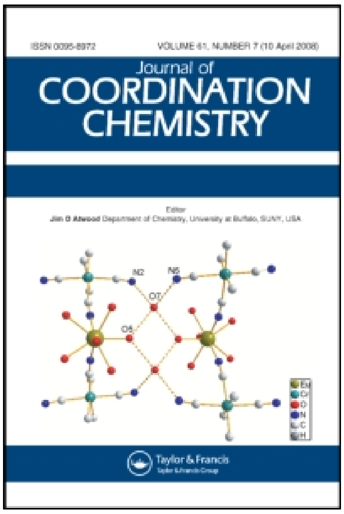

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Hydrogen Ion Affinity in Humic Acids: A Coordination Approach

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HYDROGEN ION AFFINITY IN HUMIC ACIDS: A COORDINATION APPROACH

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Potentiometric titrations of humic acids (HA) with 0.250 M $Ba(OH)_2/BaCl_2$ titrant (0.750 M constant ionic strength), have been performed at $25 \pm 0.1°C$ with a calibrated glass electrode for measuring $p[H^+]$. The pK'_w for water in this $BaCl_2$ medium is 12.899 ± 0.006 and 13.712 ± 0.006 in 0.500 M NaCl. Divalent Ba^{2+} cations force the ionization of the acid groups and improve solubility. Under such conditions derivative potentiometric curves show diverse equivalent peaks of the acidic sites of HA. The presence of 0.05% of a neutral detergent such as Triton X-100 is essential for effective dispersion of HA in the working solutions and to obtain very stable potentiometric measurements. Computer programs were used in the treatment of the potentiometric data in order to solve a number of simultaneous equations to obtain overall conditional β_{iH} formation constants, which come from a coordination model of hydrogen ions to the organic matrix and permit calculation of conditional pK data. Conductimetric titrations with $Ba(OH)_2$ or NaOH give the total acidity. A typical result in $BaCl_2$ medium for a peat HA presents seven acid groups with the following pK data: $pK_1 = 3.80 \pm 0.3$, $pK_2 = 4.67 \pm 0.02$, $pK_3 = 7.57 \pm 0.01$, $pK_4 = 8.190 \pm 0.005$, $pK_5 = 8.80 \pm 0.01$, $pK_6 = 8.91 \pm 0.02$, and $pK_7 = 8.93 \pm 0.01$.

Keywords: Humic acid; pK; Potentiometry; Acidity; Conductimetry

INTRODUCTION

The humic substances (HS) constitute a general class of biogenic organic substances, polyelectrolytes with similar properties to biocolloids. The main fraction contains the so-called humic acid (HA) and the fulvic acid (FA). The HA are considered highly functionalized biopolymers, rich in carboxylic and phenolic groups, that are "anchored" in the organic matter of peat, soils, and sediments. They are amorphous compounds, fractal material, whose structures have been modeled [1,2]. The chemistry of HA and its complexes is still obscure. Many essential functions, including chelation of metal cations, adsorption of pesticides or other pollutants in soils, are very complex and not well understood [3–6].

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Methods for solving problems of quantification and speciation of the reactive acid groups are based on analyses of its acidic properties. They depend on important parameters such as pH and ionic strength. Stability constants for acidic sites can be obtained by electrochemical methods and mathematical models [7–9]. In fact, during neutralization the growing negative charge density causes a progressive decrease in the ionization constants of the remaining carboxylic and phenolic groups, as happens with polyprotic acids. Several quantitative methods of analysis of functional groups and mathematical models of bond of the proton in HA have been examined [10–13]. Analysis of acidic functional groups in HA using potentiometric methods has been discussed in the literature [10–16]. The normal procedure is neutralization of HA with strong base to study acid properties and this causes a continuous increase in ionic strength, solubility and concentration of exchangeable cations with the consequent change in activity coefficients.

A long-term recommendation is the use of a fixed concentration of a background electrolyte in equilibrium studies. The present work deals with identification of acid groups in typical HA samples in constant ionic strength medium. This condition favors an increase of solubility by ionization and neutralization of its acid groups with a simultaneous exchange of the hydrogen ions with metal cations of the background electrolyte, viz. Ba^{2+}, Ca^{2+}, K^+, and Na^+. In such a medium conditional ionization constants of acid groups can be determined assuming that the organic matrix behaves like a polyprotic acid. A coordination approach was herein adopted in which hydrogen ions are successively coordinated to the organic matrix X to form H_1X, H_2X , …, H_JX, stepwise complexes, to which overall β_{1H}, $\beta_{2H}, \ldots, \beta_{JH}$ formation constants can be determined from proper treatment of experimental data taken from potentiometric titration with bases. These β_{iH} data can then be converted to pK_i, a more conventional form.

It has been found that derivative potentiometric curves from titration of HA samples with $Ba(OH)_2$ can be an efficient alternative methodology for a better definition of the acidic sites in conditions of constant strength ionic, held with $BaCl_2$. One reason is that $Ba(OH)_2$ is the only known fairly soluble hydroxide of a divalent cations, followed by $Ca(OH)_2$ and $Sr(OH)_2$. This has special meaning, as a divalent cation is more likely to force ionization of acidic groups by improved ion exchange with carboxylate and phenolate anions in comparison with monovalent cations such as sodium. Being divalent the exchange with one hydrogen ion of the organic matrix creates mono positive sites like $R\text{-}COOBa^+$, which improves solubility in excess of electrolyte. On this basis, the potentiometric data better identify the number of acidic sites in HA and estimate its conditional pK data. Equilibrium constants were also determined in sodium chloride medium for comparison.

EXPERIMENTAL

Chemicals and Solutions

Chemicals with A.R. specification were used (Carlo Erba, Merck, Mallinkrodt). Solutions of NaCl or $BaCl_2 \cdot 2H_2O$ for adjusting ionic strength were prepared by weighing the solids without standardization.

A saturated solution of $Ba(OH)_2$ was filtered to remove excess solid, diluted to 0.150 M OH^- with CO_2 free distilled water standardized with potassium hydrogen phytalate and stored in plastic bottles under nitrogen. Dilution to 0.025–0.01 M in the presence of $BaCl_2$, as titrant, was performed in conditions to avoid contamination with CO_2.

Similar care to avoid CO_2 contamination with NaOH standard solutions was taken. Hydrochloric acid solutions were standardized potentiometrically with the weighed primary standard tris-hydroxy-methylaminomethane (TRIS).

The HA extraction and purification procedure from vermicompost, soil, and peat deposit was carried out according to a well-accepted method from the International Humic Substances Society (IHSS), based on extraction with NaOH precipitation in acidic medium, followed by purification steps which include dialysis and lyophilization.

Electrochemical Measurements

In all experiments, a thermostated electrochemical cell at $25 \pm 0.1°C$ was used with 25.0–30.0 mL working solutions. A nitrogen flow is maintained during the titrations. For pH readings an Analion V-620 glass electrode was used with characteristics of very low alkaline error. In order to virtually eliminate junction potentials, the reference Ag/AgCl, Cl^- electrode compartment was filled with 0.250 M $BaCl_2$ or 0.500 M NaCl, which have the same concentrations used in the working solutions.

The calibration of the glass electrode was performed with two separate titrations of 24.99 mL of the electrolytes with the acidic or basic titrants (about 0.01 M for OH^- or H^+) at constant ionic strength of 0.750 M for $BaCl_2$/HCl or $BaCl_2/Ba(OH)_2$ and 0.500 M for HCl/NaCl and HCl/NaOH. The conditional $E°$, of 0.3238 V presents a standard deviation of ±0.3 mV in $BaCl_2$ electrolyte. This causes an uncertainty of ±0.006 in $p[H^+]$, a conditional pH in constant ionic medium referred to $[H^+]$ rather than the activity a_{H^+}. The slope of the glass electrode from E *vs* $-\log[H^+]$ was found to be -0.06408 ± 0.0007 V.

Titrations of HA were performed in dispersions of 0.03–0.12 g of the HA samples in volumes of electrolytes in the range of 25–60 mL. All working solutions contained about 0.05% of Triton X-100 ($C_{34}H_{62}O_{11}$, from Rohm & Hass) to work as a very efficient nonionic dispersant of the HA and to provide stable potentiometric measurements.

Conductimetric titrations of HA dispersions with standard $Ba(OH)_2$ or NaOH for determination of the total acidic reserve, were accomplished by using a digital Micronal (mod. B-330) conductivimeter, with a cell constant of 0.700 cm^{-1}.

Computer programs in QuickBASIC 4.5 (Microsoft) were used to treat the experimental data with a least square fit of the electrode response in alkaline and acidic media, for conductimetric titration data and to perform diverse treatments of $E/p[H^+]$ data from the HA titrations. The ORIGIN 5.0 graphic system was also used extensively.

RESULTS AND DISCUSSION

Electrode Calibrations

Figure 1 shows the results from one calibration of the glass electrode in 0.250 M $BaCl_2$ (0.750 M ionic strength) to be used in the HA titrations with $p[H^+]$ and $p[OH^-]$, data referred to the ionic concentrations $[H^+]$ and $[OH^-]$ instead of its ionic activities.

The intersection of the straight lines is the neutrality point of water whose conditional ionic product of water, $pK'_w = 12.899 \pm 0.006$, referred to such ionic strength at 0.250 M $BaCl_2$. Similar calibration in 0.500 M NaCl leads to a $pK'_w = 13.712 \pm 0.006$, very close to the 13.708 found by Harned and Owen [17] by using the H_2/Pt electrode.

Derivative Potentiometric Curves

Several samples of HA of different origins were studied, each one with its own characteristics. Typical derivative curves as $\Delta p[H^+]/\Delta V_{OH^-}$ or $-\Delta E/\Delta V_{OH^-}$ *vs* V_{OH^-} were obtained from diverse HA samples. Figure 2 shows a typical plot from titration in a $BaCl_2/Ba(OH)_2$ medium of a vermicompost HA_1 (0.1089 g), which is considered a

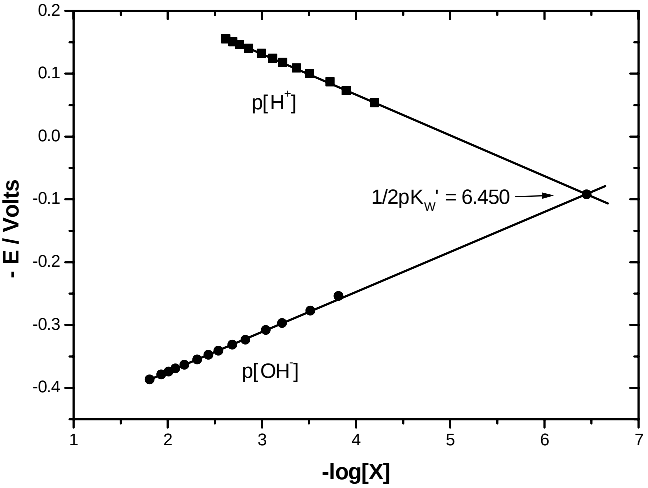

FIGURE 1 Acid–base calibration curve of the glass electrode in $BaCl_2$ medium. $[X] = [H^+]$ or $[OH^-]$.

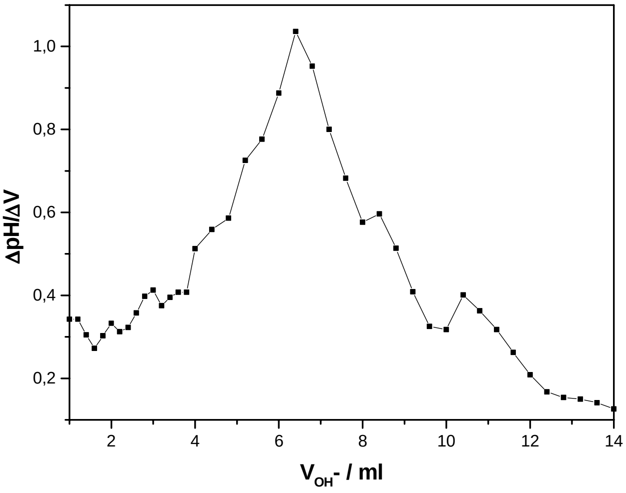

FIGURE 2 Derivative potentiometric curve of 0.1089 g HA_1, a vermicompost, in 24.99 mL of electrolyte, with 0.250 M $Ba(OH)_2/BaCl_2$ titrant, $[OH^-] = 0.04918$ M.

humic-like substance (44 experimental points). The number of peaks gives an idea of the number of acid groups titrated, although the computer treatment of the potentiometric data gives a more consistent number of such groups. The last peak indicates the total acidity reserve of the studied HA and this has been confirmed by linear plots taken from conductimetric titrations with standard solutions of $Ba(OH)_2$ and NaOH. The value of $4.692\,\mathrm{mol\,H^+\,kg^{-1}}$ was found for HA_1. The dispersion of the solid in 24.99 mL of electrolyte gives a calculated acidity of 0.02045 M which was considered in mass balances for equilibrium study during potentiometric titration with base. It is virtually soluble in the electrolyte, probably by forming hydrated $R\text{-}COOBa^+$ sites alternating with neutral $(R\text{-}COO)_2Ba$ and behaves as a homogeneous system instead of a suspension.

Figure 3 shows the derivative potentiometric curves in an NaCl/NaOH medium for a humic acid sample extracted from a native grass soil, HA_2. A similar curve was obtained in $BaCl_2/Ba(OH)_2$ medium. However the maximum derivative peak was three times higher than that in NaCl medium.

Figure 4 shows a typical plot of conductimetric titration evidencing two different acidity ranges. The second stoichiometric point gives the total acidity of the HA_2 sample and a value of $4.339\,\mathrm{mol\,H^+\,kg^{-1}}$. This value is consistent with those obtained from the potentiometric titration with NaOH or $Ba(OH)_2$.

Treatment of the Potentiometric Data for Equilibrium Constants

Computer programs were written to treat the available experimental data. The final treatment of potentiometric data from titration of HA samples at constant ionic strength leads to calculation of the well known Bjerrum's function data, $[Au]_H$, average ligand number [18,19] *vs* free ligand $[H^+]$, referred to the coordination of hydrogen ions to the organic matrix which behaves as a coordination center. Data of the Fronaeus function $F^O(L)$, "complexity" [18,19], can be calculated from the Bjerrum function

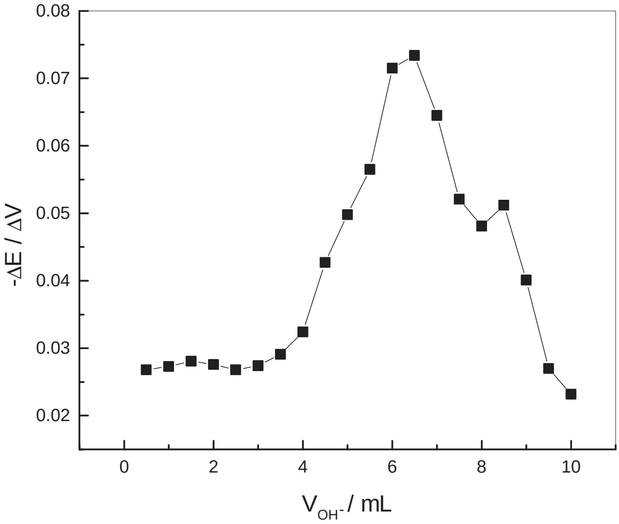

FIGURE 3 Derivative potentiometric curve by titration with NaOH 0.05150 M at constant strength (0.500 M, NaCl), of 0.1166 g HA_2 in 56.96 mL electrolyte.

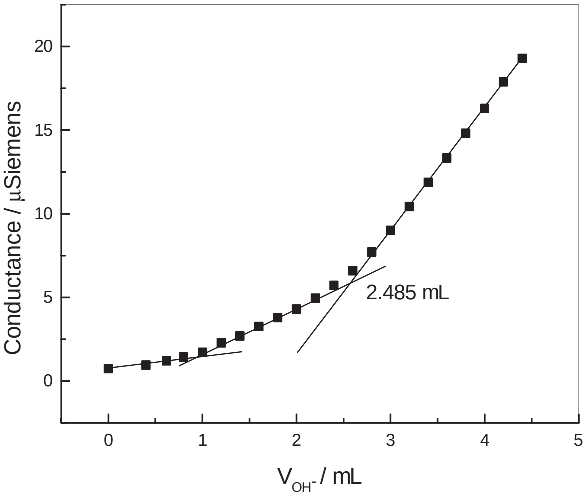

FIGURE 4 Conductimetric titration curve of 0.1166 g HA_2, of a soil, in 49.96 mL water. Titrant: NaOH 0.2036 M.

data, $[AU]_H$ *vs* $\log[H^+]$. It is a polynomium of J degree with J unknown coefficients which are the overall formation constants from 1 up to JH^+ ligands, as applied for an AH system:

$$F^O(H) = 1 + \beta_{1H}[H+] + \beta_{2H}[H+]^2 + \beta_{3H}[H+]^3 ......+\beta_{JH}[H+]^J$$

The titration with base, $C_{OH}-$ M of a mass of humic acid (0.03–0.1 g) dispersed in a volume of solution V_S (25–35 mL) in conditions of constant ionic strength leads to total acidity data C_H+ M. In the case of HA titration with several volumes in mL of the base, $V_{OH}-$, the derivative curves are an important parameter to define a number of JH^+ ligands joined to the organic matrix X, or at least to give an approximate number of acid groups, to be better defined during data treatment. Thus the concentration of the coordination center (free anion containing exchanged cations) is

$$C_X = C_{H^+}/J$$

During titration the potential E of the cell with the glass electrode is measured at each volume of titrant added and can be converted in $p[H^+]$ from a calibration curve. The free ligand $[H^+]$ is then calculated as well as the free $[OH^-]$, from K'_w. Dilution of the reactants during the titration requires corrections: F_1 is a correction factor used to correct C_{H^+} and C_X and F_2 for C_{OH^-} corrections:

$$F_1 = V_S/(V_S + V_{OH^-})$$

$$F_2 = V_{OH^-}/(V_S + V_{OH^-})$$

The hydrogen ion concentration bound to the organic matrix, $[H^+]_b$, is calculated from the following mass balance, with rigorous corrections for the measured free ligand and $[OH^-]$ released from water ionization or hydrolysis at higher $p[H^+]$ conditions:

$$[H^+]_b = F_1 C_{H^+} - F_2 C_{OH^-} + [OH^-] - [H^+]$$

The average ligand number $[\mathrm{Au}]_\mathrm{H}$ can be calculated for any point of the titration curve:

$$[\mathrm{Au}]_\mathrm{H} = [\mathrm{H}^+]_\mathrm{b}/F_1\mathrm{C_X}$$

The $[\mathrm{Au}]_\mathrm{H}$ *vs* free $[\mathrm{H}^+]$ calculated from the titration curve is a set of data that constitute the familiar formation curve, which enter the computer program for final treatment. Integration leads to $F^\mathrm{O}(\mathrm{H})$ data *vs* $\mathrm{p}[\mathrm{H}^+]$. Integration starts with the smallest $[\mathrm{Au}]_\mathrm{H}$ datum which is taken in the alkaline side of the titration curve, very close to the maximum stoichiometric point of total acidity. The first estimation of the coordination number J is then informed in order to obtain the Fronaeus function data $F^\mathrm{O}(\mathrm{H})$ after integration of $[\mathrm{Au}]_\mathrm{H}$ *vs* $\log[\mathrm{H}^+]$. In a second step the program solves by a least square fit a matrix with the weighted simultaneous equation with several $F^\mathrm{O}(\mathrm{H})_i$ polynomial data with $J\beta_{i\mathrm{H}}$ unknown parameters. Weighting factors W_i for the simultaneous equations put all $F^\mathrm{O}(\mathrm{H})_i$ data to the same magnitude,

$$W_i = F^\mathrm{O}(\mathrm{H})_\mathrm{max}/F^\mathrm{O}(\mathrm{H})_i$$

This calculation procedure was formerly described by Neves *et al.* [20] and has now been adapted to solve the equilibrium constants in HA systems. The weighted simultaneous equations for the least squares fit have the following general form, for j formation constants with $1,2,\ldots,J\mathrm{H}^+$ ligands:

$$\begin{aligned}
Y_1 &= \sum (F^\mathrm{O}(\mathrm{H})_\mathrm{max} - W_i)[\mathrm{H}^+]W_i = \beta_{1\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^2 + \cdots + \beta_{J\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^{J+1}\\
Y_2 &= \sum (F^\mathrm{O}(H)_\mathrm{max} - W_i)[\mathrm{H}^+]^2 W_i = \beta_{1\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^3 + \cdots + \beta_{J\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^{J+2}\\
&\vdots\\
Y_J &= \sum (F^\mathrm{O}(H)_\mathrm{max} - W_i)[\mathrm{H}^+]^j W_i = \beta_{1\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^{j+1} + \cdots + \beta_{J\mathrm{H}}\sum W_i^2[\mathrm{H}^+]^{2J}
\end{aligned}$$

In the computer program the initial estimate for coordination number j can be changed to a better one if an unrealistic set of equilibrium constants such as the appearance of negative parameters is found. This can happen especially in the case of titration of acid groups with very close pK data, a condition that favors overlapping of neutralization curves. A small potentiometric error can eventually present a false derivative peak in the titration curve. An estimation of $\mathrm{p}[\mathrm{H}^+]$ error can be introduced in the calculation in order to estimate the deviations of the parameters. A value of $\pm 0.006\,\mathrm{p}[\mathrm{H}^+]$ units is consistent with the standard deviation of ± 0.3 mV for the E^o, of the glass electrode (see Experimental).

Equilibrium Results

In order to check the programs and reliability of the experimental potentiometric data to obtain the protonation constants, citric acid was examined as the ionization constants of the three carboxylic groups present very close pK data [21]: $\mathrm{p}K_1 = 2.81$, $\mathrm{p}K_2 = 4.16$, and $\mathrm{p}K_3 = 5.32$, at 0.50 M ionic strength ($\mathrm{NaNO_3}$). Other $\mathrm{p}K_1$–$\mathrm{p}K_3$ data at the same ionic strength are, respectively, 2.78, 4.13, and 5.27 [22]. Present results from titration of 0.05000 M citric acid in 0.500 M NaCl with NaCl/NaOH titrant of same ionic strength gave comparable results: $\mathrm{p}K_1 = 2.56$, $\mathrm{p}K_2 = 4.15$, and $\mathrm{p}K_3 = 5.21$.

It is interesting to mention that an extended analysis of the titration data in the more alkaline region leads to find a 4th ionization attributed to the alcoholic group, a very weak acid: $pK_4 = 11.18$ for the alkoxy anion formation. A mixture of citric and phosphoric acids, simulating an HA system was also successfully analyzed for seven parameters, including the alkoxy anion formation.

Table I presents some of the calculated data for HA_2 in $BaCl_2$ medium to form the 4×4 matrix prepared with 25 weighted simultaneous equations [20] with $F^{O}(H)$ *vs* $[H^+]$ data. Four final simultaneous equations (four acid groups) were used:

$$\begin{aligned}
Y_1 &= 1.231 \times 10^{11} = 2.869 \times 10^{2}\beta_{1H} + 6.341 \times 10^{-5}\beta_{2H} \\
&\quad + 6.183 \times 10^{-10}\beta_{3H} + 4.007 \times 10^{-14}\beta_{4H} \\
Y_2 &= 2.619 \times 10^{5} = 6.341 \times 10^{-5}\beta_{1H} + 6.183 \times 10^{-10}\beta_{2H} \\
&\quad + 4.007 \times 10^{-14}\beta_{3H} + 8.122 \times 10^{-18}\beta_{4H} \\
Y_3 &= 2.919 \times 10 = 6.183 \times 10^{-10}\beta_{1H} + 4.007 \times 10^{-14}\beta_{2H} \\
&\quad + 8.122 \times 10^{-18}\beta_{3H} + 3.215 \times 10^{-21}\beta_{4H} \\
Y_4 &= 1.075 \times 10^{-2} = 4.007 \times 10^{-14}\beta_{1H} + 8.122 \times 10^{-18}\beta_{2H} \\
&\quad + 3.215 \times 10^{-21}\beta_{3H} + 1.810 \times 10^{-24}\beta_{4H}
\end{aligned}$$

Table II presents the four equilibrium data for this HA_2 and other humic acids. Figures 5 and 6 present typical distribution of the species of HA_1 (vermicompost, six acid groups) and HA_3 (humic acid from a peat deposit, seven species) as calculated with the formation constants.

TABLE I $F^{O}(H)/[H^+]$ data from HA_2 titration in $BaCl_2$ medium from computer integration to be used in weighted simultaneous equation [20]

$p[H^+]$	$[H^+]_b/M$	C_X/M	$[Au]_H$	$F^O(H)_I$	W_i
9.310	2.845×10^{-4}	2.068×10^{-3}	0.1375	1.159	2.569×10^{9}
9.053	5.024×10^{-4}	2.076×10^{-3}	0.2420	1.297	2.295×10^{9}
8.771	7.697×10^{-4}	2.083×10^{-3}	0.3695	1.582	1.948×10^{9}
8.521	1.075×10^{-3}	2.090×10^{-3}	0.5142	2.041	1.459×10^{9}
8.216	1.394×10^{-3}	2.098×10^{-3}	0.6645	3.087	9.644×10^{8}
7.850	1.725×10^{-3}	2.105×10^{-3}	0.8193	5.770	5.159×10^{8}
7.504	2.065×10^{-3}	2.113×10^{-3}	0.9774	11.80	2.523×10^{8}
7.094	2.410×10^{-3}	2.120×10^{-3}	1.137	32.03	9.294×10^{7}
6.558	2.759×10^{-3}	2.128×10^{-3}	1.297	143.8	2.070×10^{7}
5.877	3.110×10^{-3}	2.135×10^{-3}	1.456	1245	2.391×10^{6}
5.363	3.462×10^{-3}	2.143×10^{-3}	1.615	7669	3.882×10^{5}
5.062	3.815×10^{-3}	2.151×10^{-3}	1.774	2.482×10^{4}	1.199×10^{5}
4.784	4.168×10^{-3}	2.159×10^{-3}	1.931	8.125×10^{4}	3.664×10^{4}
4.493	4.515×10^{-3}	2.167×10^{-3}	2.084	3.119×10^{5}	9545
4.270	4.859×10^{-3}	2.175×10^{-3}	2.234	9.454×10^{5}	3149
4.130	5.207×10^{-3}	2.183×10^{-3}	2.386	1.991×10^{6}	1495
3.943	5.538×10^{-3}	2.191×10^{-3}	2.528	5.736×10^{6}	519.0
3.803	5.868×10^{-3}	2.199×10^{-3}	2.669	1.326×10^{7}	224.5
3.648	6.177×10^{-3}	2.207×10^{-3}	2.799	3.518×10^{7}	84.62
3.539	6.493×10^{-3}	2.215×10^{-3}	2.931	7.221×10^{7}	41.23
3.427	6.790×10^{-3}	2.223×10^{-3}	3.054	1.563×10^{8}	19.05
3.310	7.059×10^{-3}	2.232×10^{-3}	3.163	3.611×10^{8}	8.244
3.203	7.311×10^{-3}	2.240×10^{-3}	3.263	7.970×10^{8}	3.735
3.123	7.575×10^{-3}	2.249×10^{-3}	3.369	1.468×10^{9}	2.028
3.033	7.795×10^{-3}	2.257×10^{-3}	3.453	2.977×10^{9}	1.000

TABLE II Equilibrium data for this HA_1 and other humic acids

HA_1, Vermicompost in $Ba(OH)_2/BaCl_2$ $\mu = 0.750$ M		*HA_3 (peat deposit) in $Ba(OH)_2/BaCl_2$ $\mu = 0.750$ M*	
β_n	pK_n	β_n	pK_n
$\beta_1 = 1.599 \times 10^8$	$pK_6 = 8.20 \pm 0.01$	$\beta_1 = (8.547 \pm 0.3) \times 10^8$	$pK_7 = 8.93 \pm 0.01$
$\beta_2 = 1.589 \times 10^{16}$	$pK_5 = 8.00 \pm 0.07$	$\beta_2 = (6.893 \pm 0.5) \times 10^{17}$	$pK_6 = 8.91 \pm 0.02$
$\beta_3 = 1.873 \times 10^{22}$	$pK_4 = 6.07 \pm 0.02$	$\beta_3 = (4.335 \pm 0.4) \times 10^{26}$	$pK_5 = 8.80 \pm 0.01$
$\beta_4 = 1.335 \times 10^{27}$	$pK_3 = 4.85 \pm 0.07$	$\beta_4 = (6.711 \pm 0.7) \times 10^{34}$	$pK_4 = 8.19 \pm 0.005$
$\beta_5 = 1.300 \times 10^{31}$	$pK_2 = 3.99 \pm 0.006$	$\beta_5 = (2.513 \pm 0.4) \times 10^{42}$	$pK_3 = 7.57 \pm 0.009$
$\beta_6 = 9.934 \times 10^{34}$	$pK_1 = 3.88 \pm 0.06$	$\beta_6 = (1.174 \pm 0.2) \times 10^{47}$	$pK_2 = 4.67 \pm 0.02$
		$\beta_7 = (1.468 \pm 0.3) \times 10^{51}$	$pK_1 = 3.80 \pm 0.3$
HA_2 (soil) in $NaOH/NaCl$ $\mu = 0.500$ M		*HA_2 (soil) in $Ba(OH)_2/BaCl_2$ $\mu = 0.750$ M*	
β_n	pK_n	β_n	pK_n
$\beta_1 = (1.961 \pm 0.1) \times 10^9$	$pK_4 = 9.29 \pm 0.03$	$\beta_1 = (3.650 \pm 0.2) \times 10^8$	$pK_4 = 8.56 \pm 0.02$
$\beta_2 = (9.439 \pm 0.1) \times 10^{15}$	$pK_3 = 6.68 \pm 0.03$	$\beta_2 = (2.760 \pm 0.4) \times 10^{14}$	$pK_3 = 5.88 \pm 0.03$
$\beta_3 = (5.443 \pm 0.8) \times 10^{20}$	$pK_2 = 4.76 \pm 0.01$	$\beta_3 = (1.167 \pm 0.2) \times 10^{18}$	$pK_2 = 3.63 \pm 0.1$
$\beta_4 = (4.381 \pm 0.8) \times 10^{24}$	$pK_1 = 3.91 \pm 0.01$	$\beta_4 = (2.623 \pm 0.4) \times 10^{21}$	$pK_1 = 3.35 \pm 0.003$

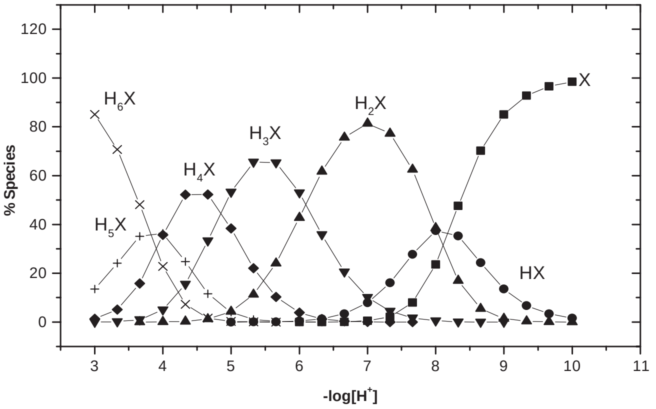

FIGURE 5 Distribution diagram of the protonated species in the vermicompost HA_1 sample, in $BaCl_2$ medium, calculated from the estimated β_{nH}, Table II.

Final Comments and Conclusions

These preliminary studies use derivative potentiometric curves in the presence of electrolyte to better identify acid groups in HA samples of diverse origin within a systematic study. Acid–base titrations in presence of Ba(II) cations has an enhancing effect in the ionization of acid groups with regard to sodium. In fact data for HA_2 in Table II show higher ionizations (smaller pK' data) in $BaCl_2$ medium in comparison with NaCl, but the same number of acid groups was found. The presence of any cation in excess forces ionization of the acid groups and promotes its solubility, specially when alkali is added. Thus the measured conditional equilibrium constants do not refer to a single ionization of a hydrogen ion but to an exchange with metal cations bound to an HA anion with a marked minimization of its effective charge. It should be added that a zwitterion structure in neutral HA exists and the protonated

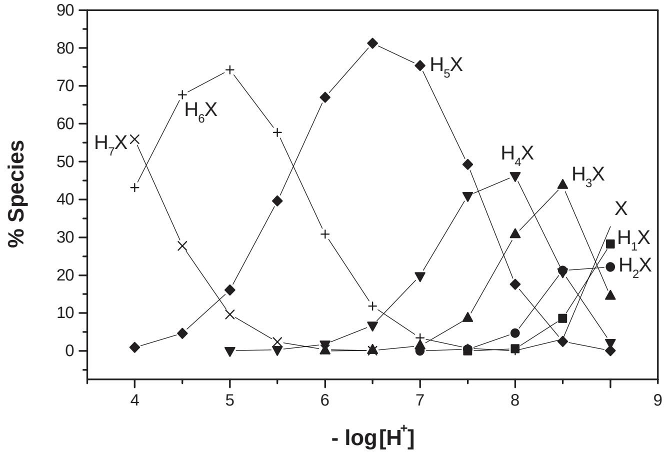

FIGURE 6 Distribution diagram of the protonated species in a humic acid HA_3 sample, from peat deposit, in $BaCl_2$ medium, Table II.

amine groups (3–4% nitrogen) behave like carboxylic or phenolic groups in the titration with bases, presenting its own p*K* of conjugated acids.

The matrix solution of properly weighted simultaneous equations of the Fronaeus function [20] looks to be a more rigorous approach and deserves special attention in future work. In fact it was possible to solve in HA_3 (Table II) three close p*K* data in the region of ionization of phenolic groups (K_i of 10^{-9} order).

This kind of titration studies requires careful setup of experimental conditions. Potentiometric measurements must be performed in conditions to minimize junction potential, to guarantee high glass electrode stability and use of adequate increases in volume of titrant. Another kind of a pH sensor in substitution of the glass electrode, as one with a silica/graphite matrix [23], must be studied for this kind of titrations due to its negligible alkaline error.

It is also important to mention that the presence of a neutral detergent, Triton X-100 (see Experimental), is essential for effective dispersion of HA in the working solutions and to obtain very stable potentiometric measurements. Studies in the presence of some water miscible solvents like dimethylsulfoxide with a marked solubilizing effect in HA substances, specially those highly polymerized humins should be interesting.

Even a normal potentiometric error can result in false potentiometric peaks in the derivative curve if small volume increments are applied during the titration. This can lead to false identification of an acid group, but the least squares fit [20] minimizes such effects during the matrix solution of weighted simultaneous equations. If the potentiometric uncertainty is ± 0.3 mV, a reasonable ΔV titrant increment (0.2–0.5 mL) should provide 10 times this value, i.e. 3 mV.

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References

[1] E.A. Ghabbour, A.H. Khairy, D.P. Cheney, V. Gross, G. Davies, T.R. Gilbert and X. Zhang, *J. Appl. Phycol.* **6**, 459 (1994).

[2] G. Davies, E.A. Ghabbour, S.A. Jansen and J. Varnum. In: P.N. Prasad, J.E. Mark and T.J. Fai (Eds.), Advanced New Materials and Emerging New Technologies (New York, 1995), p. 667.

[3] R.E. Regginal and R.V. Wandruszka, *Environ. Sci. and Technol.* **28**, 1934 (1994).

[4] C.T. Chiou, R.L. Malcom, T.I. Brinton and D.E. Kile, *Environ. Sci. and Technol.* **20**, 1934 (1986).

[5] R.L. Wershaw, Peat and Water (Amsterdam, Elsevier, 1986), p. 133.

[6] R.L. Wershaw, *Environ. Sci. and Technol.* **27**, 814 (1993).

[7] E.M. Perdue, Aquatic and Terrestrial Humic Materials (Ann Arbor Science, New York, 1983), p. 441.

[8] S.J. Marshall, S.D. Young and K. Gregson, *Eur. J. Soil Sci.* **46**, 471 (1995).

[9] J. Ephraim, S. Alegret, A. Mathuthu, M. Bicking, R.L. Malcolm and J.A. Marinsky, *Environ. Sci and Technol.* **20**, 354 (1996).

[10] M.M. Nederlof, *Environ. Sci and Technol.* **27**, 846 (1993).

[11] E. Tipping, M.A. Hurley, *Geochim. Cosmochim. Acta.* **56**, 3627 (1992).

[12] C.J. Milne, D.G. Kinniburg, J.C.M. De Wit, W.H. Van Riemsdijk and L.K. Koopal, *Geochim. Cosmochim. Acta.* **59**, 1101 (1995).

[13] R.H. Crist and J.R. Martin, *Environ. Sci. Technol.* **33**, 2252 (1999).

[14] J.C. Masini, G. Abale and E.C. Lima, *Anal. Chim. Acta.* **364**, 223, (1998).

[15] E.C. de Lima and J.C. Masini, *Quimica Nova.* **22**, 679 (1999).

[16] J.C.G.E. Silva, A.A.S.C. Machado, M.A. Ferreira and F. Rey, *Fresenius J. Anal. Chem.* **1–8**, 1402, (1998).

[17] H.S. Harned and B.B. Owen, The Physical Chemistry of Electrolyte Solutions (Reinhold Pub. Corp., New York, 1958).

[18] F.J.C. Rossotti and H. Rossotti, The Determination of Stability Constants (McGraw-Hill, New York, 1961).

[19] M.T.Beck, Chemistry of the Complex Equilibria (Van Nostrand, London, 1969).

[20] E.A. Neves, I. Gutz and R.Tavares, *J. Electroanal. Chem.* **179**, 91 (1984).

[21] M.L. Escoda, F.de la Torre and V. Salvadó, *Polyhedron* **18**, 3269 (1999).

[22] A.E. Martell, R.M. Smith and R.J. Motekaitis, NIST Critically Selected Stability Constants of Metal Complexes Database (Version 4.0, New York, 1997).

[23] M.F.Teixeira, L.A.Ramos, E.A. Neves and O. Fatibello-Filho, *Fresenius J. Anal. Chem.* **367**, 86 (2000).