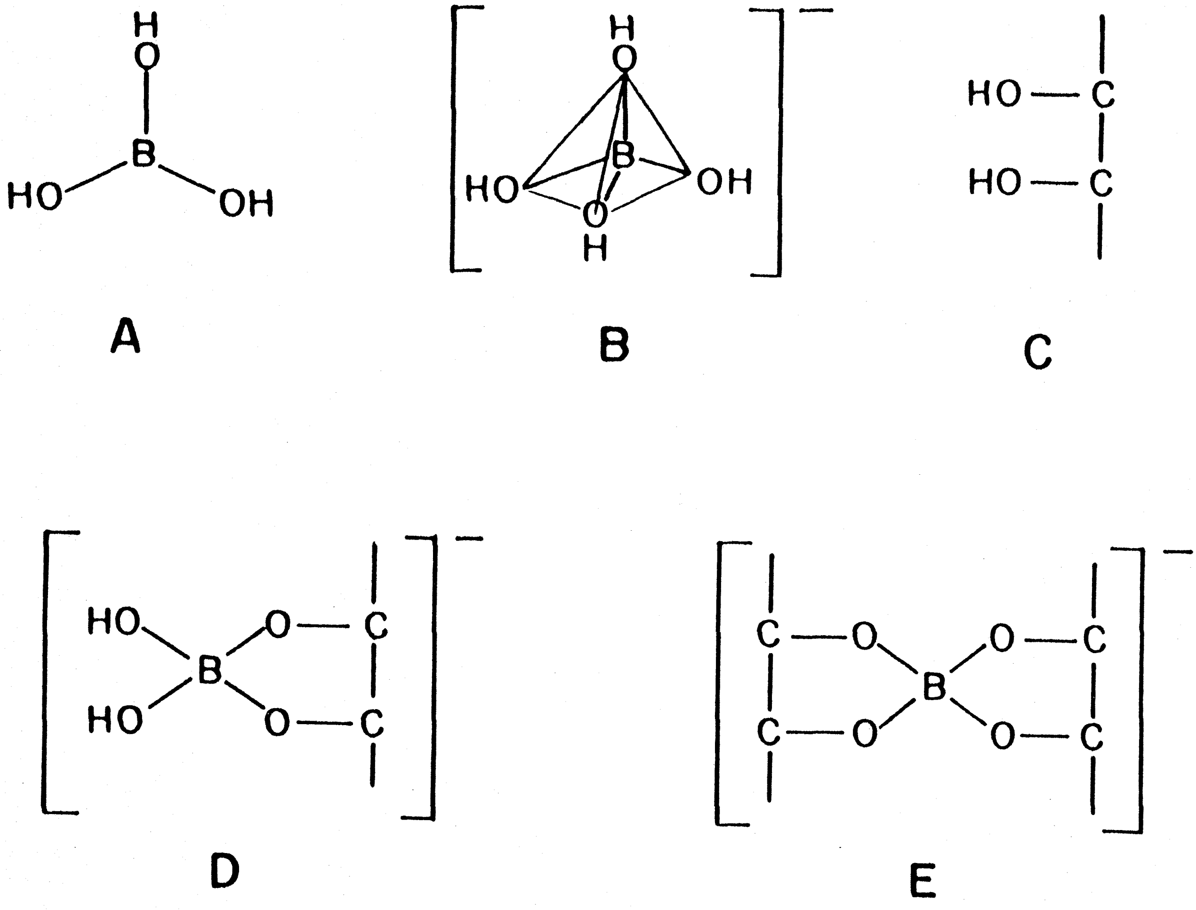
Boron in fresh natural waters is commonly in the mg L^{-1} range although many exceptions are known. Contents in soils range from 7 to 80 mg B kg^{-1}, depending on the parent material. Most of the soil boron is found in the crystal structures of the soil minerals. More available forms of boron are found in adsorbed forms associated with oxides and hydroxides of Al and Fe, edges of clay platelets, and soil organic matter. In some systems, regulation of boron solubility by solid phase calcium borate minerals have been considered possible (Evans, 1987).

Boron is an essential element for plant growth, although there is no apparent physiological need for boron in the animal kingdom. The current view of plant physiologists is that boron is not a constituent of plant enzymes but does play a role in enzymatic regulation, and thereby a role in plant growth and development, presumably by complexing with polyhydric substates, enzymes and coenzymes (Zittle, 1951; Dugger, 1983). Boron requirements in plant tissue range from 5 to 30 mg kg^{-1}; as a rule, less than 1 mg kg^{-1} hot-water extractable boron in soil or less than 0.5 mg L^{-1} B in the saturation extract may not be adequate for normal plant growth. On the other hand, boron is toxic to many plants at low levels; more than 5 mg kg^{-1} hot-water extractable boron in soil is toxic to most plants whereas as little as 1 mg kg^{-1} in the saturation extract may cause visible damage to sensitive crops (Reisenauer, 1973). In no other nutrient element is the margin between sufficiency and toxicity so small.

Considerable evidence collected for whole soils points to the role of soil organic matter in boron solubility, sorption and availability in the soil. Berger and Truog (1945) found that boron availability in 34 virgin and 48 cultivated soils was most significantly correlated with organic matter content ($r = 0.83$). Baser and Saxena (1967) found that soluble boron in 30 Indian soils was correlated with organic matter. Elrashidi and O'Connor (1982) introduced an organic carbon term into a multiple linear regression model which explained 98% of the variation of boron adsorption in a group of soils. Evans (1987) found that the boron adsorption maxima of nine Canadian soils with $pH > 6.8$ were correlated with both organic carbon ($r = 0.87$) and with water-soluble carbohydrates ($r = 0.66$). Russell (1973) speculated that the reaction between boron and soil organic matter was due to boric acid condensing with diol groups associated with carboxylic groups of humic acid. In what appears to be the only research on boron retention of pure humic acid systems, Parks and White (1952) found that calcium humate extracted with bicarbonate retained 0.6 mg B g^{-1} HA at 9 mg L^{-1} soluble B, bicarbonate-extracted humic acid retained 1.4 mg B g^{-1} HA at 7.8 mg L^{-1}, and a pyrophosphate-extracted humic acid retained 18.2 mg B g^{-1} HA at 44 mg L^{-1} B; no pH control was reported and other than the three measurements reported here, no adsorption isotherms were measured.

Boric acid is known to form well-defined complexes with polyhydric substances, particularly with cis- 1,2 and 1,3 diols by the condensation of $B(OH)_3$ with the diol to form a five-member ring complex while releasing water and a proton; condensation may continue further with an additional diol to form a second five-member ring through the boron while releasing two water molecules (Fig. 1). The condensation reaction has been observed for a number of types of diols, including alcohols (mannitol and polyvinyl alcohol), sugars (glucose, mannose, xylose), and catechol (1,2-benzenediol), and is considered to be very sensitive to differences in configuration (Conner and Bulgrin, 1967). The result of the condensation reaction is to form a borate complex with an acid strength greater than that of either the borate or the diol individually; the reaction is readily reversible in all cases (Zittle, 1951). Before the spread of HPLC techniques, Khym and Zill (1952) employed boric-borate buffers to convert uncharged saccharides into their charged borate complexes and then quantitatively separated the saccharides on an anion exchange column, and Clapp (1957) fractionated soil-derived polysaccharides by separating the charged borate complexes by electrophoresis. If a polymer contains diol groupings, as do polyvinyl alcohol and guar gum, borate is known to cause cross-linking

Fig. 1. Schematic diagram showing boric acid $B(OH)_3$ (A), borate $B(OH)_4^-$ (B), a diol ligand L (C), a 1:1 borate:ligand complex $B(OH)_2(H_{-2}L)^-$ (D), and a 1:2 borate:ligand complex $B(H_{-2}L_2)^-$ (E). The notation $H_{-2}L$ indicates the loss of two protons from functional groups of the ligand which are not usually considered to be acidic functional groups.


A B C D E

of different polymer chains and subsequent gelation (Casassa et al., 1986). In addition to reactions with diols, evidence exists for the formation of borate complexes for α-hydroxy acids such as lactic, malic, tartaric, and citric acids, aromatic o-hydroxy acids such as salicylic acid (Zittle, 1951) and even for oxalic acid, a dicarboxylic acid with no hydroxyl groups at all (Schenker and Rieman, 1953).

Traditional methods of boron analysis employ either carmine and curcumin to form colored complexes with boron or a potenitometric method with mannitol to increase the acid strength of borate (Bingham, 1982). More recently, analysis of borate has been proposed using either ion exclusion chromatography (Wilshire and Brown, 1982) or ion chromatography of BF_4^- formed by the action of 10% HF on borate on an selective adsorbent (Hill and Lash, 1980). The method employed here is a modification of a spectrophotometric method using azomethine-H popularized by Wolf (1974) for analysis of boron in soil, water, and plant samples, and subsequently adopted by the United States Department of Agriculture in 1976 and the Organization of Official Analytical Chemists in 1982.

The purpose of this study is to investigate the interaction of borate/boric acid with humic acid using three different techniques. First, adsorption of boron on humic acid will be measured by reacting known quantities of boron with humic acid in dialysis cells followed by measuring boron content in the external solution. Second, the formation of complexes between boric acid and humate will be examined potentiometrically by the release of protons upon complexation. Third, the possibility of structural changes in humic macromolecules due to cross-linking caused by borate complexation will be examined viscometrically.

MATERIALS AND METHODS

Dialysis -- Hula humic acid was dissolved with NaOH and brought to a concentration of 0.25% (w/w). Four-mL aliquots of humic acid were introduced into dialysis tubing and sealed. The dialysis cells were then immersed in distilled water and the water was brought to pH 3 with HCl; the external solution was replaced twice to remove excess salts. The dialysis cells were divided into groups, each of which was brought to a target pH and cation by titration of the external solution with either NaOH or $Ca(OH)_2$.

For the sodic system, boric acid or borax stock solutions were weighed into test tubes, followed by the addition of 1 mL 100 mN NaCl, the dialysis cells adjusted to fixed pH values with NaOH, and water to bring the total volume to approximately 10 mL fluid. The test tubes were shaken overnight to equilibrate. The dialysis cells were extracted from the cells, dried at 65°C, and weighed to permit calculation of the total liquid volume by difference. The pH of the external solution in the test tube was determined by combined glass electrode and boron was measured using the azomethine-H method. The calcium systems differed by the use of boric acid titrated with $Ca(OH)_2$ to pH 8.5 instead of borax; 10 mN $CaCl_2$ was used to establish a constant calcium concentration.

To measure boron concentration by the azomethine-H method, 0.45 g azomethine-H and 1 g ascorbic acid are dissolved in 100 mL pH-buffering and metal-masking solution, which is made by dissolving 250 g ammonium acetate and 15 g disodium EDTA in 400 mL distilled water, followed by addition of 125 mL glacial acetic acid. A 3-mL aliquot of sample or standard is transfer to a test tube. Two mL azomethine-H/buffer is added to the tube, mixed thoroughly, and allowed to stand for 30 min. Readings were made at a wavelength of 420 nm with a Pye Unicam PU 8600 UV/VIS spectrophotometer with 0.001 AU precision. The absorbance at 420 nm yields a straight calibration curve to 3 mg L^{-1} B, with a standard error of 0.03 mg L^{-1} B. Two mg L^{-1} B yields an adsorbance of about 1 absorbance unit (AU). Where the solution to be tested had a slight absorbance at 420 nm (<0.010 AU) before addition of the azomethine-H, a blank reading was made and subtracted.

Azomethine-H was synthesized using the procedure of Basson et al. (1969). Eighteen grams of H-acid (8-amino-1-naphtol-3,6-disulphonic acid, monosodium) was dissolved in 1 liter by neutralization to pH 7 with 10% NaOH and gentle heating. Concentrated HCl was added dropwise until the pH was 1.5 and the solution was heated to 90°C. Twenty mL of salicylaldehyde was added and the mixture was shaken vigorously on a mechanical shaker for 1 hr. The azomethine-H was allowed to precipitate by standing overnight, followed by filtering on a Buchner funnel and washing with ethanol. The bright orange product was dried for 3 hr at 105°C and stored in an air-tight container. In practice, 18 g H-acid yielded 20 g azomethine-H, for a yield of 87% on the H-acid.

Titration -- Hula humic acid was dissolved and brought by titration to fixed pH values of 5.0, 7.5, 8.0, and 8.5 at a concentration of 0.25% (w/w) in 0.1 M NaCl. Increments of 50 mg L^{-1} B boric acid solution were added to 10 mL of humic acid solution. After each increment of boric acid, the pH was returned to within 0.001 pH unit of the initial pH by addition of 0.1 M NaOH using a Metrohm 665 Dosimat with a minimum delivery volume of 0.002 mL. For comparison, an additional titration was performed in the same manner with 0.25% (w/w) mannitol (MW 186) at pH 7.5. The quantities of base consumed to return the humic acid solutions to the target pH after addition of boric acid were corrected for the amount of base required to titrate the boric acid to the same pH.

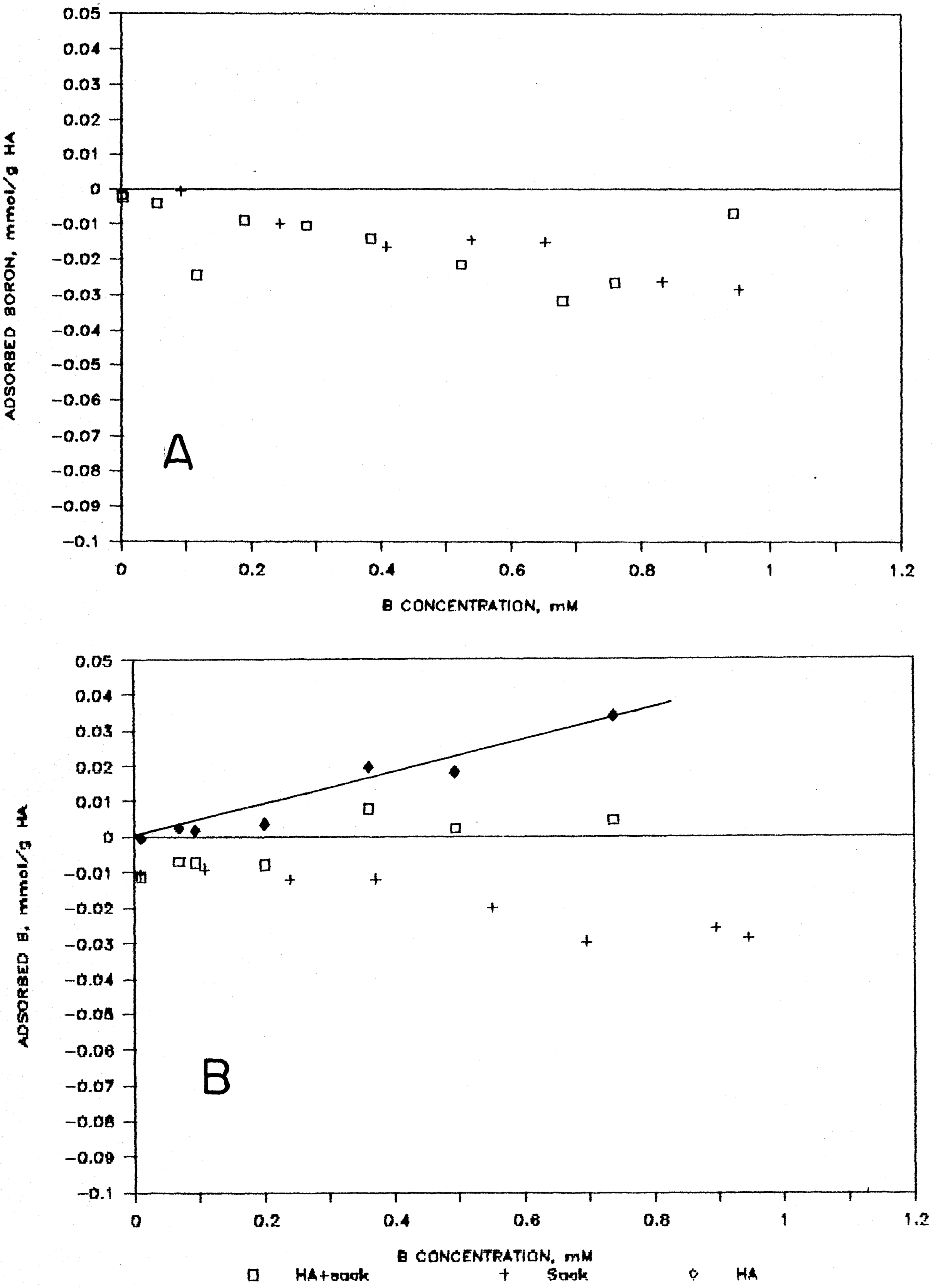
Viscosity -- To measure the possible effect of borate crosslinking on humic acid viscosity, the concentrations of humic acid and borate were higher than in preceding experiments in order to approach the 2-4% w/w polyvinyl alcohol and 0.4% borax employed by Casassa et al. (1986) to achieve a gel. Hula humic acid was brought to a concentration of 0.5% w/w at pH 9.2, with no excess electrolyte. To 15 mL of the pH 9.2 humic solution 5% w/w borax was added incrementally to a final concentration of 1.3% borax. At each increment, viscosity was measured using a modified Ostwald viscometer with a flow time of 99 sec for distilled water at 25°C. Flow times are averages of at least three readings at each increment; variations between replicate flow times were ±0.1%. As a check, the experiment was repeated with NaCl at pH 9.2 at sodium concentrations comparable to those of the borax (sodium borate) and with water at concentrations comparable to those of the boric acid.

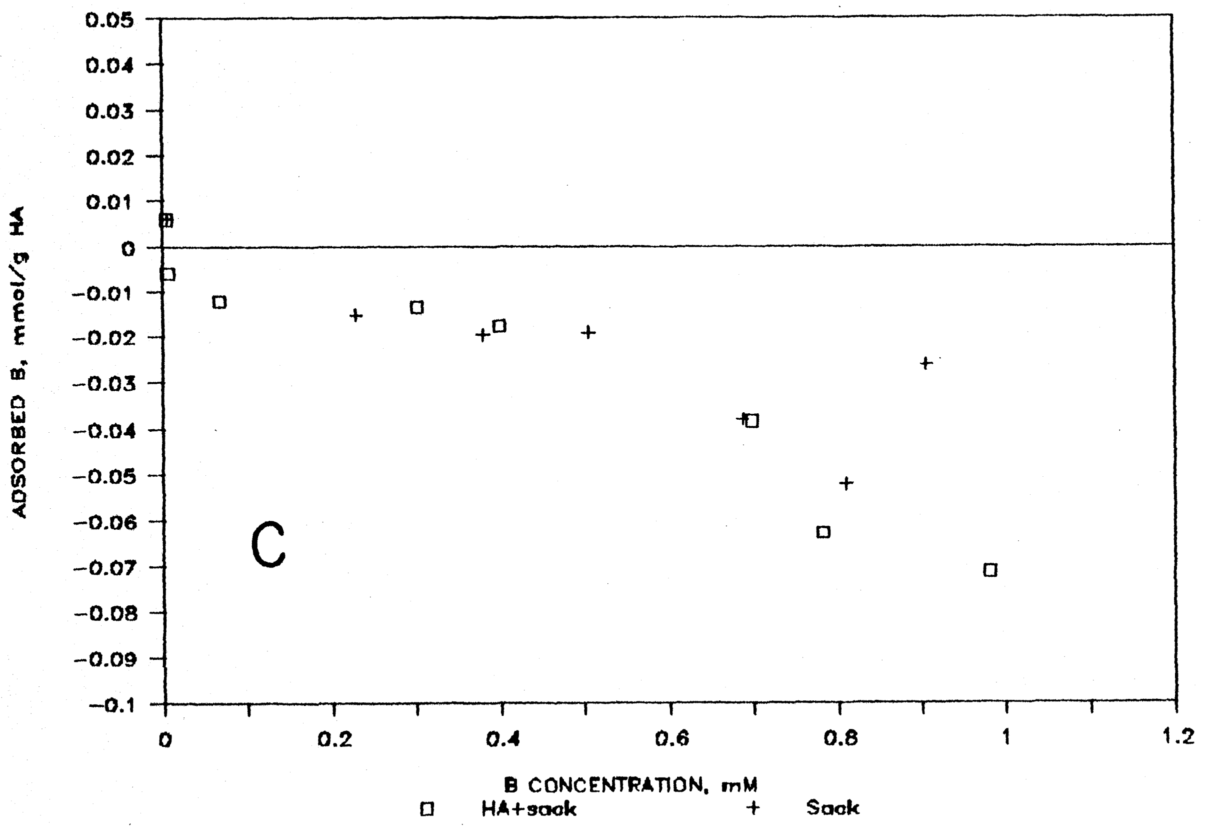
RESULTS AND DISCUSSION

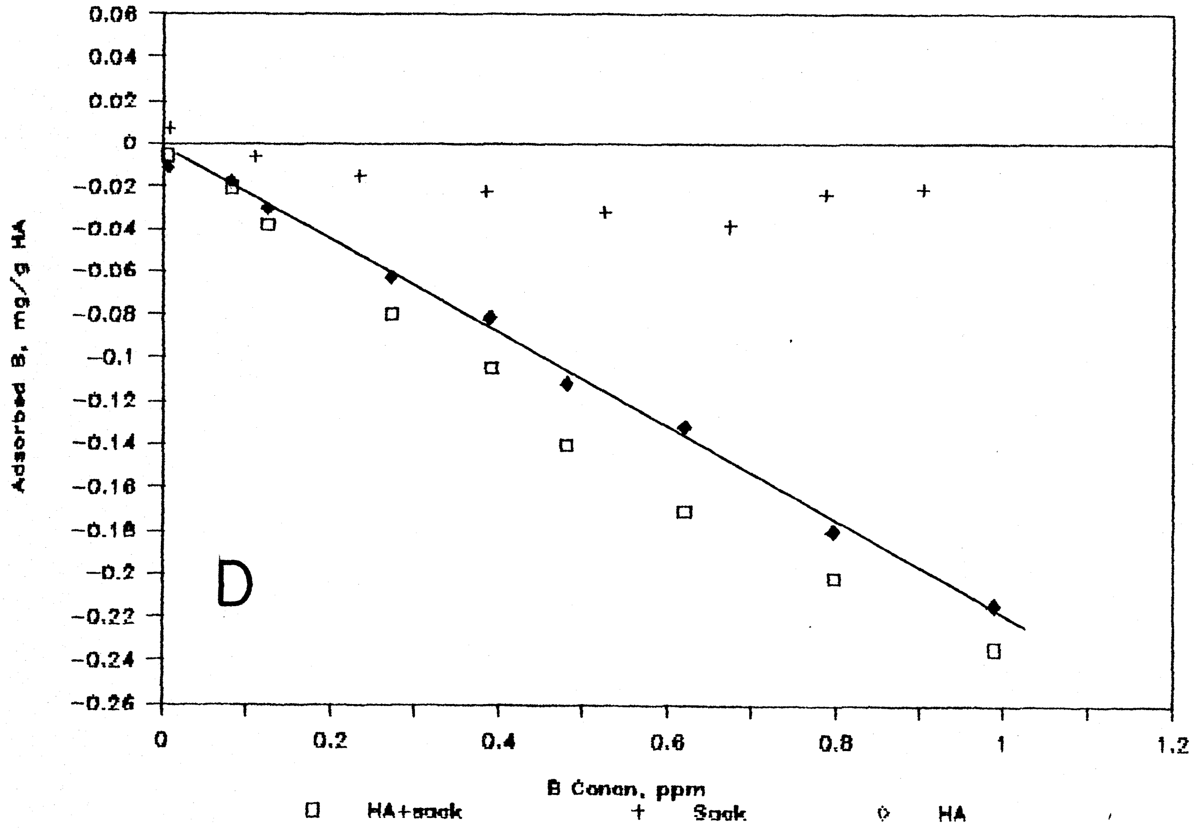
Dialysis -- Experiments separating a solution containing humic acid from an external humate-free solution were performed for both sodium and calcium humate systems at a number of pH values. At pH 5.0, the dialysis cells containing sodium humate (Fig. 2a) showed no apparent reaction with boric acid other than that of the cellophane dialysis cells alone. The cellophane cells show a slight amount of borate exclusion which can be attributed to imbition and occlusion of ~1 mL water g^{-1} dry weight in order to hydrate the cellophane. At pH 7.3 (Fig. 2b), the dialysis cells exclude borate, i.e., show negative adsorption, similar to that at pH 5.0 but the dialysis cells with humic acid show adsorption. The net behavior of the humic acid by difference at pH 7.3 may be seen to be a nearly linear isotherm, showing adsorption of 0.35 mg B g^{-1} HA at 8 mg B L^{-1}. At pH 8.7 (Fig. 2c), both the dialysis cell and the humic acid show nearly identical exclusion, indicating no significant reaction of the humic acid with borate. At pH 10.4 (Fig. 2d), the dialysis cells containing humic acid show borate exclusion much greater than that of dialysis cell alone. The net behavior of the humic acid by difference is exclusion. Such phenomena of negative adsorption are readily addressed as anion exclusion. In the case of sodium humate and borate at pH 10.4, the interaction is linear and consistent with an average exclusion of 224 ± 11 (n=8) mL g^{-1} HA.

The experiments with the sodium humate show a system with no interaction at pH 5, positive adsorption at pH 7.5, no adsorption at pH 8.7, and exclusion at pH 10.4. This behavior stems from both the

Fig. 2. Boron adsorption in sodium system by dialysis cells (+) and humic acid + dialysis cells (□) at pH 5.0 (A), 7.5 (B), 8.7 (C) and 10.4 (D). At pH 7.5 and 10.4, boron adsorption by humic acid (◆) is calculated by difference.



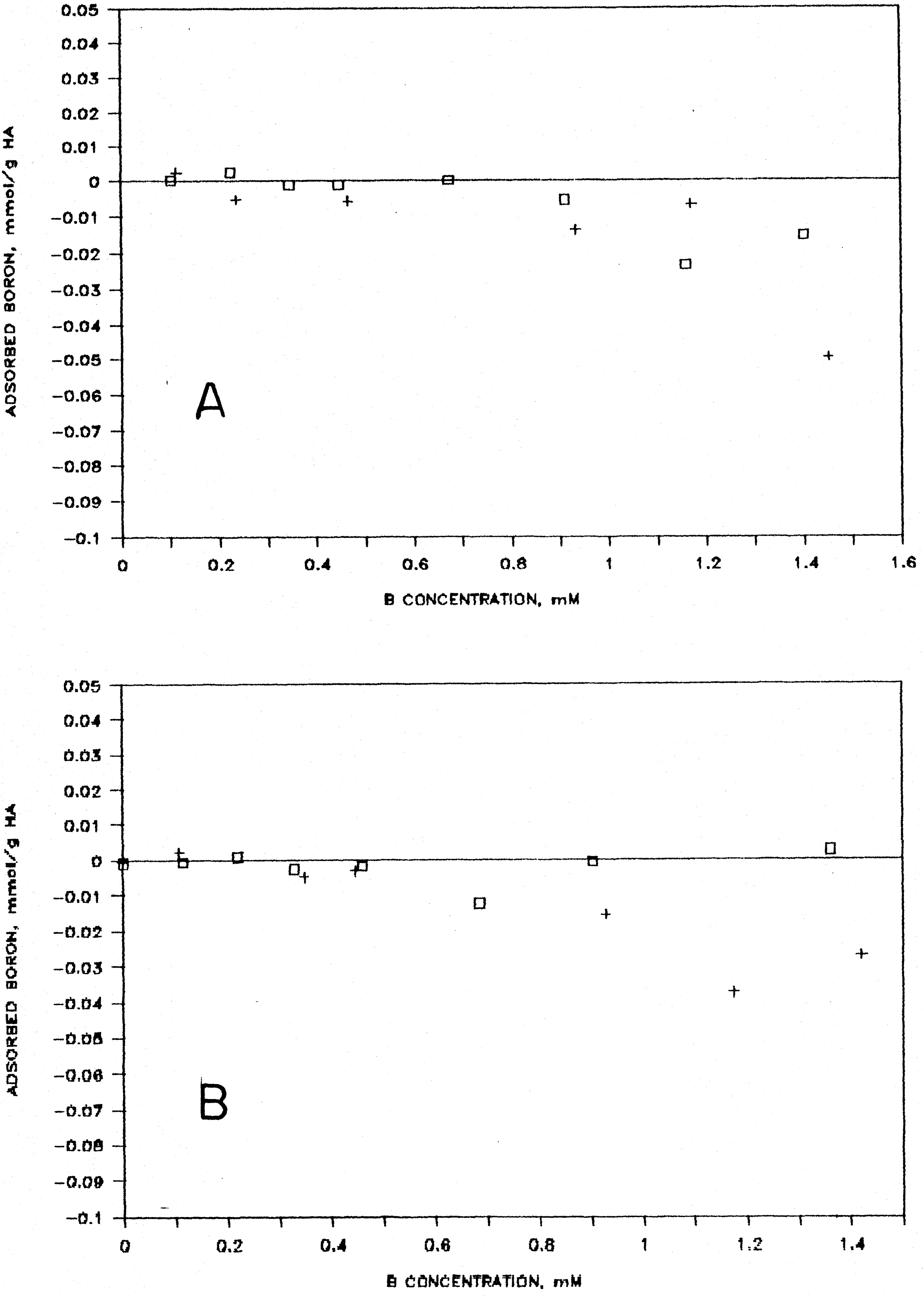

0.05
0.04
0.03
0.02
0.01
0
−0.01
−0.02
−0.03
−0.04
−0.05
−0.06
−0.07
−0.08
−0.09
−0.1
ADSORBED B, mmol/g HA
C
0
0.2
0.4
0.6
0.8
1
1.2
B CONCENTRATION, mM
□ HA+sack
+ Sack

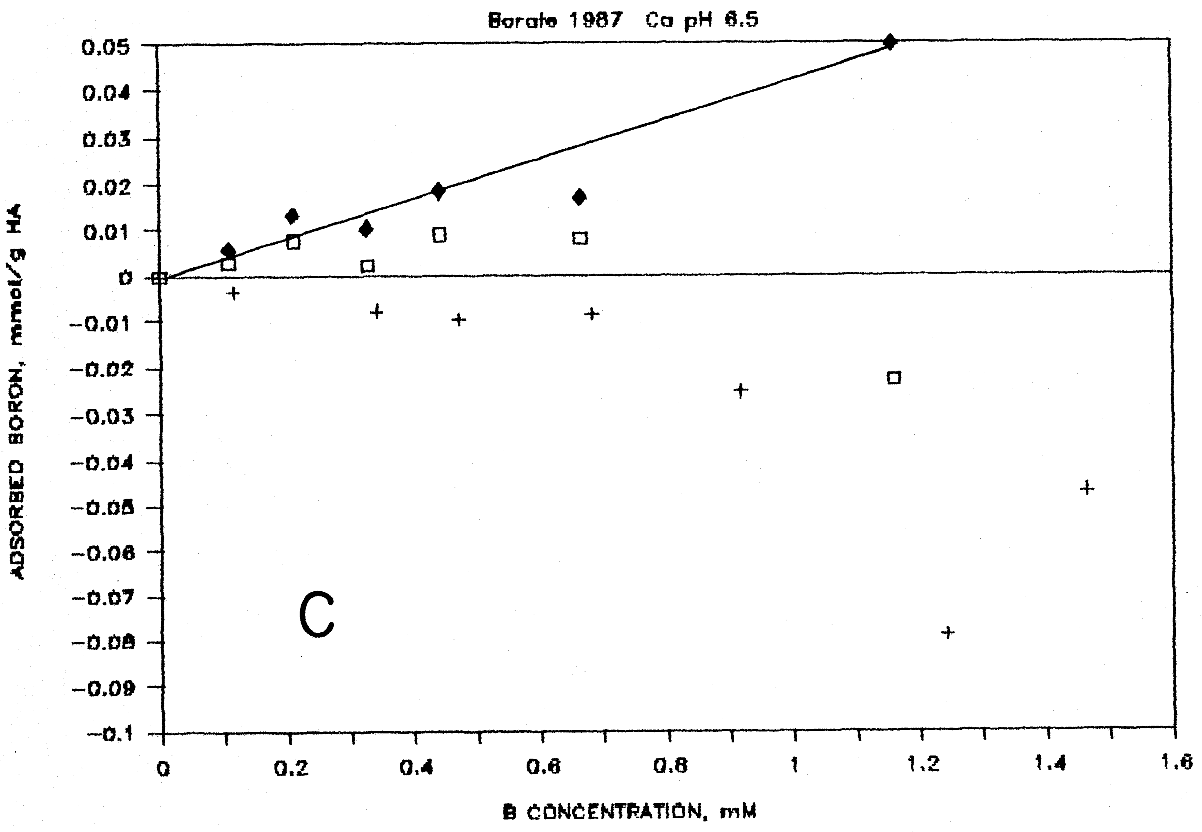

0.06
0.04
0.02
0
−0.02
−0.04
−0.06
−0.08
−0.1
−0.12
−0.14
−0.16
−0.18
−0.2
−0.22
−0.24
−0.26
Adsorbed B, mg/g HA
D
0
0.2
0.4
0.6
0.8
1
1.2
B Concn, ppm
□ HA+sack
+ Sack
◇ HA

complexing nature of the borate and the anionic behavior of both the borate and humic molecules. Because of the very weak acid nature of boric acid, the proportion of borate relative to total boron species is very pH-dependent. For example, at pH 5 only 0.01% of the boron species are ionized to borate. The formation of borate complexes is therefore in general favored by high pH but in the case of charged humic macromolecules, the higher pH levels concommitantly causes reduction of borate concentration at the humic surface due to anion exclusion, thus reducing the favorability of complexation. At pH 7.5, 2% of the boric species are anionic, at pH 8.7, 25% of the borate species are anionic, and at pH 10.4, 94% of the borate species are anionic. Furthermore, at pH 10.4, most of the phenolic groups which were favorable to complexation have been ionized and the borate species are 95% anionic and repelled.

Experiments for borate-complexing by humates in an environment containing calcium were conducted at pH values of 3.0, 5.0, and 6.5. Experiments set up with higher initial pH values of both the humate and added borate drifted to values of pH 6.5 and lower overnight; part of the difficulty may have been due to precipitation of carbonates in the test tubes, resulting in lower pH values. The results of the experiments in Fig. 3 show little or no boron adsorption by the dialysis tubes (~0.36 g cellophane per tube) or by the dialysis tubes filled with 10 mg Hula humic acid at concentrations up to 1.5 mM B (16 mg L^{-1} B) at pH values measured for the calcium humate system.

Fig. 3. Boron adsorption in calcium system by dialysis cells (+) and humic acid + dialysis cells (□) at pH 3.0 (A), 5.0 (B) and 6.5 (C). At pH 6.5, boron adsorption by humic acid is calculated by difference.




Borate 1987 Ca pH 6.5
ADSORBED BORON, mmol/g HA
0.05
0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
-0.1
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
B CONCENTRATION, mM
C

Titrations -- The type of interaction between borate and a reactive substance is demonstrated by the results of a titration of mannitol, a compound with six hydroxyl groups among which are two cis- diols. When a 0.25% w/w solution of mannitol (= 13.4 mM) was brought to 1 mg L^{-1} B (0.092 mM) with boric acid, the pH dropped from 7.50 to 7.13 (Fig. 4). To return the pH to 7.50, base equivalent to 0.038 mM OH^- was consumed, whereas to simply bring the boric acid to pH 7.50 required 0.001 mM OH^-. In short, it was possible to determine that 40% of all the boric acid in the first increments reacted with the mannitol, releasing one proton for each boron complex formed. The titration data for mannitol and humic acid may be summarized as an adsorption isotherm in Fig. 5 which shows that up to 14 mg L^{-1} B approximately half of the borate reacted to release protons and half remained in solution.

It may be shown that this technique returns reliable information on borate complexation by comparing the empirical binding curve of Fig. 5 with measured boric acid/mannitol stability constants collected by Conner and Bulgrin (1967), which are as follows:

$$K_1 \ \{B(OH)_4^- + L \longleftrightarrow B(OH)_2(H_{-2}L)^- \} = 1100$$

$$K_2 \ \{B(OH)_4^- + 2L \longleftrightarrow B_2(H_{-2}L_2)^- \} = 137{,}000$$

where L is the concentration of the uncomplexed mannitol ligand and the symbol $H_{-2}L$ indicates that the ligand loses two protons which do not otherwise normally participate in acid-base reactions, in this case from aliphatic hydroyxl groups. The equilibrium concentrations of all species

Fig. 4. Titration curve of 10 mL 0.25% w/w mannitol solution with 0.1 M NaOH to maintain a pH of 7.50 after repeated 0.2 mL additions of 4.62 mM $B(OH)_3$. Each arrow represents boric acid addition.

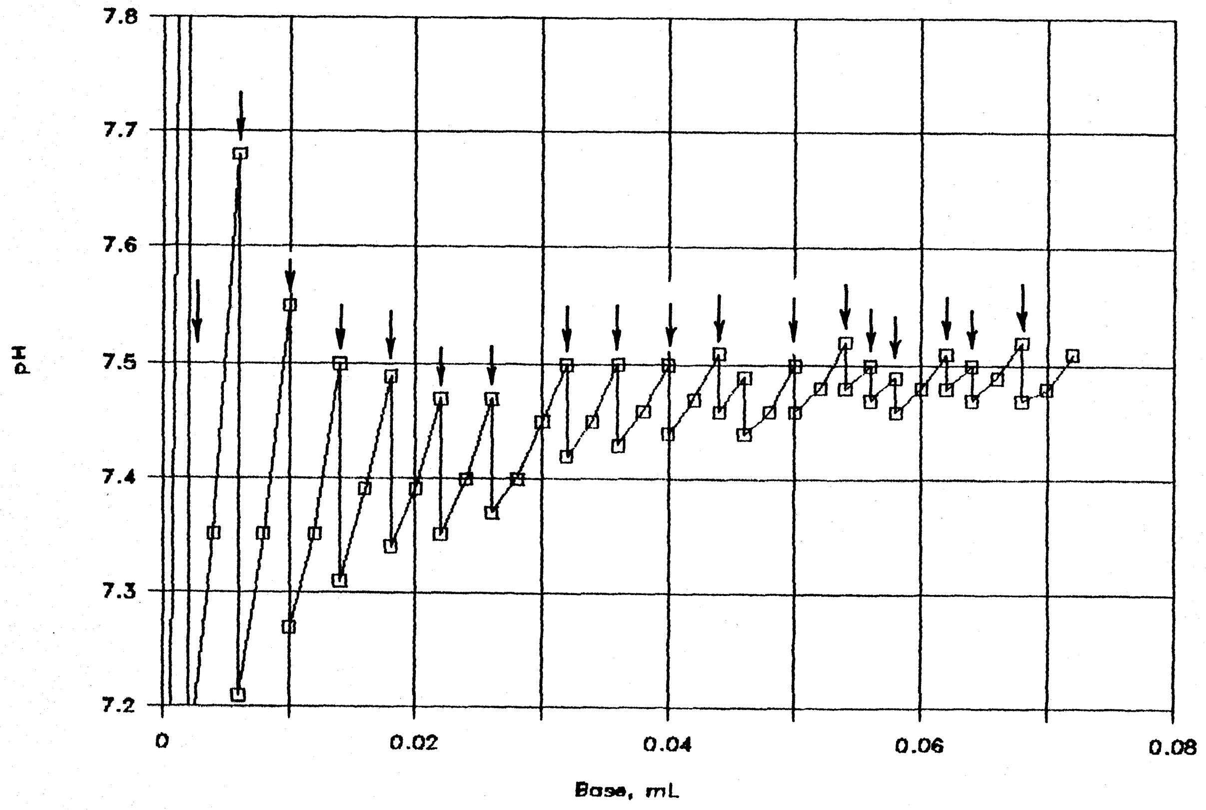
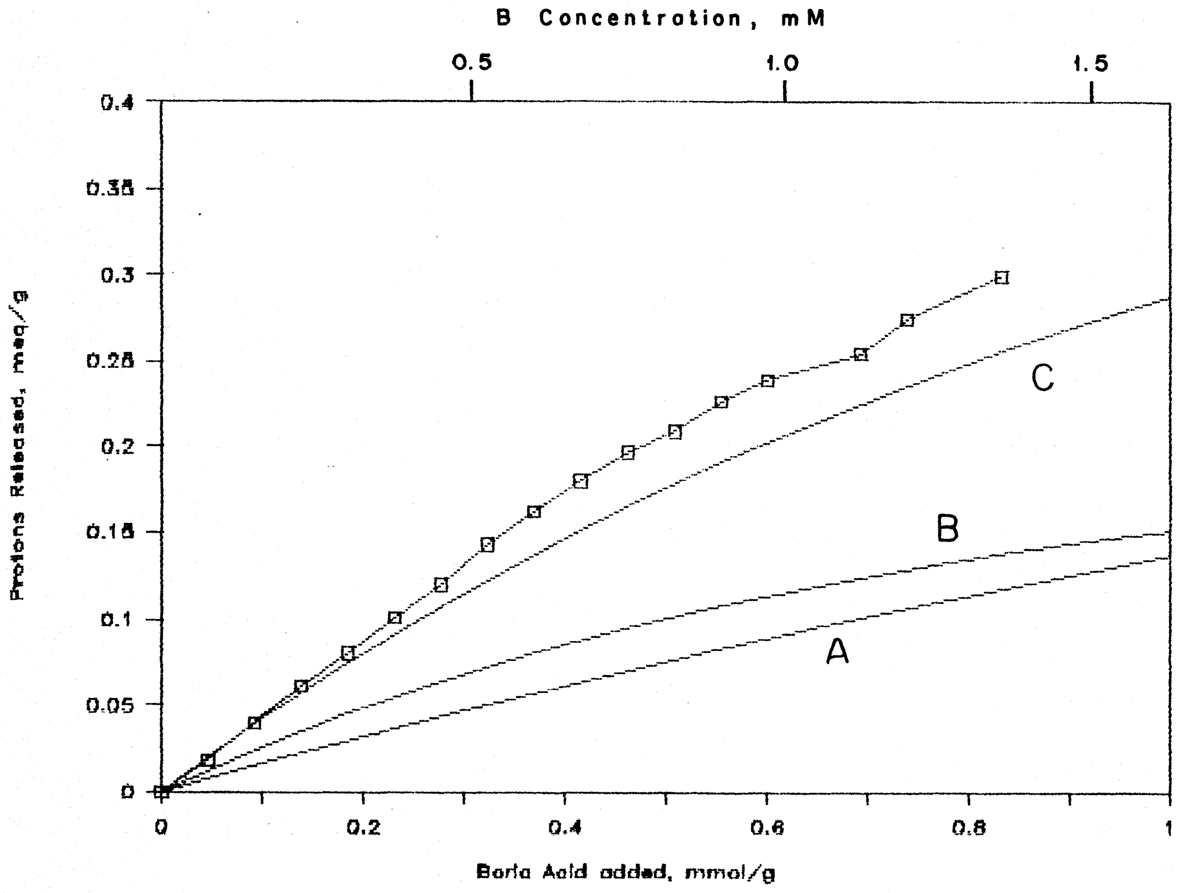


Fig. 5. Proton release curve (□) of mannitol at pH 7.5 determined from the data in Fig. 4, indicating formation of charged borate:mannitol complexes. Smooth lines represent formation of $B(OH)_2(H_{-2}L)^-$ (A), $B(H_{-2}L_2)^-$ (B) and $B(OH)_2(H_{-2}L)^-$ + $B(H_{-2}L_2)^-$ (C), predicted by the constants of Connor and Bulgrin (1967).



may be calculated from the constants, pH and the total concentrations of boric acid and mannitol using mass balance equations as follows:

$$\begin{aligned} \Sigma B &= B(OH)_3 + B(OH)_4^- + B(OH)_2(H_{-2}L)^- + B(H_{-2}L^2)^- \\ &= B*H/K_a + B + K_1*B*L + K_2*B*L^2 \end{aligned}$$

$$\begin{aligned} \Sigma L &= L + B(OH)_2(H_{-2}L) + 2*B(H_{-2}L_2) \\ &= L + K_1*B*L + 2*K_2*B*L^2 \end{aligned}$$

where ΣB and ΣL indicate the total concentrations of borate and mannitol species, respectively, B is uncomplexed $B(OH)_4^-$ and K_a is the acid dissociation constant of boric acid; the above mass balance equations may be rearranged to factor B and L and then solved recursively as follows:

$$\begin{aligned} B &= \Sigma B/(1 + H/K_a + K_1*L + K_2*L^2) \\ L &= \Sigma L/(1 + K_1*B + K_2*B*L) \end{aligned}$$

The above equations converge within several iterations to constant values for free borate or mannitol, permitting calculation of the quantity of $B(OH)_2(H_{-2}L)^-$ and $B(H_{-2}L_2)^-$, each of which releases one proton during its formation during the course of the titration. From the solid line C in Fig. 5, it may be seen that the sum of negatively charged borate complexes predicted by constants does not differ by more than 10% from the measured proton release curve, indicating that the titration method employed here is consistent with data generated by other researchers for well-defined materials. Further, it may be seen that at the concentrations employed here that the 1:2 complex (B) is almost equal in concentration with the 1:1 complex (C).

Titrations of humic acid with boric acid were conducted primarily at the point of least buffering of the humic acid, i.e., pH 7.5-8.5, at the break between the titration of the carboxylic groups and the phenolic groups. This pH range simultaneously provides the greatest possible pH changes upon proton release and base addition and is close to normal soil pH values. Even so, the addition of boric acid to humic acid solutions produced very little pH drop (Fig. 6 a-c), requiring relatively small volumes of base to restore the target pH. After minor corrections for the amount of base required to titrate the boric acid itself to target pH values, the proton release curve in Fig. 7 was obtained. No proton release is evident at pH 5.0 but complexing is evidenced at pH 7.5 and increases at pH 8.0 and 8.5, which show similar behavior. The data that complexing is identical at pH 8.0 and 8.5 is noteworthy since at pH 8.5 the $B(OH)_4^-$ concentration is 2.8 times greater than at pH 8.0 at the same ΣB, a fact which should increase complexation by an equal measure if the reaction were one simply of complexation. However, if the humic acid were to electrostatically repel the $B(OH)_4^-$ species, then the concentration of boric species for complexation at the humic surface would be expected to be lower than the concentration of borate species in the equilibrium solution. The extent of boron interaction over the boron concentration range considered, which covers almost the entire range in agricultural soils, indicates that the reaction is on the order of 0.05 mmol B g^{-1} HA, which is 0.5 mg

Fig. 6. Titration curve of 10 mL 0.25% w/w Hula humic acid solution with 0.1 M NaOH to maintain a pH of 7.5 (A), 8.0 (B) and 8.5 (C), after repeated 0.2 mL additions of 4.62 mM $B(OH)_3$; at pH 5.0, no base was required and titration curve is not shown. Each arrow represents boric acid addition.

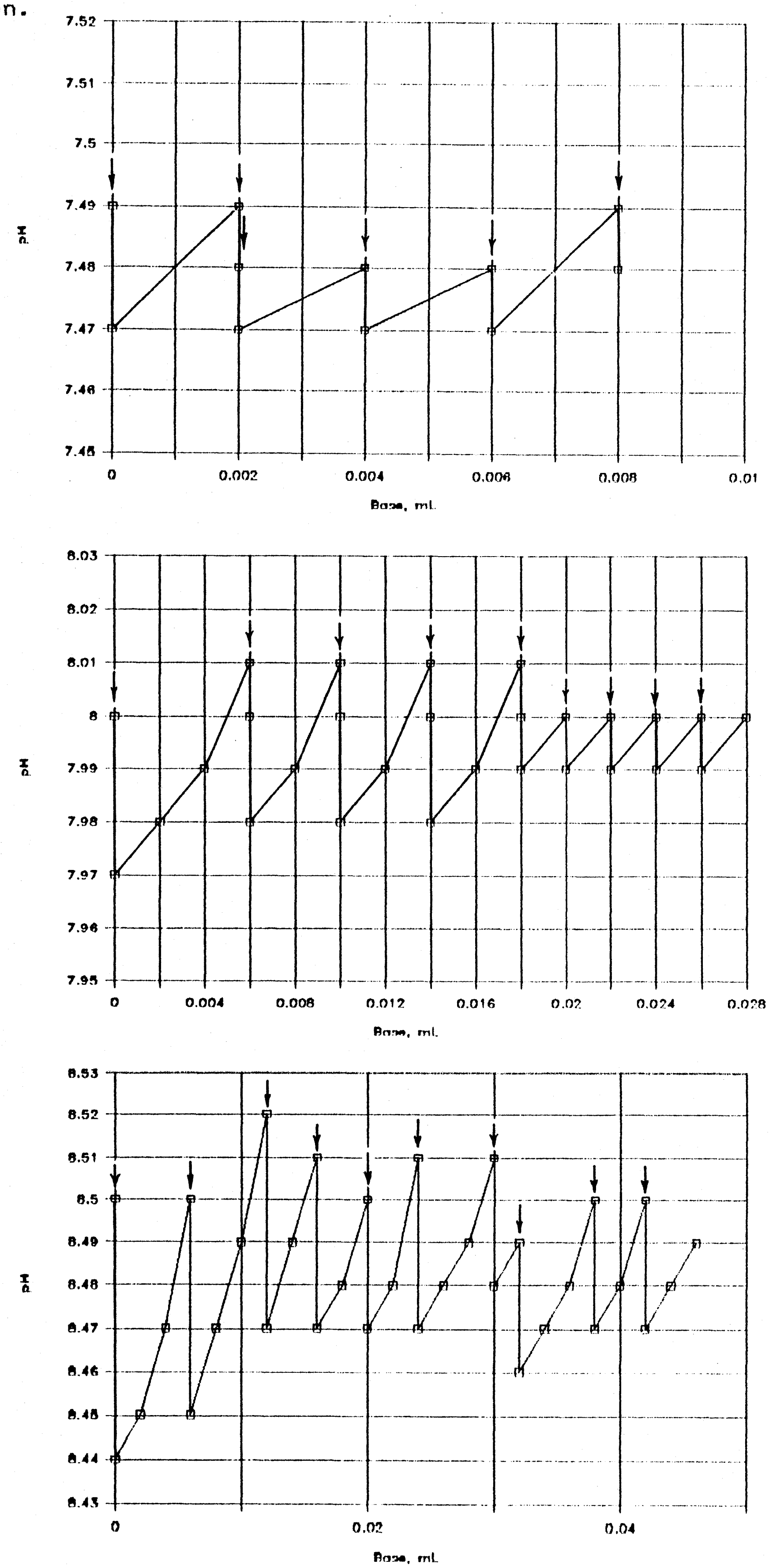
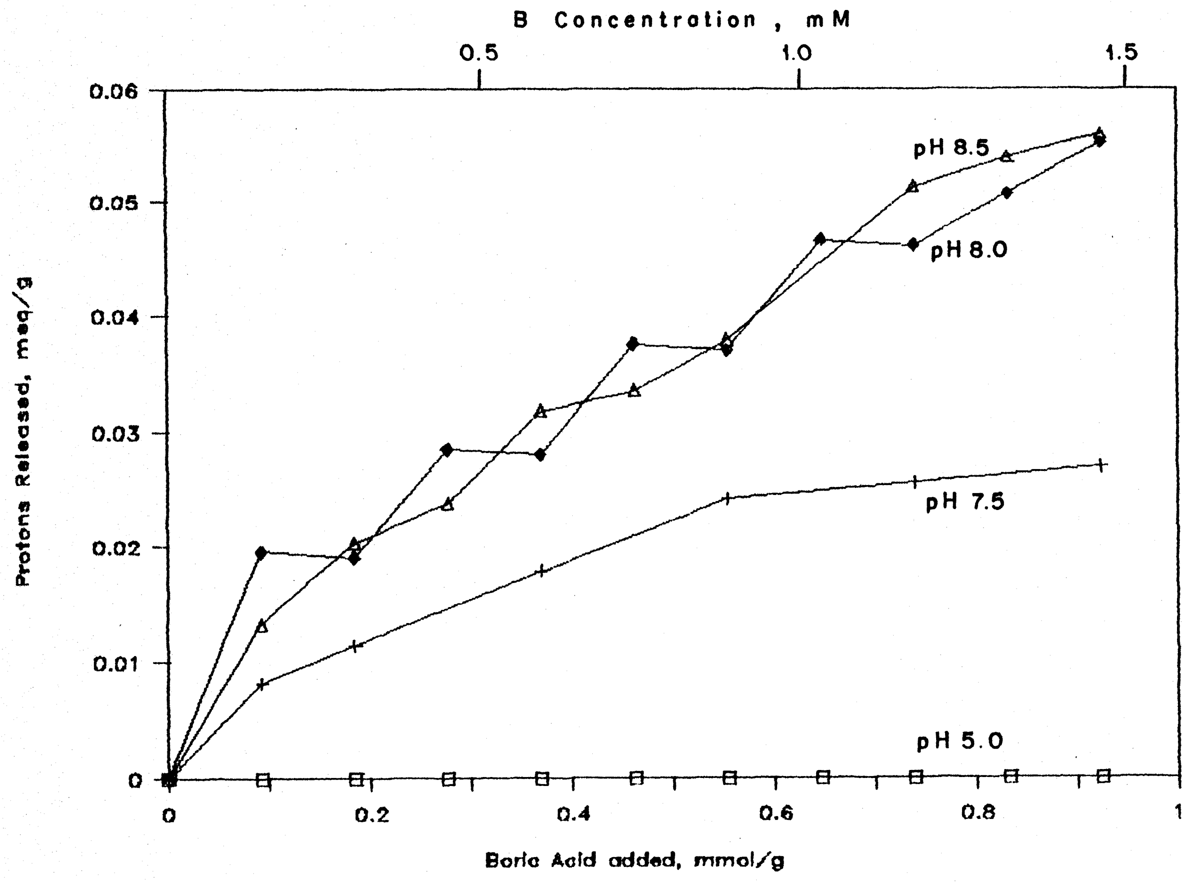


Fig. 7. Proton release curve of Hula humic acid determined from the data in Fig. 6, indicating formation of charged borate:humic acid complexes.



B g^{-1} HA, as measured by both the dialysis and titration technique at pH 7.3-8.5. By way of comparison, it should be noted that the mannitol over the same range of boron concentrations adsorbed 0.27 mmol B g^{-1}.

Viscosity -- The addition of borax to macromolecules containing 1,2 diol groups is known to cause changes in viscosity. Casassa et al. (1986) reported gelation with 2 or 4% polyvinyl alcohol and 0.4% borax; the polyvinyl alcohol used had a molecular weight of at least 100,000 and 1-2% 1,2 diol structures among the more the predominant pattern of 1,3 diols, a much less favored geometry for borate complexation. In the course of this study, even polyvinyl alcohol (B.D.H Chemicals Ltd., Poole, England) with a nominal molecular weight of only 10,000 resulted in the formation of a stiff gel at these concentrations. The process of gelation is due to the formation of complexes of the sort labelled above $B(H_{-2}L_2)^-$, where L now represents a ligand and each ligand is on a different macromolecule, and borate acts as a cross-linking agent between the two macromolecular ligands. The concentration of a number of natural substances required to obtain a gel reaction with borax may be as low as 0.2%, while other substances require concentrations up to 10% or form only viscous, ropy solutions (Zittle, 1951).

No gel formation was noted over the range of 0.4% Hula humic acid with 1% borax to 0.5% HA and 0.2% borax at pH 9.2. Viscometric measurements were conducted to search for structural changes more subtle than gelation. The reduced viscosity may be given as:

$$\eta_{red} = (\eta/\eta_0 - 1)/c$$

where η = the viscosity of the solution

η_0 = the viscosity of the solvent

c = the concentration of the solute

At pH 9.2, the addition of borax was found to actually reduce the viscosity of the humic solution (Fig. 8), instead of increasing the viscosity due to the structural changes in the direction of gel formation. The cause of the decrease became clear when NaCl was added to the humic solution at equimolar concentration of sodium. The conclusion by Ricci (1952) that borax ($Na_2B_4O_7$) in solution may be considered as boric acid half-titrated with NaOH is relevant here since it appears that it is the increase in ionic strength, whether due to NaCl or $NaB(OH)_4$, which is causing the reduction in viscosity; indeed, the reduction of viscosity of colloidal systems with a double electric layer with increasing ionic strength has long been noted. When boric acid was added to humic acid at pH 5.2, only slight changes in viscosity were measured; the addition of the same volumes of water also produced little or no change in the reduced viscosity.

These viscometric measurements show no evidence for cross-linking of humic acid molecules due to complexes of the 1:2 borate:ligand type even at very high concentrations of borate at pH 5.2 and pH 9.2 under conditions which form easily discernable complexes with known borate-complexing ligands. These results are consistent with dialysis and titration experiments which show no complexation type at these pH

Fig. 8. Reduced viscosity of Hula humic acid solution at pH 9.2 during addition of either borax (□) or NaCl (+). Two replicates for each treatment.

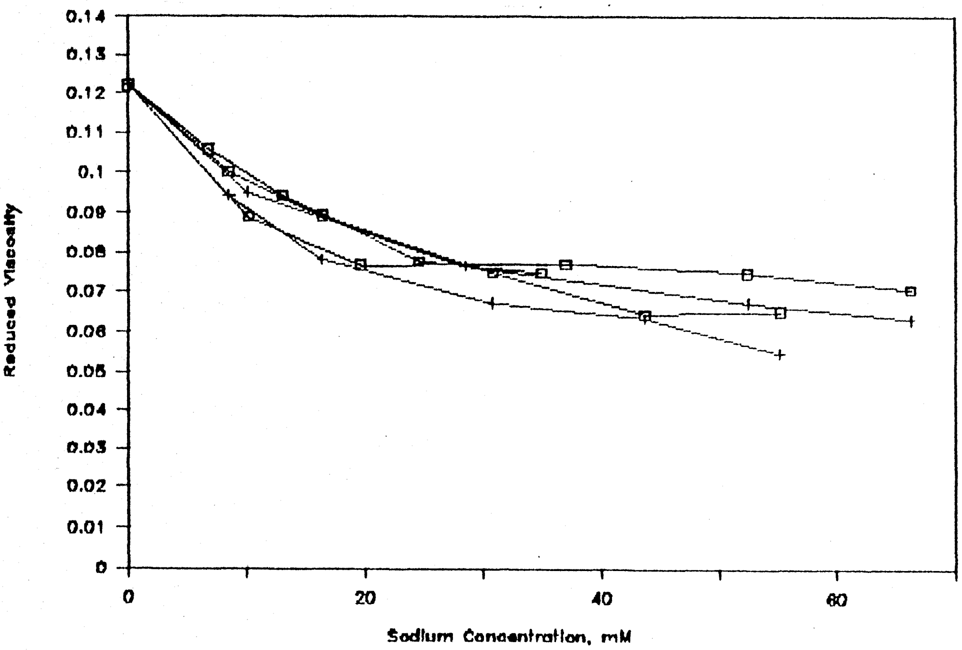
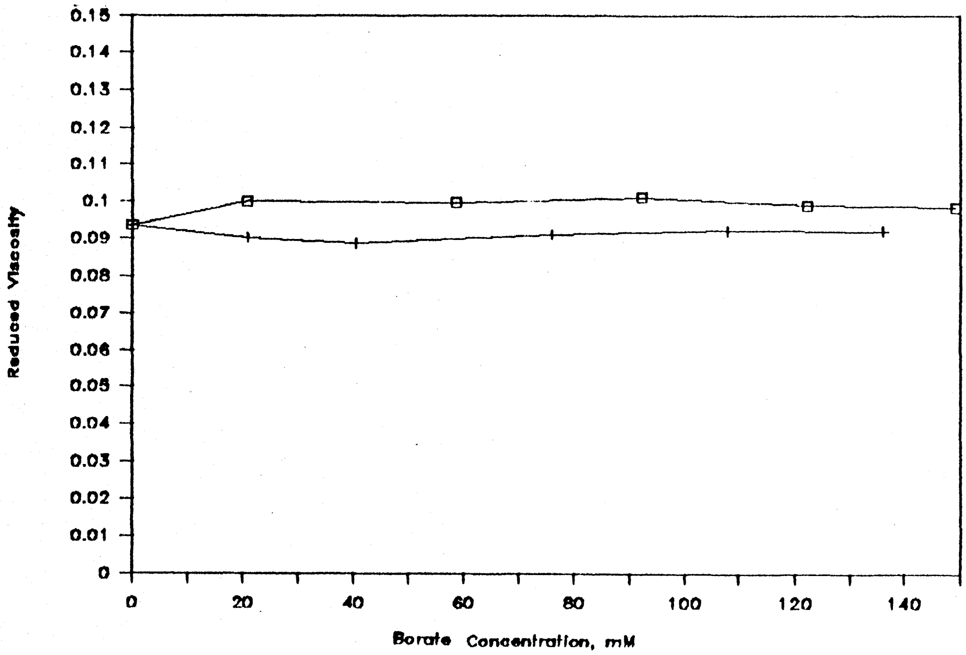


Fig. 9. Reduced viscosity of Hula humic acid solution at pH 5.2 during addition of either boric acid (□) or equivalent volume of water (+).



values. The low levels of B adsorption at pH levels 7.5-8.5 as compared to mannitol made cross-linking very unlikely; therefore measurements of viscosity at these pH values were not conducted.

CONCLUSIONS

The interaction of humic acid with boric acid/ borate has been studied here by three different methods. Dialysis experiments have shown that the sodium humate is inert to borate at pH 5, linearly adsorbs borate at least until 0.032 mmole B g^{-1} HA at pH 7.5 and 0.74 mM B, is relatively inert at pH 8.7, and shows exclusion of borate solution to the extent of 220 mL g^{-1} HA at pH 10.4. Calcium humate shows no interaction at pH 3.0 or 5.0, but at pH 6.5 boron is linearly adsorbed at least until 0.032 mmole B g^{-1} HA at 1.2 mM B. A potentiometric method, whose accuracy was initially tested using mannitol, provided similar information. No interaction was found at pH 5, 0.023 mmole B g^{-1} HA was adsorbed at 1.32 mM B and pH 7.5, and 0.055 mmol B g^{-1} HA was adsorbed at 1.32 mM B at both pH 8.0 and 8.5. The data point to an interaction of a type which is a balance between more favorable reaction as pH increases due to formation of $B(OH)_3$ and yet as $B(OH)_3$ is ionized to $B(OH)_4^-$, the reaction is increasingly limited by anion exclusion phenomena which in the extreme cause exclusion of as much as 200 mL borate solution g^{-1} humic acid. Viscometric data indicates that the extent of borate complexation, even at high concentrations of humate and borate, is insufficient to cause changes in the tertiary structure due to cross-linking.

Overall, it may be concluded that humic acid is not extremely reactive with borate when compared to molecules with well-defined cis-diol groups such as mannitol or polyvinyl alcohol. Martin et al. (1963) and Dubach el al. (1964) have presented evidence based on active-H measured by diborane that indicates that the alcoholic OH content of humic acids may be very low, in which case the content of cis- 1,2 or 1,3 diols is even lower. The catechol ligand, due to its geometry which aligns two aromatic carbons and two adjacent hydroxyl groups in a planar configuration, has a markedly favorable geometry for borate complexation with stability constants higher than aliphatic diol hydrocarbons (Table 1). Perdue (1985) has expressed reservations about the presence of more than one phenol substitution per aromatic ring on the grounds that dihydroxyaryl compounds are relatively rare in proteins and lignins and are virtually absent among the oxidative degradation products of humic substances and lignin. Assuming ~2 mmol g^{-1} benzene rings for Hula humic acid, as calculated by Perdue for a fulvic acid, and 1.1 mmol g^{-1} phenolic groups as measured by titration, then there is on average only 0.5 hydroxyl group per benzene ring and the probability of a 1,2-dihydroxybenzene (catechol) moiety may be very low. Furthermore, the Hula humic acid has been for the most part purified of soil polysaccharides, polymers of borate-complexing ligands of the type of glucose and mannitol in Table 1, by the standard IHSS (1982) procedure of precipitation twice by acid, leaving most polysaccharides soluble and separatable together with fulvic acid. Humic

Table 1. Stability constants (25°C) for polyol-borate complexes (Conner and Bulgrin, 1967).

Compound	K_1	K_2
1,2-propanediol	4.0	3.8
1,3-propanediol	1.3	0.1
1,2,3-propanetriol	14	57
cis-1,2-cyclohexanediol	1	0.7
trans-1,2-cyclohexanediol	0	0
cis-1,2-cyclopentanediol	26	142
trans-1,2-cyclopentanediol	0	0
1,2-benzenediol (catechol)	8000	17,300
glucose	135	870
D-mannitol	1100	137,000

acids are therefore relatively unreactive with borate because of the low contents of the cis-diol, catechol, and saccharide ligands.

The role of the borate-complexing nature of the humic acid as measured above may be compared with the measured boron adsorption isotherms measured for whole soils. Mezuman and Keren (1981) measured isotherms for four soils at different pH values. The soil with the highest organic matter content, 1.2%, adsorbed 3 µmol g^{-1} B at a boron concentration of 0.5 mM and at pH 9. At the same boron concentration but at pH 8.0-8.5, a maximum of 30 µmol B g_{-}^{1} was adsorbed by Hula humic acid, which is ten times higher than that of the soil. If the 1.2% organic matter of the soil is roughly equivalent to Hula humic acid in boron adsorbing properties, then the organic matter would be expected to contribute about 0.3 of 3 µmol g^{-1} adsorbed B. Mezuman and Keren (1971) reduced the organic matter content to 0.6% by oxidation with H_2O_2 without apparent change in the adsorption isotherm. Taken together, it would seem that the sorption properties of boron in whole mineral soils would seem to be only partially due to humic substances. The consistent reports of statistical correlations between boron content, boron availability, and boron solubility with total soil organic matter would seem to be based less on the 60-70% of soil organic matter which is humic acid, fulvic acid, and humin than on the 5 to 25% of carbohydrates, including oligosaccarides and polysaccharides of varieties with known borate-binding properties (Clapp, 1957).

SUMMARY AND CONCLUSIONS

Humic substances are natural, organic substances ubiquitous in soil and sediments. Humic substances have average molecular weights of 500 to 100,000 and are believed to be randomly coiled linear electrolytes. Soil-derived humic substances are generally considered to have a high aromatic carbon content and a high content of weakly acidic (carboxylic groups) and very weakly acidic (phenolic and amine) functional groups. The acidic nature of the humic substances gives rise to hydrophilic properties when the humic substances are ionized and is responsible for linear extension of the polyelectrolyte when ionized due to mutual repulsion of ionized sites. In addition to the hydrophilic nature due to ionized acidic groups, humic substances also have been found to possess hydrophobic sites. Unlike most other macromolecules of biological origin such as proteins and nucleic acids, humic molecules are heterogenous in size and structure and of necessity must be dealt with in the aggregate by means of averages.

Considerable research has been conducted in the past on the nature and strength of interactions of humic substances with cations of metals ranging from macroelements to rare radionucleotides. A variety of interactions with ions and molecules other than cationic adsorption may be expected of humic substances, but very little is known of such interactions. For example, the literature contains two reports of chloride exclusion in humate solutions, one report of borate adsorption on humic substances, and reports of fluoride adsorption on Al-humate and phosphate adsorption on Fe-humate. More information exists for the adsorption of pesticides and herbicides, which are mostly hydrophobic chemicals, on humic substances. It is the intent of this study to expand the literature for interaction of humic substances with nitrate, borate, and EDDHA (ethylenediamine di(o-hydroxyphenylacetic acid, a iron-chelating synthetic amino acid of agricultural significance).

The interaction of nitrate with a number of humic substances extracted from soils of Israel and abroad was initially investigated by titration of humic solutions with nitrate stock solutions, with measurement of nitrate activity using ion selective electrodes (ISE) selective for nitrate, similar to accepted techniques for studies of cation interaction with humic substances. Two different manufactures of electrodes gave two different results which either indicated strong nitrate adsorption or nitrate exclusion from the humic molecules. The results of both types of nitrate electrode were considered to be unreliable in concentrated solutions of humic acid (0.25% w/w).

Subsequent experiments were conducted with batch experiments using dialysis cells, permitting chemical analysis of a humate-free solution. Existing ion chromatographic techniques were modified to permit rapid, accurate analysis of nitrate in the sample. Measurements consistently showed concentrations of nitrate in the humate-free cell greater than that expected if the added nitrate had simply mixed into the entire volume of solution, thereby indicating exclusion of nitrate from the dialysis cell containing the humic substance. Measurements show the presence of nitrate exclusion over the entire range tested -- pH 3.5 to pH 6, and ionic strength from 1 mM to 100 mM. At high pH and low ionic strength, exclusion was as much as 400 mL g^{-1}. The nature of the exclusion phenomenon is in general consistent with a polyelectrolyte with weakly acidic groups which when ionized cause a double electric layer which excludes anions and expands and contracts in response to electrolyte concentration.

To provide greater understanding of the nitrate exclusion data, detailed acid-base titrations of humic acid extracted from Hula peat soil were conducted at different levels of electrolyte strength up to 100 mM. Titration curves were strongly affected by the ionic strength, as was the apparent acid strength of the carboxylic groups, but the acid content of the carboxylic group and of the phenolic group was constant. The extended linear polyelectrolyte was considered to be best modelled as an infinite charged cylinder. A published seminumerical model and an original numerical model were employed to solve the double electric layer equation in differential form for a cylinder having a charged surface. For a number of discrete levels of dissociation of the carboxylic group, radii were calculated which best fit the measured dependence of pH on ionic strength based on the changes in acid strength due to surface potentials on an ideal charged cylinder. The fit of the cylindrical double electric layer model to the titration data was 0.03 pH units or less. The apparent radii determined by this method range from 0.85 nm at 12.5% ionization to 0.35 nm at 75% ionization.

Exclusion data for nitrate permits a different test of the double electric layer model since the acid titration data reflects explicitly only the effect of the surface potential on the acid strength of the humic substance whereas anion exclusion is a phenomenon which is the summation of co-ion repulsion from the surface to an infinite distance from the surface; exclusion is thus dependent upon the distribution of electric potential in space rather than at a single point. The exclusion volumes predicted by the radii of the titration data were used to construct a single-value estimation of the exclusion volume as a function of radius and ionic strength. Using the nitrate exclusion data measured by ion chromatography together with measured ionic strength, an apparent radius was extracted for each nitrate data point.

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 (neutral pH). The decrease in radius with dissociation may have its origin in the uncoiling and extension of the polyelectrolyte which is not explicitly included in the model. The dimensions of the humic molecule fall in the range of a number of other fibrous biomacromolecules such as DNA and the alpha helix constituent of proteins. The narrow radius explains both the elusiveness of the humic macromolecule upon examination by electron microscopy and their great flexibility and ability to undergo conformational changes in the the presence of high salt concentrations and low concentrations of di- and trivalent metal ions.

The EDDHA molecule, particularly in its ferrated form NaFeEDDHA, is of agricultural significance as the most effective iron fertilizer known for use on calcareous soils. FeEDDHA has been found to undergo a certain amount of fixation immediately upon contact with soil, followed by a slow reduction of availability with time which reduces overall fertilizer recovery. Previous reports attributed the fixation of FeEDDHA to reactions with organic matter, especially considering that FeEDDHA, as an anion, is thought not to be adsorbed by soil clays. For this project, a new method of measuring EDDHA was developed based on ion chromatography as a substitute for published solvent extraction and non-specific spectroscopic methods. Experimental data showed that at pH

3.5 in an environment of Na-humate and NaCl, FeEDDHA could be adsorbed at rate of 30 µmol g^{-1} when the equilibrium concentration was 25 µmol L^{-1} FeEDDHA in 180 mM NaCl or as little as 12 µmole g^{-1} at 65 µmol L^{-1} when the electrolyte level was 0.8 mM. Some adsorption is still found at pH 4.5, but at pH 6.8 to 7.5, no adsorption is evident even at 180 mM NaCl; instead, the data clearly shows exclusion of FeEDDHA from the humic substances up to 350 mL g^{-1}; the exclusion phenomenon is also present at pH 11. Experiments with $Ca(FeEDDHA)_2$ and Ca-humate with $CaCl_2$ as added electrolyte show that FeEDDHA is adsorbed at pH 4-5 but that at pH 6-7 there is neither significant adsorption nor exclusion. Experiments with unferrated EDDHA show behavior much the same as that of FeEDDHA, i.e., adsorption at pH values less than 5 and net exclusion at values greater than pH 6. The mechanism of exclusion is almost certainly exclusion of the $FeEDDHA^-$ or $EDDHA^{2-}$ anion by ionized acidic groups on the humic molecule; as with nitrate, exclusion would be expected to be greater as ionization increases. The mechanism of adsorption of FeEDDHA and EDDHA at low pH is not known, but could result from either interaction of the hydrophobic parts of the EDDHA molecule with hydrophobic sites on the humic molecule or hydrogen bonding under acid conditions between amine groups of EDDHA and unionized carboxyl groups of the humic substance. Although it is difficult to extrapolate from humic solutions to soil humates in situ, it would appear that under conditions prevailing in a calcareous soil – basic pH and low ionic strength – the experiments above would not suggest strong adsorption of FeEDDHA on humates; on the other hand, a preliminary experiment performed with calcium montmorillonite at pH 7 showed that up to 0.7 µmol FeEDDHA g^{-1} adsorbed at 250 µM FeEDDHA and 25 mM $CaCl_2$. It would appear that soil mineral colloids are not as inert to FeEDDHA as previously held and montmorillonite, or other clay minerals, may be the FeEDDHA-fixing phase, probably through edge sites.

The interaction of humic acid with boric acid/ borate has been studied here by three different methods. Dialysis experiments have shown that the sodium humate is inert to borate at pH 5, linearly adsorbs borate at least until 0.032 mmole B g^{-1} HA at pH 7.5 and 0.74 mM B, is relatively inert again at pH 8.7, and shows clearcut exclusion of borate solution to the extent of 220 mL g^{-1} HA at pH 10.4. Calcium humate shows no interaction at pH 3.0 or 5.0, but at pH 6.5 boron is linearly adsorbed at least until 0.032 mmole B g^{-1} HA at 1.2 mM B. A potentiometric method, whose accuracy was initially tested using mannitol, provided similar information. No interaction was found at pH 5, 0.023 mmole B g^{-1} HA was adsorbed at 1.32 mM B and pH 7.5, and 0.055 mmol B g^{-1} HA was adsorbed at 1.32 mM B at both pH 8.0 and 8.5. The data point to an interaction of a type which is a balance between more favorable reaction as pH increases due to ionization of $B(OH)_3$ and yet as $B(OH)_3$ is ionized to $B(OH)_4^-$, the reaction is increasingly limited by anion exclusion phenomena which in the extreme cause exclusion of as much as 200 mL borate solution g^{-1} humic acid. Viscometric data indicates that the extent of borate complexation, even at high concentrations of humate and borate, is insufficient to cause changes in the tertiary structure due to cross-linking.

Overall, it may be concluded that humic acid, after precipitation in acid by which polysaccharides are excluded, is not extremely reactive with borate due to the low contents of the <u>cis</u>-diol, catechol, and saccharide ligands in the humic acid fraction of soil organic matter.

The consistent reports of statistical correlations between boron content, boron availability, and boron solubility with soil organic matter would seem to be based less on the 60-70% of soil organic matter which is humic acid, fulvic acid, and humin than on the 5 to 25% of carbohydrates, including oligosaccarides and polysaccharides of varieties with known borate-binding properties.

In conclusion, this work has expanded the knowledge of the interactions of humic substances with anions. In the course of the research, analytical techniques for nitrate and EDDHA have been developed and published. Nitrate was found to be excluded by sodium humate solutions under all conditions; the extent of exclusion is dependent upon pH and ionic strength. FeEDDHA was adsorbed on humic acid at low pH and excluded at neutral pH. Boric acid was found to be unreactive with humic acid at slightly acid pH, to be adsorbed at neutral and slightly alkaline pH, and excluded at high pH values. Adsorption mechanisms were either condensation between borate and humate or hydrogen bonding between EDDHA and humate. Exclusion was due to electrostatic repulsion. Nitrate exclusion data was combined with acid-base titration data using double electric layer theory with cylindrical geometry to yield an apparent radius of 0.35 to 0.85 nm, depending upon degree of ionization, for the humic macromolecule.

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APPENDIX I -- PREPARATION AND CHARACTERIZATION OF HULA HUMIC ACID

PREPARATION:

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; 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.

CHARACTERIZATION:

Elemental Content

C	46.7%
N	3.73%
ash	<0.5%

Functional Group Analysis (meq g^{-1})

CO_2H	2.39 ± 0.04
Phenolic OH	1.11 ± 0.06
Total Acidity	3.51 ± 0.07

Spectroscopic Analysis		
	E_4/E_6 (pH 9)	7.40
	E_4/E_6 (pH 6)	7.96

Infrared Adsorption Bands (Fig. 1)

Frequency (cm^{-1})	Assignment (Stevenson, 1982)	
3378	3400-3300	O-H stretching, N-H stretching
3221		
2920	2940-2900	Aliphatic C-H stretching
2608		
1721	1725-1720	C=O stretching of COOH and ketones
1630	1660-1630	C=O of amide groups, quinone C=O, or C=O of H-bonded conjugated ketones
	1620-1600	Aromatic C=C, strongly H-bonded C=O of H-bonded ketones
1422	1460-1450	Aliphatic C-H
1227	1280-1200	C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers
1125	1170-950	C-O stretching of polysaccharide or polysaccharide-like substances, Si-O of silicate impurities
1084	"	
1040	"	

Reduced Viscosity (η_{sp}/c)		
	pH 5.2	0.094
	pH 9.2	0.122

Summary -- The C content of Hula humic acid is somewhat lower than most humic acids (50-60%) and is in the range typical of fulvic acids (40-50%). The N content is in the range typical of humic acids (2-6%). The E_4/E_6 value is lower than for most humic acids (<5.0) and is in the range of fulvic acids (6.0-8.5), perhaps indicating a low molecular weight. The contents of acidic functional groups and their acid strength are normal for humic acids (Takamatsu and Yoshida, 1978). Infrared spectrum shows evidence of aromaticity, carboxyl and hydroxyl groups, as well as some polysaccharide impurities. Standard values, unless specified, are those of Stevenson (1982).

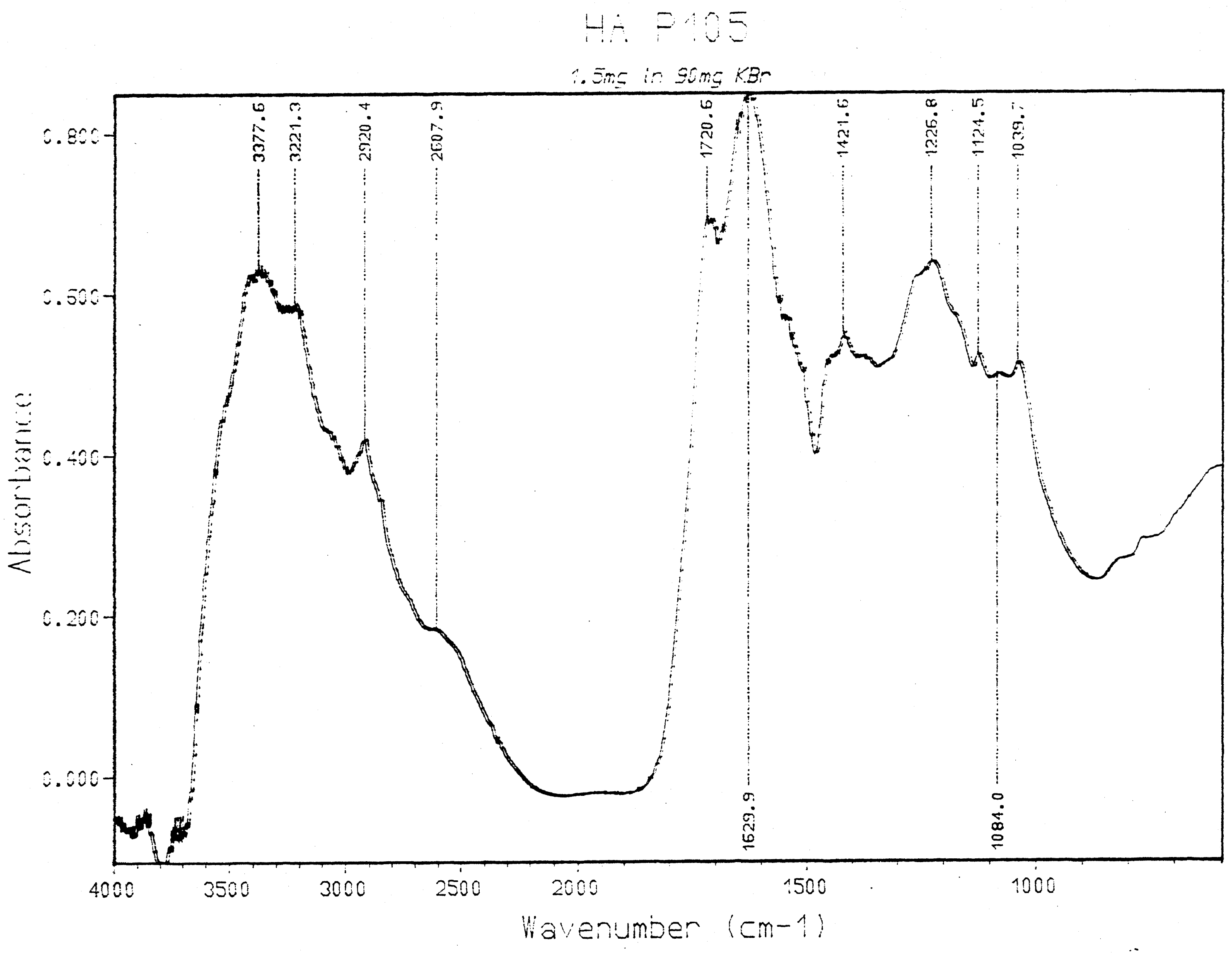


Fig.1 Infrared spectrum of Hula humic acid.

APPENDIX II -- NUMERICAL SOLUTION OF THE DOUBLE ELECTRIC LAYER

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 dϕ/dx reverses sign, whereupon the estimate of ϕ_s is reduced and calculations are restarted. If, during the course of calculations, ϕ or dϕ/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 ϕ = dϕ/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/cm^3 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).

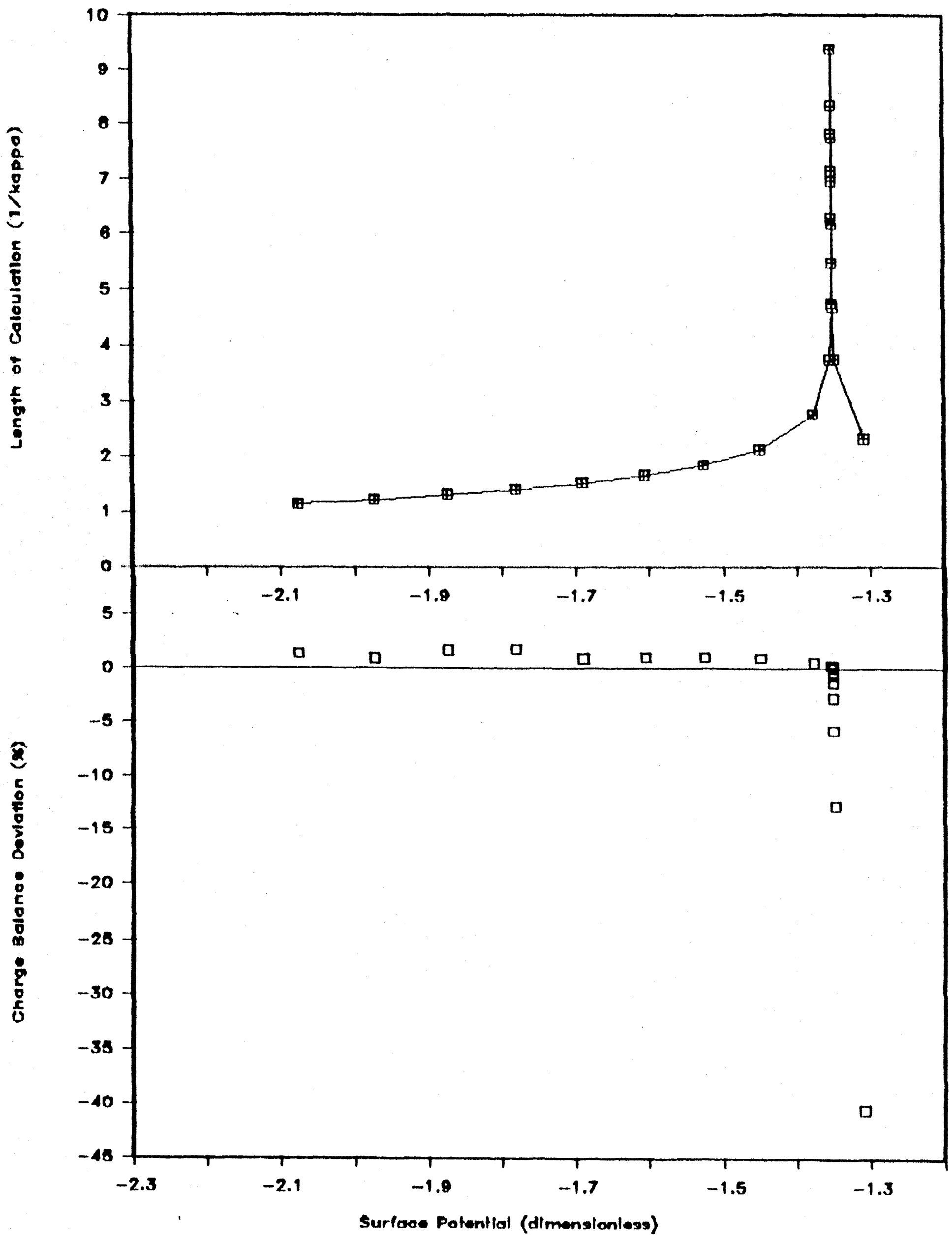
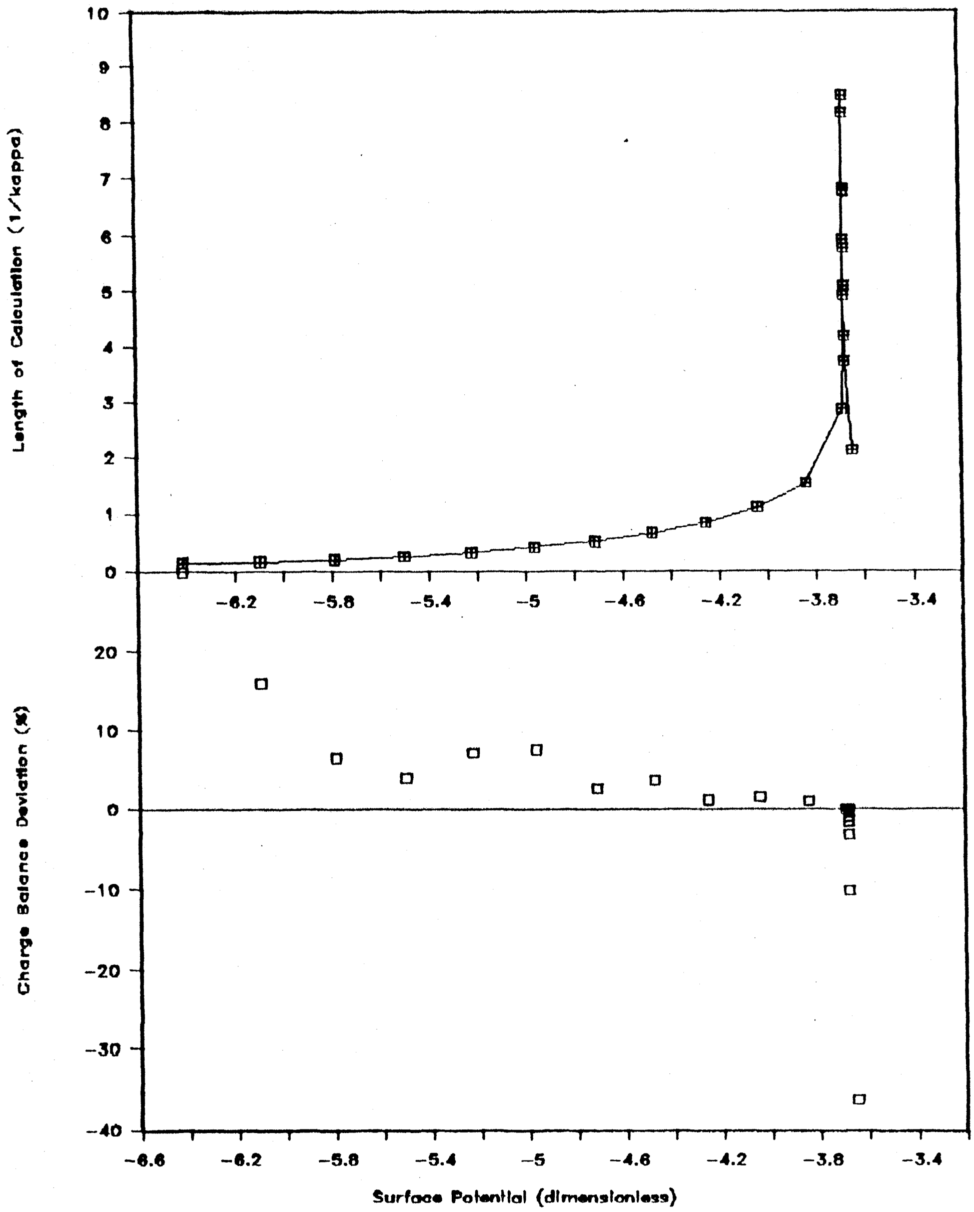


Fig. 2. Results of the algorithm for calculation of the double electric layer for an infinite cylinder of 0.5 nm radius and a charge density of 13811.75 esu (equivalent to 2.39/2 meq/g charge and 1.61 g/cm^3 density) in the presence of 0.001 mol/L 1:1 electrolyte. The best dimensionless surface potential 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).



APPENDIX III -- LIST OF 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	$(8\pi n e^2/\epsilon kT)^{1/2} = 3.29.107 \sqrt{I}$ 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 = $\Phi e/kT$
x	distance from axis of cylinder
a	distance of cylinder surface from axis
p	density of water (w) or humic acid (HA), 1.00 or 1.61 g cm^{-3}, respectively

יחסי גומלין של אניונים עם חומרים הומיים

חיבור לשם קבלת תואר
דוקטור לפילוסופיה

מאת

פיליפ ברק

הוגש לסינט האוניברסיטה העברית בירושלים

אב תשמ"ז אוגוסט 1987

עבודה זו נעשתה בהדרכתו
של פרופ' יונה חן

תוכן עניינים

מבוא כללי

פיתוח שיטות למדידת יחסי גומלין של אניונים
עם חומרים הומיים

דחיית חנקה על-ידי חומצה הומית

רדיוס מולקולות הומיות על-פי טיטרציית חומצה/בסיס
ודחיית חנקה

יחסי גומלין של EDDHA ו-FeEDDHA עם חומרים הומיים

יחסי גומלין של חומצה בורית ובורט עם חומרים הומיים

נספחים

תקציר

יחסי הגומלין הכימיים בין חומרים הומיים ובין האניונים השונים בסביבת הקרקע כמעט לא מוכרים בספרות המקצועית מלבד דיווחים בודדים על ספיחת זרחה, סיליקה, בורט ופלואריד, ודחיית כלוריד. מטרת מחקר זה להרחיב את המידע בנושא יחסי הגומלין של האניונים חנקה, ethylenediamine di(o-hydroxyphenyl acetic acid) [EDDHA] ובורט עם חומרים הומיים. במהלך המחקר פותחו שיטות אנליטיות מתקדמות עבור חנקה ו-EDDHA המתבססות על כרומטוגרפיה יונית.

נמצא שיון החנקה נדחה (ספיחה שלילית) על ידי תמיסות חומצה הומית בכל התנאים שנבדקו (pH 3.5-6.0 וחוזק יוני 1-100 מילימולר). שיעור הדחיה הגיע עד כדי 400 מ"ל \ג' חומצה הומית.

ספיחת EDDHA על חומצה הומית תלויה ב-pH ובחוזק היוני של התמיסה. ב-pH 3.5 וריכוז 25 מיקרומולר EDDHA, נספחו 30 מיקרומולר EDDHA \ג' בחוזק יוני (NaCl) של 180 מילימולר, ו-12 מיקרומול \ג' בחוזק יוני של 0.8 מילימולר. תופעת הספיחה עדיין קיימת ב-pH 4.5 אך בערכי pH מ-6.8 עד 11 התופעה השלטת היא דחיית EDDHA על ידי חומצה הומית. שיעור הדחיה מגיע עד כדי 350 מ"ל \ג' חומצה הומית. מוצע שמנגנון הספיחה הוא קשירת מימנים עם קבוצות קרבוקסיליות של החומצה ההומית.

חומצה בורית אינה מגיבה עם חומצה הומית ב-pH 5, נספחת ב-pH 7.5-8.5, ונדחית ב-pH 10.5 בהיותה בעיקר האניון בורט. שיעור הספיחה ב-1 מילימולר חומצה בורית (בורט) אינו עולה על 0.05 מילימול \ג' חומצה הומית. שיעור הדחיה ב-pH 10.5 היה 220 מ"ל \ג' חומצה הומית. מנגנון הספיחה הוא כנראה קונדנסציה בין יון הבורט ובין ליגנדים מסוג cis-diol או catechol הנמצאים בשיעורים קטנים כמרכיבי החומצה ההומית.

תופעת הדחיה נגרמת על ידי דחיה אלקטרוסטטית בין האניון המומס ובין המקרומולקולה ההומית המיוננת וטעונה מטען שלילי עקב התפרקות קבוצות מסוג החומצה החלשה. דחיה נמדדה עבור כל שלושת האניונים שנבדקו לפחות בתנאי pH

נייטרלים או בסיסיים. מדידות דחיית חנקה וכן נתוני טיטרציה חומצה-בסיס שימשו ליישום תיאוריית השכבה החשמלית הכפולה בתנאי גיאומטריה גלילית של חלקיקים על מנת להוות מודל לפוליאלקטרוליט ליניארי. תוצאות חישובי המודל מצביעות על כך שהמקרומולקולה ההומית מתנהגת כגליל טעון בעל קוטר של 0.85-0.35 ננומטר בתלות בדרגת היינון. תוצאות דומות התקבלו כאשר המודל נבחן מן ההיבט של התנהגות הקבוצות החומציות ומן ההיבט של דחיית אניונים. מימדים אלה דומים למימדי ביומולקולות אחרות כגון DNA ו-α-helix וגם מתאימים למודלים המקובלים של הרכבם הכימי של חומרים הומיים.

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