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Proton-binding study of standard and reference fulvic acids, humic acids, and natural organic matter

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Abstract—The acid–base properties of 14 standard and reference materials from the International Humic Substances Society (IHSS) were investigated by potentiometric titration. Titrations were conducted in 0.1 M NaCl under a nitrogen atmosphere, averaging 30 min from start to finish. Concentrations of carboxyl groups and phenolic groups were estimated directly from titration curves. Titration data were also fit to a modified Henderson-Hasselbalch model for two classes of proton-binding sites to obtain "best fit" parameters that describe proton-binding curves for the samples. The model was chosen for its simplicity, its ease of implementation in computer spreadsheets, and its excellent ability to describe the shapes of the titration curves. The carboxyl contents of the IHSS samples are in the general order: terrestrial fulvic acids > aquatic fulvic acids > Suwannee River natural organic matter (NOM) > aquatic humic acids > terrestrial humic acids. Overall, fulvic acids and humic acids have similar phenolic contents; however, all of the aquatically derived samples have higher phenolic contents than the terrestrially derived samples. The acid–base properties of reference Suwannee River NOM are surprisingly similar to those of standard Suwannee River humic acid. Results from titrations in this study were compared with other published results from both direct and indirect titrations. Typically, carboxyl contents for the IHSS samples were in agreement with the results from both methods of titration. Phenolic contents for the IHSS samples were comparable to those determined by direct titrations, but were significantly less than estimates of phenolic content that were based on indirect titrations with $Ba(OH)_2$ and $Ca(OAc)_2$. The average phenolic-to-carboxylic ratio of the IHSS samples is approximately 1:4. Models that assume a 1:2 ratio of phenolic-to-carboxylic groups may overestimate the relative contribution of phenolic groups to the acid–base chemistry of humic substances. *Copyright © 2003 Elsevier Science Ltd*

1. INTRODUCTION

Since 1981, the International Humic Substances Society (IHSS) has collected, maintained, and distributed standard and reference samples of humic acids and fulvic acids to scientists throughout the world. Such a collection of standard and reference humic substances facilitates intercomparisons of laboratory measurements and the development of new methodologies for studying the physical and chemical properties of humic substances. Additionally, scientists throughout the world have subjected the IHSS samples to a variety of measurements and analyses. To date, however, there has been no systematic analysis or comparison of the acid–base properties of the entire collection of these standard and reference materials.

The objective of this study is to present and analyze the results of acid–base titrations of IHSS standard and reference fulvic and humic acids and the recently collected natural organic matter (NOM) from the Suwannee River. The 14 samples in this collection include six fulvic acids, seven humic acids, and one sample of NOM. Alternatively, the samples may be classified according to the source materials from which they were isolated. On this basis, there are six aquatic samples (isolated from rivers and lakes) and eight terrestrial samples (isolated from soil, peat, and leonardite). Primary emphasis is placed on comparison of the concentrations of carboxyl and phenolic groups in these samples. These results are also com-

pared with a large number of previously published measurements of carboxyl and phenolic contents.

The acid–base properties of humic substances are of intrinsic interest because these substances contribute to the acid–base balance of natural waters. In addition, the complexation of metal cations by humic substances is strongly dependent on pH (Randhawa and Broadbent, 1965; Van Dijk, 1971; Sposito et al., 1978; Sposito and Holtzclaw, 1979; Perdue and Lytle, 1983; Marinsky and Ephraim, 1986; Cabaniss and Shuman, 1988). Any successful thermodynamic model of metal complexation by humic substances must therefore also be able to model proton binding. Numerous models have been developed to describe metal-binding and proton-binding equilibria with humic substances and NOM. A few of the more common models are the Non-Ideal Competitive Adsorption (NICA) (Benedetti et al., 1995) and NICA-Donnan models (Koopal et al., 1994; Benedetti et al., 1996; Milne et al., 2001), Models V and VI (Tipping and Hurley, 1992; Tipping, 1998), and the competitive gaussian distribution model (Perdue and Parrish, 1987; Dobbs et al., 1989). The titrations in this paper were all conducted at the same temperature, ionic strength, and concentration of dissolved organic carbon, and any mathematical model that reproduces the titration curves can, in principle, be used to describe them. Given that the main objective of this study is not modeling per se, a very simple model has been used, primarily to obtain fitting parameters that can be used to generate titration curves of the IHSS samples using only a simple computer spreadsheet. The fitting parameters also pro-

vide, at least to some extent, a basis for comparing the acid–base properties of the samples.

2. BACKGROUND

The concentrations of carboxyl and phenolic functional groups are among the most widely measured and reported properties of humic substances. Methodologically, there are two basic approaches—indirect titrations and direct titrations. Indirect titrations have the advantage of simplicity—titration of a filtered reaction mixture to a fixed pH end point after a 24-h equilibration with either $Ba(OH)_2$ or $Ca(OAc)_2$ (Blom et al., 1957; Brooks and Sternhell, 1957; Schnitzer and Gupta, 1965). Some of the limitations of these indirect methods of analysis have been discussed previously (Dubach et al., 1964; Van Dijk, 1966; Holtzclaw and Sposito, 1979; Perdue et al., 1980; Perdue, 1985). Direct titrations provide much more detail about the thermodynamics of proton binding by humic substances, because pH is monitored continuously as increments of a titrant are added.

Simple proton-transfer reactions between Brönsted acids and bases in aqueous solution are extremely fast, approaching the diffusion-controlled limit (Bell, 1973). In the titrations of humic substances, however, it is usually evident that pH tends to drift downward after additions of base titrant, suggesting that slow reactions of some type are generating additional acidity. This hypothesis is supported by frequent reports of significant hysteresis in forward-reverse titrations of humic substances (Davis and Mott, 1981; Varney et al., 1983; Paxeus and Wedborg, 1985; Bowles et al., 1989; Antweiler, 1991; Leenheer et al., 1995; Marshall et al., 1995; Santos et al., 1999). Some investigators report no hysteresis (Borggaard, 1974; Plechanov et al., 1983). It has been reported that repeated titration of a humic substance to high and low pH gradually eliminates hysteretic effects (Marinsky et al., 1982; Paxeus and Wedborg, 1985; Marshall et al., 1995). If side reactions do occur and generate additional acidity, they are not apparent in indirect methods in which pH is not monitored during the equilibration time. There seems to be no consensus regarding the cause of observed pH drift and hysteresis (Antweiler, 1991; Santos et al., 1999), nor is there an established protocol for direct titrations of humic substances.

The lack of distinct inflection points in the titration curves of humic substances and the overlap of wide ranges of pK_a values of acidic functional groups make it impossible to quantify unambiguously the separate contributions of carboxyl and phenolic groups to the total acidity of a humic substance (Dubach et al., 1964; Reuter, 1980; Perdue et al., 1980; Perdue et al., 1984; Bowles et al., 1989). It has been common practice to rely upon some simple, empirical rules to estimate the concentrations of carboxyl and phenolic groups. For example, it is often assumed that all carboxyl groups (and no phenolic groups) are titrated by pH 7.0 (Reuter, 1980; Cabaniss, 1991; Santos et al., 1999) or pH 8.0 (Bowles et al., 1989; Perdue, 1990; Cabaniss, 1991; Santos et al., 1999), and that one-half of the phenolic groups are titrated between pH 8.0 and pH 10.0 (Thurman, 1985; Bowles et al., 1989). Alternatively, numerical models of the acid–base chemistry of humic substances can be fit to titration data, and the carboxyl and phenolic contents are obtained as model fitting parameters. In two recent publications,

Model VI (Tipping, 1998) and the NICA-Donnan model (Milne et al., 2001) were fit to relatively large numbers of titration data sets (19 and 48, respectively). Milne et al. (2001) were able to fit the NICA-Donnan model to 18 of Tipping's data sets; however, the phenolic content had to be fixed manually for six of Tipping's data sets and for four additional data sets. Nonetheless, the strong dependence of model-based estimates of carboxyl and phenolic content on the model used to fit the data is nicely illustrated by a comparison of the results from the two models. The NICA-Donnan model yielded a phenolic-to-carboxyl ratio of 32:68 for the fulvic acids and a ratio of 46:54 for the humic acids. Tipping assumed a phenolic-to-carboxyl ratio of 33:67 for both fulvic and humic acids in Model VI. The Model VI carboxyl contents are positively correlated with the NICA-Donnan carboxyl contents, with an r^2 of 0.90. The regression slope and intercept are 0.60 and 1.5, respectively. In contrast, the Model VI phenolic contents are negatively correlated with the NICA-Donnan phenolic contents, with an r^2 of only 0.44. The regression slope and intercept are −0.32 and 2.7, respectively. It is clearly impossible to determine which set of model estimates, if either, is correct. It is sufficient to note that they are different. This is the nature of modeling.

In this study, forward titrations for all 14 IHSS samples were conducted rapidly and under the same experimental conditions. An empirical pH-based method was used to obtain estimates of carboxyl (charge density at pH 8.0) and phenolic groups (2 times the change in charge density between pH 8.0 and 10.0). These model-independent estimates provide the basis for much of the analysis and comparisons that follow. Titration data were also fit to a modified Henderson-Hasselbalch model for two classes of proton-binding sites to obtain "best fit" parameters that describe proton-binding curves for each humic and fulvic acid. The model was chosen for its simplicity, its ease of implementation in computer spreadsheets, and its excellent ability to describe the shapes of the titration curves.

3. EXPERIMENTAL

3.1. Preparation and Titration of IHSS Samples

A 50-mL stock solution of each IHSS sample was prepared. Each stock solution contained 0.36 ± 0.01 g L^{-1} (on a dry ash-free basis) of an IHSS sample in 0.1 M NaCl, which was used as a background electrolyte. The stock solutions of soil, peat, leonardite, and Summit Hill humic acids also contained 0.002 M NaOH, which was needed to effectively disperse/dissolve those samples. Next, a 10.00-mL aliquot of each stock solution was transferred into a 25-mL Teflon vessel. Sufficient HCl was added to the soil, peat, leonardite, and Summit Hill humic acids to exactly neutralize the initially added 0.002 M NaOH. This step caused the initial volumes of those samples to increase by slightly less than 2%.

The Teflon vessel was then submerged in a water bath whose temperature was maintained at 25.00 ± 0.02 Celsius. An Orion combination pH electrode that was previously calibrated with pH 4.00, 7.00, and 10.00 standard buffers was used to monitor pH. A Gilmont 2-mL microburette filled with carbonate-free 0.100 M NaOH was inserted into the vessel. The sample was continually stirred under a nitrogen atmosphere. The initial pH of the solution was recorded.

The NaOH titrant was added to the 10-mL aliquot of sample in increments of 6 to 8 μL, estimated to the nearest 0.1 μL. Both the added volume of titrant and the stable pH (stable for 7 s with drift of no more than $\pm$ 0.001 pH units) were recorded after each addition of titrant. Each 10-mL aliquot of sample was titrated from its initial pH of 3.0 to 3.3 to a maximum pH of 10.5 to 10.7 in 25 to 35 min. The measured pH was assumed to be the direct reading of hydrogen ion

activity in bulk solution. Electrode junction potentials, though perhaps important, were not considered. Three replicate titrations were performed on each humic and fulvic acid sample. A fourth replicate titration was performed if there was unusual behavior of pH or a malfunction of the equipment.

3.2. Calculations

Although the ionic strength was expected to remain near 0.1 due to the presence of 0.1 M NaCl background electrolyte, the ionic strength of the solution was calculated at each point in the titration. The contribution of ionized functional groups of an IHSS sample to ionic strength was approximated by assuming that the IHSS sample is a mixture of monoprotic acids. It follows that the organic charge at any point in a titration is given by Eqn. 1 (the electroneutrality equation)

$$\sum_i [Org_i^-] = [Na^+] + [H^+] - [Cl^-] - [OH^-] \quad (1)$$

where $[Na^+]$ and $[Cl^-]$ are dilution-corrected concentrations of added NaOH and HCl, and $[Org_i^-]$ is the dilution-corrected concentration of the ith organic anion in the sample. The corresponding ionic strength is given by Eqn. 2.

$$I = \frac{1}{2}\left([Na^+] + [Cl^-] + [H^+] + [OH^-] + \sum_i[Org_i^-]\right) \quad (2)$$

Combining these equations, the ionic strength is approximately given by

$$I = [Na^+] + [H^+] \quad (3)$$

The dilution-corrected $[Na^+]$ included initial and added Na^+. The $[H^+]$ was calculated from pH, using the Davies equation with $B = 0.30$ (Davies, 1962) to calculate the activity coefficient for hydrogen ion. This sequence of calculations was repeated iteratively until the change in $[H^+]$ was less than 1%. As might be expected, ionic strength never differed significantly from 0.1. The calculated organic charge from Eqn. 1 was normalized to the dilution-corrected concentration of dissolved organic carbon (DOC) to obtain Q_{TOT} in units of meq gC^{-1}.

Once the pH-dependence of Q_{TOT} was known, carboxyl content was estimated as the value of Q_{TOT} at pH 8, and phenolic content was estimated as two times the change in Q_{TOT} between pH 8 and pH 10.

3.3. Modeling

The titration curves of humic substances occasionally exhibit unexplained curvature at the low- and high-pH ends of a titration (Sposito et al., 1977; Marshall et al., 1995; Robertson and Leckie, 1999; Milne et al., 2001). The pH-based estimates of carboxyl and phenolic content that were described earlier in this article are largely unaffected by this phenomenon. In contrast, model-derived estimates of these parameters can be seriously biased by inclusion of these unusual data points. In this study, the individual data sets for the titration curves for each sample were plotted as Q_{TOT} vs. pH. Next, any potentially erroneous data at the low- and high-pH ends of each titration curve were temporarily deleted, and the individual data sets were combined to generate a single aggregated data set per sample. A modified Henderson-Hasselbalch model (Katchalsky and Spitnik, 1947) that describes proton binding by two classes of binding sites was fit to the aggregated data sets, and a single set of fitting parameters was obtained for each IHSS sample.

$$Q_{TOT} = \left(\frac{Q_1}{1 + (K_1[H^+])^{1/n_1}}\right) + \left(\frac{Q_2}{1 + (K_2[H^+])^{1/n_2}}\right) \quad (4)$$

For consistency with the conventional interpretation of the acid–base properties of humic substances, it is assumed in this study that the two classes of acidic functional groups in this model are carboxyl and phenolic groups, and their concentrations (meq gC^{-1}) are represented by the fitting parameters Q_1 and Q_2, respectively. The log K_1 and log K_2 are fitting parameters that represent mean log K values for proton binding by the two classes of binding sites. The parameters n_1 and n_2 are empirical constants ($n \geq 1$) that reflect the range of log K values within each distribution of proton-binding sites. The optimum set of

fitting parameters for each IHSS sample was obtained using nonlinear regression to minimize a weighted sum of squares of residuals, for which the weighting factor is the reciprocal of the experimental Q_{TOT} (Perdue and Parrish, 1987; Perdue et al., 1984). The Fletcher-Powell minimization algorithm was used (Fletcher and Powell, 1963). The fitting parameters of this model can be used to calculate titration curves that very closely approximate the actual titration curves of the IHSS samples. Note that Eqn. 4 is evaluated using the concentration of hydrogen ion, even though all titration curves shown subsequently in this paper use pH as the independent variable.

4. RESULTS AND DISCUSSION

4.1. Raw Titration Results

Titrations of all IHSS samples were conducted from an initial pH of 3.0 to 3.3 to a maximum pH of 10.5 to 10.7. Below pH 5, chemical equilibrium was established rapidly in all titrations, i.e., the drift in pH was no more than $\pm$ 0.001 pH unit in 7 s. Above pH 5, the addition of an aliquot of NaOH titrant resulted in an initially rapid increase in pH, followed by a slow downward drift in pH. This phenomenon was especially evident in the relatively unbuffered pH range of 6.5 to 9.7. In this range, the typical decrease in pH was 0.03 to 0.08 pH unit, being greatest around pH 8.5. For the concentrations of IHSS samples used in these titrations, this decrease in pH is equivalent to less than 1% of the observed organic anion concentration within this pH range (Perdue, 1990). At pH 8.5, a stable pH was achieved in 2 to 4 min, depending on the IHSS sample being titrated. Even though this phenomenon is quantitatively insignificant on the time scale of these experiments (Santos et al., 1999), it may be indicative of pH-related changes in the IHSS samples other than simple dissociation of acidic functional groups (Antweiler, 1991).

4.2. Titration Curves

The pH-based estimates of carboxyl and phenolic content and the optimized fitting parameters of the modified Henderson-Hasselbalch model for each IHSS sample are given in Table 1. All experimental titration data and the model-generated titration curves are shown in Figure 1 for the standard Suwannee River fulvic acid (1S101F) and for the reference Summit Hill soil humic acid (1R106H). The experimental data in Figure 1 include the potentially erroneous data at low and high pH, which were omitted when fitting the modified Henderson-Hasselbalch model to the data. The excellent agreement between the model and the data is typical for all data sets, so only the model results will be shown in subsequent plots. The model-generated titration curves for all 14 IHSS samples are shown in Figure 2. This plot bears a striking resemblance to Figure 3 in Milne et al. (2001), which contains titration curves for 11 fulvic acids and 15 humic acids at ionic strengths ranging from 0.001 to 1. Those authors pointed out that fulvic acids had generally greater negative charge and a smaller degree of in-group variability than humic acids. The results in Figure 2 of this paper also indicate that fulvic acids have greater charge densities across the entire pH range of the titrations; however, the in-group variability among the fulvic acids is at least as great as among the humic acids. Furthermore, it is evident in Figure 2 that charge densities of the IHSS samples across the pH range of the titrations are in the general

Table 1. pH-based and model-based estimates of the carboxyl and phenolic contents of the IHSS samples.

Sample Description	IHSS Code	%C	Q_1'	Q_2'	Modified Henderson-Hasselbalch Model						
					Q_1	$\log K_1$	n_1	Q_2	$\log K_2$	n_2	r^2
Std. Suwannee FA	1S101F	52.44	11.44	2.91	12.00	3.80	3.21	1.48	9.52	1.00	0.9992
Std. Soil FA	2S102F	50.12	13.24	2.27	14.12	3.67	3.62	0.74	9.53	1.00	0.9970
Std. Peat FA	1S103F	50.45	13.34	2.32	14.22	3.99	3.33	0.76	9.57	1.00	0.9989
Ref. Suwannee FA	1R101F	53.04	12.23	3.11	12.94	3.81	3.36	1.60	9.62	1.00	0.9991
Ref. Peat FA	1R103F	52.12	15.24	1.78	16.25	3.73	3.54	1.31	10.74	1.00	0.9995
Ref. Nordic FA	1R105F	52.31	11.16	3.18	12.15	3.79	3.95	1.49	9.67	1.00	0.9995
Ref. Suwannee NOM	1R101N	52.47	9.85	3.94	10.57	3.94	3.60	2.61	9.74	1.19	0.9994
Std. Suwannee HA	1S101H	52.55	9.59	4.24	10.69	4.42	3.79	2.28	9.68	1.11	0.9996
Std. Soil HA	1S102H	58.13	8.28	1.87	8.90	4.36	3.16	0.85	9.80	1.00	0.9984
Std. Peat HA	1S103H	56.37	9.01	1.91	9.64	4.22	3.20	0.94	9.86	1.00	0.9993
Std. Leonardite HA	1S104H	63.97	7.46	2.31	8.17	4.59	3.32	1.13	9.72	1.31	0.9996
Ref. Peat HA	1R103H	56.84	8.87	2.05	9.54	4.26	3.25	1.01	9.85	1.00	0.9995
Ref. Nordic HA	1R105H	53.33	9.06	3.23	10.32	4.32	4.22	1.64	9.89	1.11	0.9986
Ref. Summit Hill HA	1R106H	54.00	7.14	2.42	7.53	4.47	3.12	1.31	9.23	1.57	0.9958

Std. = standard sample; Ref. = reference sample; FA = fulvic acid; HA = humic acid; NOM = natural organic matter; %C = mass percent of carbon in a sample, on a dry, ash-free basis; Q_1' and Q_2' = pH-based estimates of carboxyl and phenolic content; Q_1 and Q_2 = model-based estimates; log K's and n's = additional model parameters (see text); r^2 = coefficient of regression of predicted versus observed charge density.

order: terrestrial fulvic acids > aquatic fulvic acids > Suwannee NOM > aquatic humic acids > terrestrial humic acids.

4.3. Concentrations of Acidic Functional Groups

As stated previously, the two classes of proton-binding sites in the IHSS samples are assumed to be carboxyl and phenolic groups. All pH-dependent methods of estimating the concentrations of carboxyl and phenolic functional groups are inher-

ently operational, and the results vary from method to method (Perdue et al., 1980). Table 1 contains both the pH-based estimates (Q_1' and Q_2') and the model-based estimates (Q_1 and Q_2) of carboxyl and phenolic content. A comparison of the values obtained by these two methods reveals several consistent trends. First, there is a very strong linear correlation between Q_1' and Q_1 values ($r^2 = 0.993$), and Q_1' values are always slightly less than Q_1 values ($Q_1'/Q_1 = 0.93 \pm 0.02$).
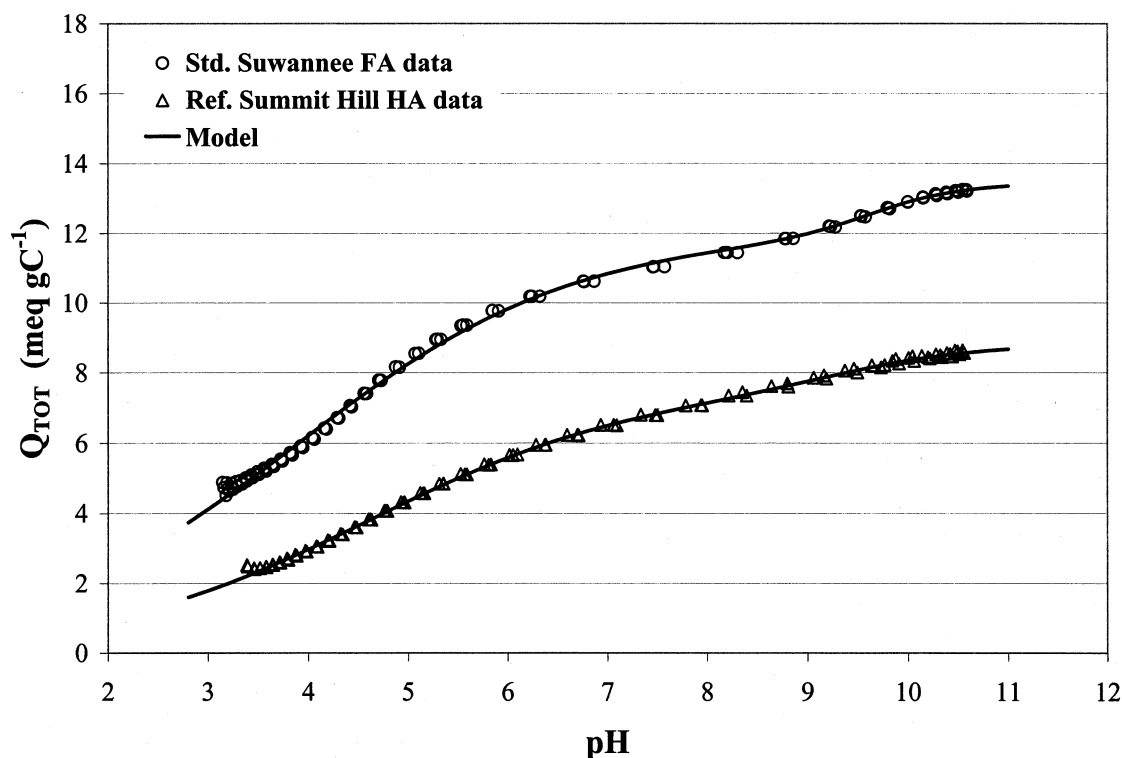

Fig. 1. Titration data and "best fit" model curves for standard Suwannee fulvic acid and reference Summit Hill soil humic acid.
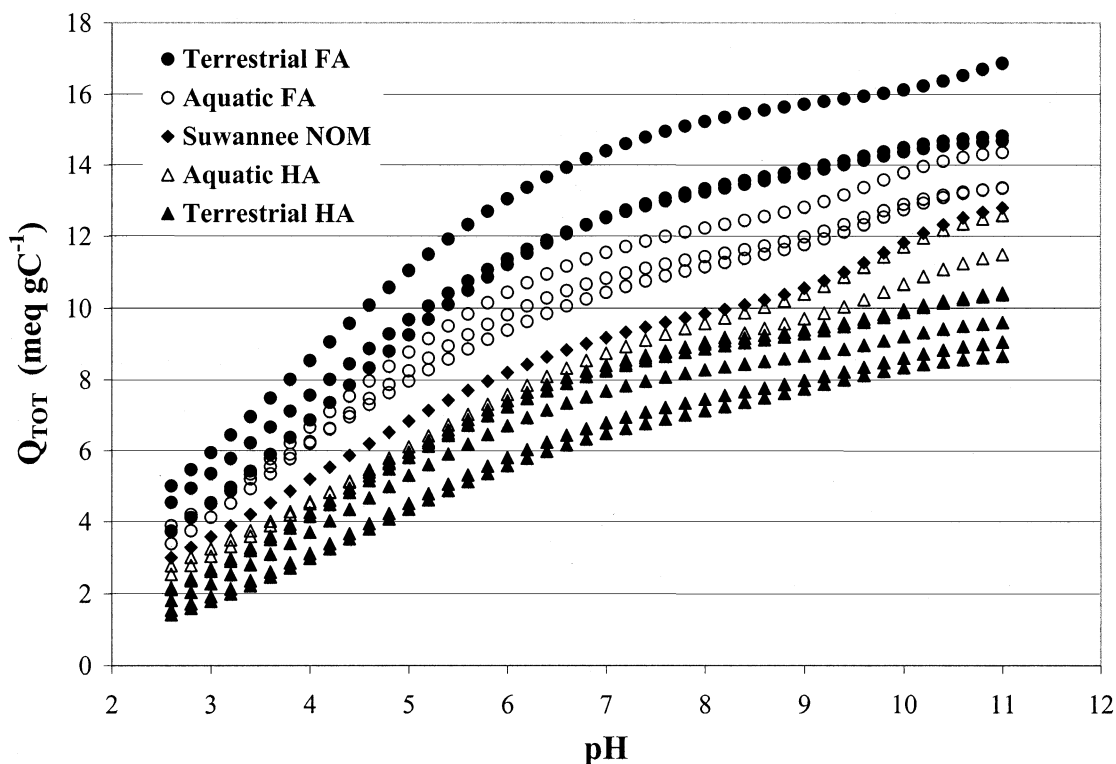
Fig. 2. Titration curves for 14 IHSS samples.

There is a weaker linear correlation between Q_2' and Q_2 values ($r^2 = 0.794$), and Q_2' values are always substantially greater than Q_2 values ($Q_2'/Q_2 = 2.1 \pm 0.5$). The corresponding total acidities ($Q_{TOT}' = Q_1' + Q_2'$; $Q_{TOT} = Q_1 + Q_2$) are linearly correlated ($r^2 = 0.980$), and the two estimates of total acidity are quite similar ($Q_{TOT}'/Q_{TOT} = 1.04 \pm 0.03$). Given the highly linear correlations that exist between results of the two methods, an analysis of either set of concentrations of carboxyl and phenolic functional groups of the IHSS samples will lead to the same general conclusions. Given that the pH-based estimation method is much more prevalent in the literature than is the model-based method used in this study, the Q_1' and Q_2' values of the IHSS samples will be used for further analysis.

Overall, carboxyl contents range from 7.1 to 15.2 meq gC^{-1}, thus accounting for ~9% to 18% of the carbon in the IHSS samples. The latter value is surprisingly high, because it indicates that almost one in five carbons of the reference peat fulvic acid must be a carboxyl group. The fulvic acids contain 12.8 ± 1.5 meq gC^{-1} of carboxyl groups. In contrast, the humic acids contain 8.5 ± 0.9 meq gC^{-1} of carboxyl groups. The corresponding ratio ($8.5/12.8 = 0.66$) is similar to other recently reported ratios of 0.59 and 0.69 (Milne et al., 2001 and Tipping, 1998, respectively), which were estimated from fitting numerical models to a wide variety of data sets. These relative differences in carboxyl content are reflected directly in the titration curves shown in Figure 2, because most of the acidity in the IHSS samples is attributable to carboxyl groups.

Overall, phenolic contents range from 1.8 to 4.2 meq gC^{-1}. The phenolic contents of fulvic acids (2.6 ± 0.6 meq gC^{-1}) and humic acids (2.6 ± 0.9 meq gC^{-1}) are not statistically different. Samples of aquatic origin, however, have significantly

greater phenolic contents (3.4 ± 0.5 meq gC^{-1}) than their terrestrial counterparts (2.1 ± 0.2 meq gC^{-1}). The phenolic content of Suwannee River NOM (3.9 meq gC^{-1}) is similar to that of aquatic humic acids and greater than that of aquatic fulvic acids.

Carboxyl groups constitute 78% to 90% of the total acidity for fulvic acids and 69% to 82% for humic acids. Phenolic groups are much less abundant. In fact, the average ratio of phenolic-to-carboxyl groups for the IHSS samples is only ~21: 79. The rapid titration protocol presumably minimizes the contribution of acid-generating side reactions (which are especially noticeable in the alkaline pH range) to the apparent phenolic content. The results reported here for the IHSS samples also depend directly, of course, on the assumption that all carboxyl groups (and no phenolic groups) are titrated at pH 8, and that half of the phenolic groups are titrated between pH 8 and pH 10.

4.4. Other Estimates of Carboxyl and Phenolic Content

The scientific literature contains many reports of the concentrations of carboxyl and phenolic groups in humic substances and NOM. It is desirable to examine those results to obtain a broader statistical basis for comparing the carboxyl and phenolic contents of the IHSS samples with other estimates from the literature. Results vary with the nature of the sample, the method of analysis, and the dimensional units in which the results are reported. In most studies, the concentrations of acidic functional groups have been normalized to the mass of dry, ash-free organic matter rather than to the mass of organic
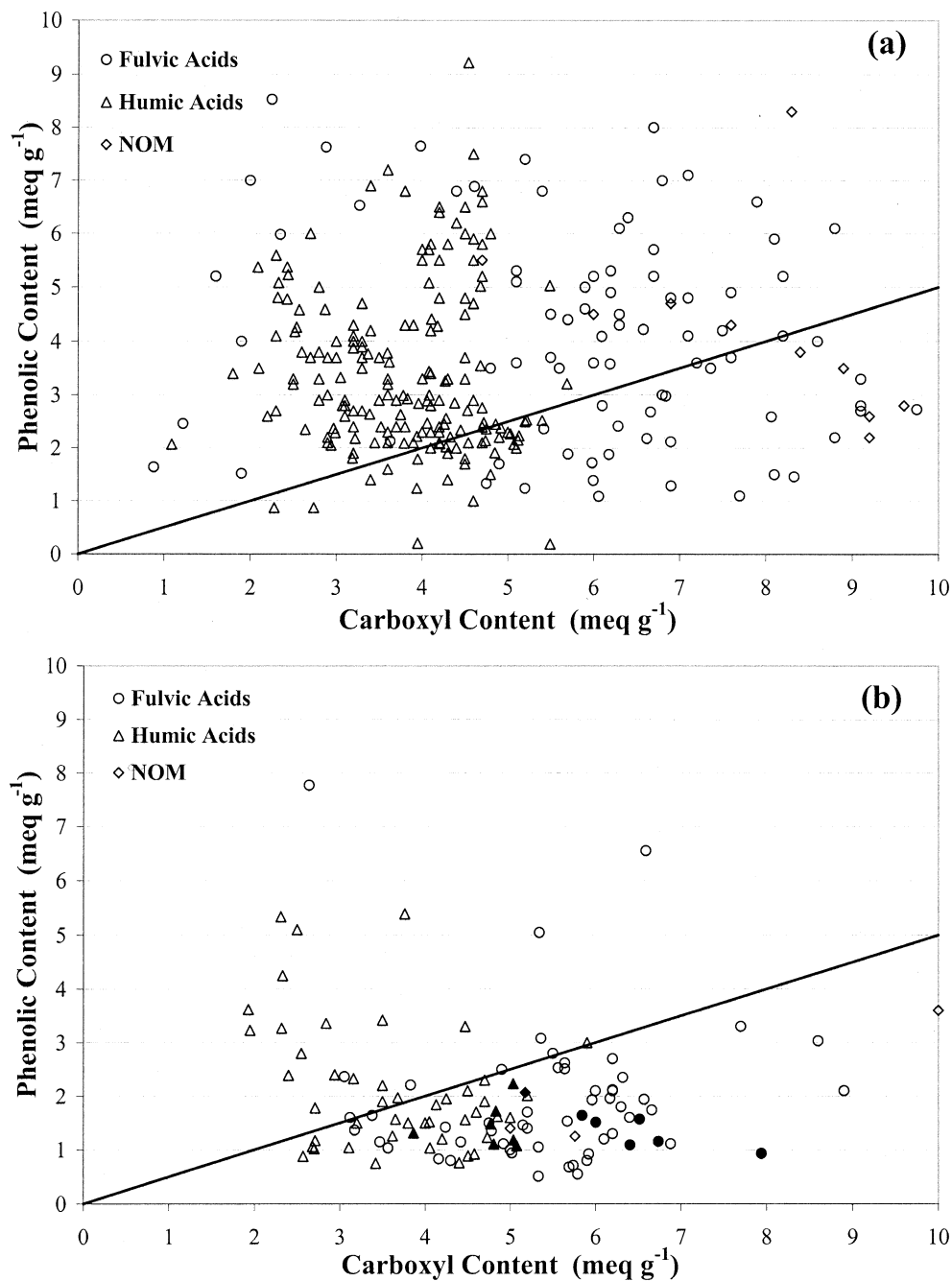
Fig. 3. (a) Carboxyl and phenolic contents for 83 fulvic acids, 189 humic acids, and 12 freshwater natural organic matter samples from the literature as determined by indirect titrations with $Ba(OH)_2$ and $Ca(OAc)_2$. (b) Carboxyl and phenolic contents for 53 fulvic acids, 47 humic acids, and 3 freshwater natural organic matter samples from the literature (open symbols), and the 14 IHSS samples (solid symbols), as determined by direct titrations. Literature data for direct titrations include 18 fulvic acids and 20 humic acid data points from Milne et al. (2001). The line in both plots is the 1:2 assumed ratio of phenolic-to-carboxyl groups in Model V and Model VI (Tipping and Hurley, 1992; Tipping, 1998). All concentrations are in units of meq g^{-1}.

carbon (e.g., in units of meq g^{-1}). The %C parameter in Table 1 of this paper is the mass percent of carbon in each sample (on a dry, ash-free basis). These values can be used to convert the Q_1' and Q_2' (and Q_1 and Q_2) values in Table 1 from meq gC^{-1} to meq g^{-1}.

The majority of published estimates of the concentrations of

carboxyl and phenolic groups have been obtained using indirect titrations. Those results are used in this study as originally reported. A smaller number of titration data sets were available, from which estimates of carboxyl and phenolic contents have been or can be derived using a variety of pH-based and model-based methods. In selecting titration data sets for inclusion in

this study and for extracting carboxyl and phenolic contents from those data sets, the following protocol has been used:

1. Only titration data for terrestrial (sediment, peat, soil) and freshwater (lakes, streams, groundwater, swamps, bogs) samples were considered.
2. Only titrations that were conducted in the ionic strength range of 0.01 to 1.0 were considered. If a paper contained titrations at more than one ionic strength, then only the titration data for the ionic strength nearest to 0.1 were used.
3. If models were used to estimate carboxyl and phenolic contents, then only results obtained from models that estimate these parameters independently were used. In particular, results obtained from Models V and VI, which force the phenolic content to be one-half of the carboxyl content, were not included.
4. When multisite models (more than two binding sites) were used, all binding sites with $pK_a < 8$ were considered to be carboxyl groups, and the remaining sites were considered to be phenolic groups.
5. When a model included only one binding site, that site was assumed to be carboxyl groups, and phenolic content was assumed to be zero.
6. If carboxyl and phenolic contents were estimated by an arbitrary pH cutoff or by analysis of the second derivative of the titration curve, those values were used directly.
7. Only proton-binding data for the whole NOM and humic and fulvic acids were used. No data for fractionated humic classes were used.
8. If the same sample was used in more than one study, the results of each study were used, in compliance with the preceding criteria.

The results of 387 published estimates of carboxyl and phenolic contents, together with the 14 results from this study (converted to meq g^{-1}), are plotted in Figure 3. Figure 3a includes 284 estimates from the literature that were obtained using indirect titrations ($Ca(OAc)_2$ and $Ba(OH)_2$ methods). Figure 3b includes 103 estimates from the literature that were obtained from direct titrations and the 14 results from this study. Included in Figure 3b are 38 results of Milne et al. (2001) that were obtained using the NICA-Donnan model to fit titration data sets without constraining the phenolic contents. Both Figures 3a and 3b include a solid line with a slope of 1:2, which is the ratio of phenolic-to-carboxyl groups used in Model V (Tipping and Hurley, 1992) and Model VI (Tipping, 1998). If Figures 3a and 3b are compared, it is seen that indirect and direct methods yield a similar range of carboxyl content. In contrast, indirect methods yield a much wider range of phenolic content than do direct methods. Indirect titrations using the $Ca(OAc)_2$ and $Ba(OH)_2$ methods yield much higher estimates of phenolic content than do direct titrations such as those reported in this study. It is very likely that acid-generating side reactions occur when total acidity is measured after a 24-h exposure of a humic substance to extremely alkaline conditions.

Most of the results from indirect titrations lie above the 1:2 line, and most results from direct titrations lie below this line. Omitting the 38 results of Milne et al. (2001), only 6% of the results from direct titrations lie above the 1:2 line. About 53% of the results of Milne et al. (2001) plot above the 1:2 line.

If, for any reason, indirect estimates of phenolic content are too high, then Models V and VI overestimate the importance of phenolic groups. If so, then the models presumably compensate internally by giving phenolic groups a distribution of pK_a values that predicts substantial dissociation of phenolic groups in the pH range where carboxyl groups are more likely to dissociate. If a portion of the carboxyl content is thus defined as phenolic content, then these models may tend to underestimate carboxyl content. This possibility is supported by the regression slope of 0.60 between the carboxyl contents that were predicted by Model VI and the NICA-Donnan model when applied to the same data sets (see earlier discussion).

Table 2 provides a statistical summary of the data in Figure 3 and a few additional observations of carboxyl content (without a corresponding phenolic content), expressed in terms of mean values and standard deviations. The data are grouped according to the type of sample (fulvic acid, humic acid, or NOM) and the general method of analysis (direct titrations or the $Ba(OH)_2/Ca(OAc)_2$ methods). The average results of this study (converted to meq g^{-1}) are included for comparative purposes. In addition, the average results obtained by fitting the NICA-Donnan model (Milne et al., 2001), Model VI (Tipping, 1998), and the Stockholm humic model (Gustafsson, 2001) to 38, 19, and 18 titration data sets, respectively, are provided. The most obvious trend in Table 2 is the very large phenolic contents that are obtained using the $Ba(OH)_2/Ca(OAc)_2$ methods. This trend is evident for all three types of samples. It seems highly probable that these values are too high. In contrast, carboxyl contents that are obtained using the $Ca(OAc)_2$ method are clearly within the range of the estimates that are obtained from direct titrations, except in the case of NOM samples. Additional evidence for the possible underestimation of carboxyl contents by Model VI is found in Table 2. The lowest estimates of carboxyl contents of fulvic acids are those obtained using Model VI. The second-lowest carboxyl contents of humic acids are obtained using this model. Given the range of values that were obtained by each method, this evidence is, at best, suggestive. Interestingly, the modifications of Model VI by Gustafsson (2001), in which the fixed ratio of phenolic-to-carboxyl content is decreased from 0.5 to 0.3 for fulvic acids, yields carboxyl and phenolic contents that are in much better agreement with other results of direct titrations in Table 2. The results reported in this study for the IHSS samples are the highest estimates of carboxyl contents and the lowest estimates of phenolic contents for the fulvic and humic acids. This is a consequence of the use of the pH-based method, which assumes, as stated previously, that all carboxyl groups (and no phenolic groups) are titrated at pH 8, and that half of the phenolic groups are titrated between pH 8 and pH 10.

4.5. Suwannee River Samples

The IHSS samples include four samples from the Suwannee River in southeast Georgia, U.S.A. The standard fulvic acid, standard humic acid, and reference fulvic acid were isolated using the XAD-8 method (Malcolm et al., 1989). The reported recovery of organic carbon was 75%, and the fulvic acid:humic acid ratio was 87:13 (Malcolm et al., 1989). The reference NOM was concentrated 20-fold in the field using the reverse

Table 2. Summary of carboxyl and phenolic contents for fulvic acids, humic acids, and natural organic matter (NOM) from the literature and from this study.

Sample type	Method of analysis	Carboxyl groups, meq g^{-1}			Phenolic groups, meq g^{-1}		
		Average	Std. Dev.	n	Average	Std. Dev.	n
Fulvic acids							
IHSS samples	Direct titration	6.6	0.8	6	1.4	0.3	6
Others[a]	Direct titration	5.4	1.3	40	1.6	0.7	35
NICA-Donnan[b]	Direct titration	5.4	1.2	18	2.6	1.9	18
Model VI[c]	Direct titration	4.8	0.7	10	2.4	0.4	10
Stockholm Model[d]	Direct titration	5.3	0.9	9	1.6	0.3	9
Others[e]	Ba(OH)$_2$/Ca(OAc)$_2$	6.1	2.0	83	4.1	1.9	83
Humic acids							
IHSS samples	Direct titration	4.8	0.4	7	1.4	0.4	7
Others[a]	Direct titration	3.8	0.9	35	1.6	0.5	27
NICA-Donnan[b]	Direct titration	3.2	0.9	20	2.7	1.4	20
Model VI[c]	Direct titration	3.3	0.5	9	1.7	0.2	9
Stockholm Model[d]	Direct titration	3.6	0.8	9	1.8	0.4	9
Others[e]	Ba(OH)$_2$/Ca(OAc)$_2$	3.8	0.9	198	3.4	1.6	189
NOM samples							
IHSS samples	Direct titration	5.2	—	1	2.1	—	1
Others[a]	Direct titration	5.6	2.1	8	2.1	1.3	3
Others[e]	Ba(OH)$_2$/Ca(OAc)$_2$	8.3	1.8	12	3.8	1.9	12

All concentrations are in meq g^{-1} on a dry, ash-free basis.

[a] Reuter, 1980; Miles et al., 1983; Steelink et al., 1983; Steinberg and Muenster, 1985; Malcolm, 1985; Collins et al., 1986; Ephraim et al., 1986; Malcolm and MacCarthy, 1986; Portal et al., 1986; Bowles et al., 1989; Ephraim et al., 1989; Aleixo et al., 1992; Wang et al., 1992; de Wit et al., 1993; Machesky, 1993; Nederlof et al., 1993; Fukushima et al., 1995; Westall et al., 1995; da Silva et al., 1996; Christensen et al., 1998; Masini et al., 1998; Avena et al., 1999; Robertson and Leckie, 1999; Santos et al., 1999; Ma et al., 2001.

[b] Milne et al., 2001.

[c] Tipping, 1998.

[d] Gustafsson, 2001.

[e] Wright and Schnitzer, 1960; Hansen and Schnitzer, 1966; Khan, 1971; Riffaldi and Schnitzer, 1972; Ortiz de Serra and Schnitzer, 1973; Beck et al., 1974; Schnitzer and Vendette, 1975; Weber and Wilson, 1975; Holcomb, 1979; Hatcher et al., 1981; Schnitzer and Preston, 1986; Baes and Bloom, 1989; Frund et al., 1989; Stearman et al., 1989; Lobartini et al., 1991; Novak and Smeck, 1991; Raysid et al., 1992; Orlov, 1995; Barancikova et al., 1997; Celi et al., 1997; Martin et al., 1998; Ceppi et al., 1999; Monteil-Rivera et al., 2000; Novak et al., 2001.

osmosis (RO) method of Serkiz and Perdue (1990). The overall recovery of organic carbon was 92.9%.

Titration curves for the four different IHSS samples that were obtained from the Suwannee River are shown in Figure 4, and both pH-based and model-based estimates of carboxyl and phenolic contents are given in Table 1. The reference fulvic acid has the greatest charge density at all pH, with the standard fulvic acid being slightly lower, and the standard humic acid being lowest at all pH values. The relative charge densities seen in the titration curves are also reflected in the carboxyl contents of these samples. The titration curves for the reference NOM and the standard humic acid closely parallel each other below pH 8, and they almost merge above pH 9. Their carboxyl and phenolic contents agree within ± 0.3 meq gC^{-1}, and their total acidities are essentially the same. Given that these samples were collected by different researchers at different times using different methods, any attempt to analyze these similarities and differences is almost entirely speculative. Nonetheless, some speculation is warranted. The relatively good recovery of organic carbon in the XAD-8 method, together with the high fulvic-to-humic acid ratio, suggests that the Suwannee River contains predominantly fulvic acids. The even higher recovery of organic carbon in the RO method suggests that the NOM includes not only most of the fulvic acids and humic acids, but also some solutes that were not isolated in the XAD-8 method. It follows that the titration curve of NOM is expected to resemble more closely that of the fulvic acids, which it obvi-

ously does not. Among the possible explanations are the following:

1. The chemical properties of dissolved organic matter in the Suwannee River vary substantially over time, rendering this analysis inappropriate.
2. The organic matter that is not isolated by the XAD-8 method contains less carboxyl and more phenolic groups than the humic acid.
3. Fulvic acids are generated from more humic-like substances during their isolation using the XAD-8 method, perhaps through acid-catalyzed or base-catalyzed reactions such as those acid-generating processes which have been suggested to explain pH drift and hysteresis in the titration curves of humic substances.
4. Humic acids are generated from fulvic acids during the RO process, perhaps through acid-catalyzed condensation reactions that eliminate acidic functional groups (e.g., esterification).

Much additional research would be needed to determine the relative merits of each of these possible explanations. At present, it is sufficient to note that the acid–base properties of the NOM and humic acid are remarkably similar.

4.6. Proton-binding Affinities

Although the model fitting parameters in Table 1 are empirical, such parameters are often interpreted in a thermodynamic
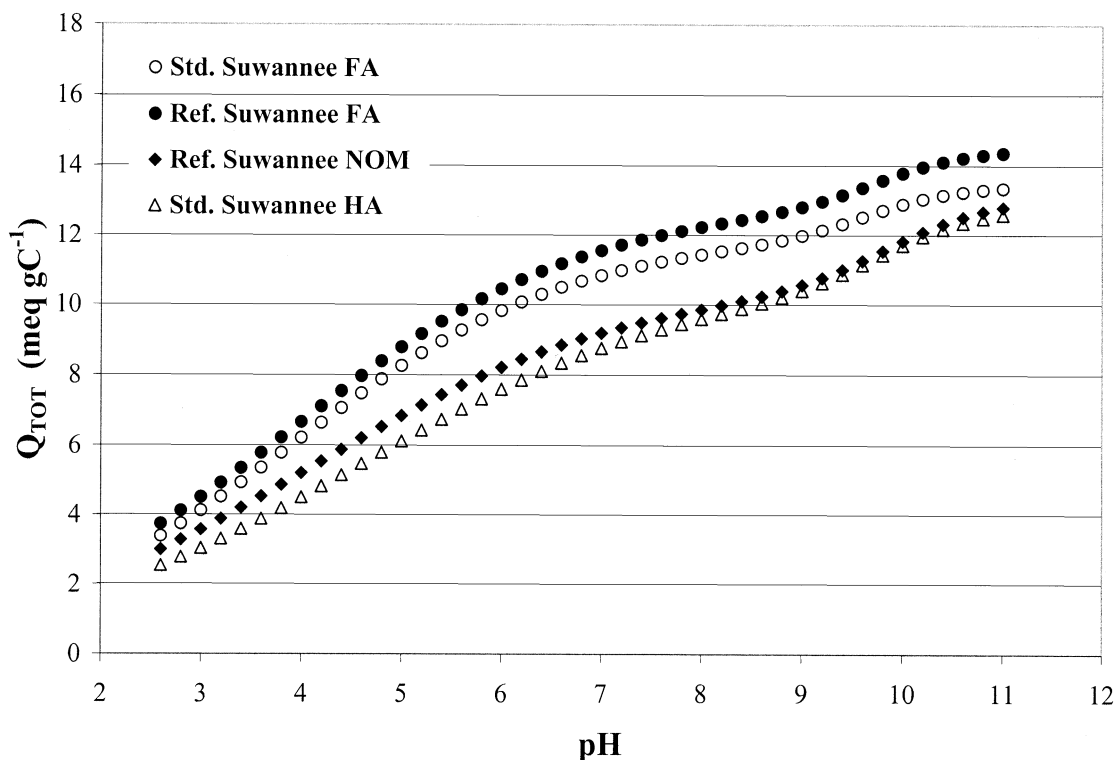
Fig. 4. Titration curves for standard and reference Suwannee River fulvic acid, standard Suwannee River humic acid, and reference Suwannee River natural organic matter.

sense. As stated previously, log K_1 and log K_2 are fitting parameters that represent mean log K values for proton binding by the carboxyl and phenolic groups, respectively. It is evident from Table 1 that the log K_1 values fall into two tight groups, one for fulvic acids (log K_1 = 3.8 ± 0.1) and one for humic acids (log K_1 = 4.4 ± 0.1). Although these log K values are not directly comparable with those of Model VI and the NICA-Donnan model, all three models predict that the carboxyl groups of fulvic acids are generally more acidic than those of humic acids. Interestingly, the log K_1 for the reference Suwannee River NOM is closer to the average value for fulvic acids, even though its concentrations of carboxyl and phenolic groups are nearly identical to those for the standard Suwannee River humic acid. The log K_2 values of the IHSS fulvic acids, humic acids, and NOM are not statistically different.

The parameters n_1 and n_2 are empirical constants (n ≥ 1) that reflect the range of log K values within each class of functional groups. The width parameters (n_1) for the carboxyl groups of the IHSS fulvic acids, humic acids, and NOM are not statistically different. All samples have rather broad distributions of carboxyl groups. The model predicts that there is very little heterogeneity in the phenolic groups. In fact, most of the IHSS samples have n_2 values of 1.00, which corresponds to a single binding site. This is a surprising result. Both Model VI (Tipping, 1998) and the NICA-Donnan model (Milne et al., 2001) predict substantial heterogeneity in phenolic groups. Earlier applications of the gaussian distribution model to proton binding by humic substances also indicate substantial heterogeneity in the phenolic groups (Perdue and Lytle, 1983; Perdue et al., 1984).

5. CONCLUSIONS

Carboxyl and phenolic contents of 14 IHSS samples from terrestrial and aquatic environments have been estimated from alkalimetric titration data using both pH-based and model-based methods. The carboxyl contents of these samples are in the general order: terrestrial fulvic acids > aquatic fulvic acids > Suwannee NOM > aquatic humic acids > terrestrial humic acids. These relative differences in carboxyl content are reflected directly in the titration curves of the samples, because most of the acidity in the IHSS samples is attributable to carboxyl groups. Overall, the IHSS fulvic acids and humic acids have similar phenolic contents; however, all of the aquatically derived samples have higher phenolic contents than the terrestrially derived samples. The acid–base properties of reference Suwannee River NOM are surprisingly similar to those of standard Suwannee River humic acid. The average phenolic-to-carboxyl ratio of the IHSS samples is 21:79, which is somewhat lower than values that have been published previously.

The carboxyl and phenolic contents of the IHSS samples have been compared with reported estimates from 284 indirect titrations. The carboxyl contents of the IHSS samples are generally consistent with these literature values. Phenolic contents from the indirect methods are much higher than phenolic contents of the IHSS samples. The carboxyl and phenolic contents of the IHSS samples have also been compared with 103 literature estimates were obtained by direct titration, including 38 estimates that were recently obtained using the NICA-Donnan model. These results have also been compared with recent estimates that were obtained by fitting Model VI

and the Stockholm humic model to large numbers of titration data sets. The comparison confirms the relatively high carboxyl contents and relatively low phenolic contents of the IHSS samples. It is also noteworthy that Model VI gives significantly lower estimates of carboxyl contents, which may be a consequence of the model's assumed 1:2 ratio of phenolic-to-carboxyl groups.

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