

Solution Interaction of Humic Acids with Calcium Ions Involves a Two-Phase System

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Humic acid particles, dissolved into a homogenous solution, interact with calcium ions via binding sites existing both within the particles and on their surfaces. This is shown by three series of studies in 0.1 M KCl and 24 °C involving kinetic data, and two sets of steady-state data: pH titrations (*Z*-data) and data obtained using a calcium-ion-sensitive electrode. The steady-state was controlled via a redox electrode. The diffusion of calcium ions into the humic acid particles is substantial, and the intraparticle bound fraction constitutes 24–42 mol % of the total Ca^{2+} concentration (*B*) at $B \leq 1$ mM. The electric field produced within the particles by the bivalent calcium ions leads to the release of up to two protons beyond those dissociating in the absence of calcium ions, yielding internal binding sites formally described as CaH_{-1}A and CaH_{-2}A (A is a humic acid ligand). The reactions occurring on the surfaces of the particles are consistent with the formation of a major complex, CaA , and a minor complex, Ca_2A , with the apparent $\log \beta_{11} = 3.6$ and $\log \beta_{21} = 6.7$, respectively. The concentration of humic acids was $1\text{--}2 \text{ g l}^{-1}$, *B* varied from 0.13 to 1.73 mM and the pH from 2.5 to 7. Analysis of the acid–base data confirms our previous study in 0.1 M NaClO_4 medium [*Acta Chem. Scand.* 53 (1999) 172] yielding three apparent *pK*-values (5.79, 3.77, and 1.7), as well as the ‘dimer’ HA_2 supposed to involve hydrogen bonds of the particles; the apparent $\log^* \beta_{12} = 8.58$.

The capacity of humic substances to interact strongly with the metal ions present in soil and natural waters makes them important agents for the speciation and mobility of metal ions into the biosphere. The importance of this matter has encouraged a large number of studies intended to predict the role of humic substances in the utilization and storage of metal ions; for a summary on this subject, see the compilations by Frimmel and Christman,¹ and by Hayes *et al.*² Calcium ions are of particular interest for such a prediction, since their complexes formed with humic substances are the source for the calcium ion uptake by the plant kingdom. Also, carbonate in the form of $\text{CaCO}_3(\text{s})$ is often used to restore the neutral pH of acidified soil and natural water systems, and most of these calcium ions will interact with humic acids.

Earlier studies on the metal complexes of humic acids and fulvic acids have been reviewed repeatedly; see for instance Refs. 3–5. Some of the more recent studies were discussed in some detail in our previous communication.⁶ Earlier work on calcium ion complexes was discussed by Sposito *et al.*,⁷ who studied the calcium ion complexes of fulvic acids. They measured the formation function as

a function of the free Ca^{2+} concentration at pH 7, and the results indicated two different kinds of calcium ion binding.⁷

In this study we make use of our previous results^{8–10} indicating that humic acids consist of fractal particles of colloidal size 100–200 nm. The fractal dimension, 2.3, indicates that these particles contain >90% water and thus metal ion diffusion into these solvent-rich particles seems quite likely. This high water content has also been found in peat,¹¹ which consists of about 60% humic acids.

Metal ion diffusion into peat and humic acid particles has been studied in some detail by Kadlek and Keolian¹² and by Bunzl *et al.*,¹³ it was assumed that these particles behaved as synthetic ion exchange particles.^{12,13} According to these authors,^{12,13} and the mathematical analyses of metal ion diffusion in corresponding systems,^{14,15} large particles would favour intraparticle diffusion and small particles (of the present size) would favour film diffusion. The film is supposed to be the boundary layer between a particle and the bulk solvent. Although a synthetic ion exchanger is a simplified model for the complex humic acid particles, which may contain ligand groups both inside the particles as well as on their surfaces, the kinetic data of our previous study obeyed those of a film diffusion model.⁶

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This study deals with kinetic data as well as steady-state data on the Ca^{2+} -humic acid system. All the data were recorded anaerobically using humic acids of colloidal size; the steady states were controlled by a redox electrode. The colloidal size of humic acids was provided first by repeated dialysis and then by the fact that the system was allowed to reach a steady state. In this work we titrated a dialysed sample of humic acids of pH 7 containing calcium ions with HCl, thus avoiding the use of OH^- ions which may cause side reactions.¹⁶ The pH and the Ca^{2+} ion concentration were measured using glass and Ca^{2+} -sensitive electrodes. The results are consistent with the idea that binding sites exist both within the humic acid particles and on their surfaces.

Notations

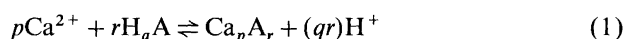
A^{2-}	formal notation of humic acid ligand tentatively given the charge '-2'; the charge is often omitted.
A	total molar concentration of humic acid ligands obtained from C_A via $A = 10^{-3} C_A f$, where $f = 1.392$ (see Section 2.1, p. 977).
A^*	value of A after eliminating the calcium species, defined by eqn. (7d).
a	molar concentration of free A^{2-} .
B	molar concentration of total calcium ions.
B_i	molar concentration of intraparticle bound calcium ions.
b	molar concentration of free calcium ions.
$F(h)$	function defined by eqn. (7e).
$F(T)$	function defined by eqn. (14).
$F(\beta)$	function defined by eqn. (8c).
H	total molar concentration of protons over A^{2-} .
h	molar concentration of free H^+ .
K_1, K_2, K_3	apparent stepwise stability constants for proton binding, cf. p. 978.
p, r	coefficients in complexes Ca_pA_r , defined by eqn. (5b).
R	parameter defined by eqn. (13).
q	coefficient in H_qA_r , defined by eqn. (5a).
s	coefficients of protons released beyond those dissociating in the absence of calcium ions, for instance, in the intraparticle species $(\text{CaH}_{-s}\text{A})_i$.
T	empirical parameter defined by eqn. (14).
U	normalized model function; $U = Y(\beta_{11}R)^{-1}$.
v	normalized variable, $v = hK_2$, see eqn. (12).
Y	defined by eqn. (8d).
Z	average number of protons bound per C_A defined by eqn. (4) (in unit mmol g^{-1}).
$^*\beta_{qr}$	apparent stability constant for proton binding, defined by eqn. (5a).

β_{pr} apparent stability constant for calcium complexes, defined by eqn. (5b).

Method

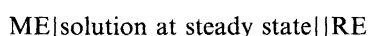
Special approach for studying humic acid metal complexes. The humic acid system is a redox system characterized by a high sensitivity to oxygen and by non-equilibrium behaviour.^{9,17,18} However, under carefully controlled anaerobic conditions the system can be brought to a steady state, where the measured redox potential is a linear function of pH (see Fig. 2 in Ref. 18). An anoxic condition is necessary, since polymerization catalyzed by oxygen (phenolic coupling)¹⁹ may change the structure of the humic acids during a titration, and thus invalidate a proper analysis of the data. A redox electrode is a far more sensitive indicator than a glass electrode concerning whether or not a steady state is reached; thus the electromotive forces (EMF) of the redox electrode cell usually reach the steady state a fairly long time (2–10 h) after that of the glass electrode cell is fairly stable. In addition, the redox electrode is highly sensitive to oxygen and to redox impurities, such as Fe ions.

Theory. We assume that Ca^{II} and humic acids react according to the general reaction:



where A denotes a ligand of a humic acid particle (for simplicity the charges are omitted). The ligand A is supposed to be situated either inside the particle or on its surface. In the former case, the Ca^{2+} ions reach the ligands via diffusion into another phase (the humic acid particle phase) separated from the bulk solvent; then, the bound Ca^{2+} ions are not in 'equilibrium' with the free Ca^{2+} concentration in the bulk solvent. The intraparticle exchange of Ca^{2+} ions depends on the diffusion flux densities of the ions, Ca^{2+} , H^+ , Cl^- and K^+ , which are functions of their concentration gradients, the induced electrical field within the particles, and the diffusion coefficients of the ions (cf. Refs. 12, 14 and 15). The ligand A may contain one or more ligand atoms situated on the same organic residue; for instance, a malonate residue forming a chelate via its two carboxylates. In addition, eqn. (1) is supposed to indicate complex formation via different organic residues within the same particle or via organic residues situated on two or more different particles, then yielding complexes CaA_2 , CaA_3 and so on. In order to enable the release of protons [eqn. (1)], it is assumed that the pH is sufficiently low compared to the pK value(s) of the ligand(s). If calcium ions release protons from groups which do not dissociate in the absence of Ca^{2+} ions, the complex is given a negative coefficient, such as CaH_{-s}A ($s = 1, 2, \dots$); the last term in eqn. (1) will then be $(rq + s)\text{H}^+$.

The calcium ion reactions were measured using cells of the following type:



where ME is a glass electrode (Ingold no. U262-S7) or an electrode sensitive to calcium ions (Mettler-Toledo S/N:647413). RE is a commercial Ag, AgCl electrode with double junctions (Metrohm no. 60726) of the type:

0.1 M KCl|0.1 M KCl|AgCl, Ag

At 24 °C the electromotive force (EMF) can be represented by the following equations for the cells of the calcium ion electrode and the glass electrode, respectively:

$$E_{Ca} = E_{0Ca} + 29.48 \log b + E_j \quad (2)$$

$$E_G = E_{0G} + 58.96 \log h + E_j \quad (3)$$

Here, b and h are the free concentrations of Ca^{2+} and H^+ ions in the bulk solvent. For details regarding the redox measurements, Refs. 6 and 18 should be consulted.

The liquid junction potential, E_j , was determined in a separate experiment, where a 0.1 M KCl solution was titrated with HCl. The result was $E_j \approx 400$ h (cf. Ref. 17). A solution of 1 mM HCl in 0.1 M KCl was generally used for calibration of the glass electrode cell. The slope of the line, E_G versus pH, for the glass electrode cell [then using a (Ag, AgCl, 3.0 M KCl) reference electrode (Ingold no. 363-S7)] was controlled by a series of standard buffers.²⁰ The EMF values of the calcium electrode cell were calibrated by three standard solutions, 0.1, 1 and 10 mM $CaCl_2$ containing 0.1 M (K)Cl. The EMF values of the cells studied were measured using Guildline digital nanovoltmeters (model 4880A).

We have assumed, considering eqns. (1)–(3) as valid, that the activity factors are approximately constant in the 0.1 M KCl medium, so that they can be included in the E_0 constants.

Experimental

Materials. Humic acids were prepared from A1 horizon podzol soil taken from a meadow in a suburb of Uppsala. The preparation used in this study is the same one as that used in the previous work.⁶ Thus, during the initial phase of the preparation we precipitated humic acids with HCl and then dissolved the precipitate in a 0.15 M pyrophosphate buffer of pH 7. Materials which did not dissolve were discarded by centrifugation (mostly inorganic products); this procedure was repeated four times. The final step in the preparation procedure⁶ involved dialysis against a 0.15 M pyrophosphate buffer of pH 7 followed by prolonged dialysis against bidistilled water; samples of the final humic acid solutions were frozen and kept at $-80^\circ C$. Lyophilization or other kind of drying procedures were avoided, and so was the use of strong alkali (cf. Refs. 8 and 16). Spectrapor membrane tubing (Los Angeles, USA) was used, 3500 MW cutoff. The humic acid preparation contained <1% silicate, <0.1% Fe, <0.02% Ca and <0.001% Cu as calculated on a dry-weight basis. The C/N atomic ratio was 14.6. The concentration of each solution was obtained via C analysis assuming a carbon content of 53% for humic acids.²¹

The calcium ion solutions, (0.1 M in Cl^-) used in this study were prepared from $CaCl_2$ and KCl (Merck, analytical grades). Standard solutions as well as the final solutions of the titrations were controlled by analysis of calcium. All the other chemicals used in this study were also of analytical grades.

Procedure. Complex formation was measured by successively adding samples of HCl to a solution of humic acid containing Ca^{II} and 0.1 M KCl. In separate titrations, only humic acids in 0.1 M KCl were titrated. An anaerobic atmosphere was maintained using argon from which traces of oxygen were removed by passing it over hot activated copper²² and then through a solution of Cr^{II} ; then the argon gas was freed from possible acid and base impurities by passing it through solutions of 10% NaOH and 10% H_2SO_4 and then, finally, through two bottles of 0.1 M KCl before reaching the titration vessel. The solutions were protected against light, and the titrations were made in a dark room kept at $24.00 \pm 0.05^\circ C$.

The titrations were represented on the form $Z(pH)_B$, where Z is the average number of protons bound per C_A as defined by

$$Z = (H - h)/C_A \quad (4)$$

H is the total molar concentration of protons and C_A is the total concentration of humic acids in $g\ l^{-1}$. (Note that the millimolar scale is used for $(H-h)$ in Figs. 2 and 7; thus the unit for Z is $mmol\ g^{-1}$).

From each titration curve recorded in the absence of calcium ions, the precise value of the inflexion point at about pH 7.5 was determined, and at this point the value of H (and thus the value of Z) was set to zero. The titrations with HCl, represented as $Z(pH)$ curves, would then show the uptake of protons by humic acids as normalized to the analyzed humic acid concentration [eqn. (4)]. The data collected from the measurements in the presence of calcium ions were scaled to a set of $Z(pH)_B$ curves using the $Z(pH)$ curve obtained in the absence of calcium ions. Then the initial solution only contained humic acids (a dialysed sample of neutral pH) and 0.1 M KCl. This solution was first adjusted to a pH close to 7; from the precise measured pH at steady-state we then obtained the Z -value from the $Z(pH)$ acid–base curve. Then the calcium ion solution was added and the Z -value adjusted for the dilution; this datum became the first point on the $Z(pH)_B$ plot. (B is the total molar Ca^{2+} concentration).

For each measured point, it was carefully controlled that the EMF of the redox electrode cell obeyed the steady-state line corresponding to that of Fig. 2 in Ref. 18. Then the EMF of the calcium ion electrode cell was measured. Usually, a steady state was reached after 8–24 h. During the titration as well as after each titration was completed the solution was carefully examined for any precipitate giving a negative result.

Time resolved data were recorded for the initial reactions of Ca^{II} ions with humic acids. Then the protons set

free, as a result of the calcium ion reactions, were recorded by measuring E_G [eqn. (3)] as a function of time; a Hewlett-Packard timer-counter-DVM (5326B) was used. Although stirring was provided by the Ar bubbling, magnetic stirring was also used at the injection of the calcium ion solution into the titration vessel containing humic acid and 0.1 M KCl.

Results

1. Kinetic data. Figure 1 shows time-resolved data for the stepwise addition of Ca^{2+} ions to a humic acid solution of 1 g l^{-1} yielding the accumulated total calcium concentration of 0.39, 0.52, 1.14 and 1.73 mM. In the previous study⁶ time-resolved data were shown for the first initial addition of Ca^{2+} ions to solutions containing only humic acids and 0.1 M KCl. The data of Fig. 1 obey traditional relaxation kinetics; thus, for the stepwise additions a, b, c, and d of Fig. 1 we obtain the τ -values 350, 189, 6.7 and 3 s. The value of τ corresponds to the time when the difference between the observed maximum value of E_G and that at the time, t , decreased to 63.2% of its value at $t=0$ (cf. Ref. 18).

The process that might control the overall rate of humic acid-metal ion interactions includes: (1) film diffusion through the boundary layer surrounding the humic acid particles; (2) intraparticle diffusion in the matrix of the particles; (3) reactions at the binding sites of the particles. Since the reactions of calcium ions in general are very fast ($\ll 1 \text{ s}$),²³ the generally slow rates shown in Fig. 1 indicate that the Ca^{2+} interactions with humic acids are diffusion-controlled rather than reaction-controlled; the possible exception is the last step shown in Fig. 1. As shown in the previous study (Fig. 1 in

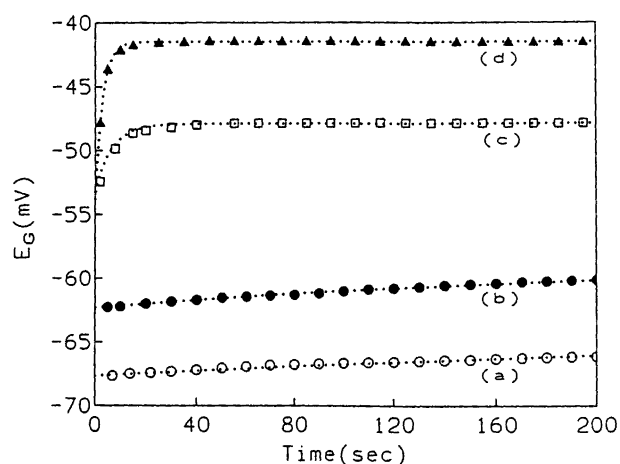


Fig. 1. Time-resolved data for Ca^{2+} interactions with humic acids ($C_A = 1 \text{ g l}^{-1}$). The protons set free by the Ca^{II} reactions are recorded via the increase in E_G [eqn. (3)] as a function of time. After the stepwise addition of calcium ions the accumulated total concentrations of Ca^{2+} were: (a) 0.39, (b) 0.52, (c) 1.14 and (d) 1.73 mM. The symbols are experimental data and the dotted curves have been calculated from the τ -values given in the text.

Ref. 6), the initial rate of the interaction shows a marked increase in the rate of released protons with increasing calcium ion concentration. These data⁶ obey very similar courses as those reported by Bunzl *et al.*¹³ (Fig. 2e of their publication). Based on a thorough mathematical analysis,¹⁴ Bunzl *et al.*¹³ concluded that this increasing rate for increasing Ca^{2+} concentration is consistent with film diffusion rather than intraparticle diffusion. As a result it is indicated from our previous study⁶ that Ca^{2+} ions diffuse into the humic acid particles, where they react and release protons. Likewise, the present data involving stepwise addition of calcium ions show an increasing rate of releasing H^+ ions from the humic acid particles with increasing calcium concentration. 'Saturation' of calcium ions within the particles may essentially be reached before the last step, since the rate of protons set free is of the same order of magnitude at 1.14 mM ($\tau = 6.7 \text{ s}$) as it is at 1.73 mM (3 s).

2. Steady-state data.

2.1. Acid-base data. Humic acids at approximately constant $C_A = 1.0$ and 2.0 g l^{-1} were titrated with HCl. In agreement with our previous study⁶ (Fig. 2 in Ref. 6) the data showed a plateau at about pH 7.5 and a slight tendency to an inflexion point at pH 2.9. Between these two points 2.8 mmol H^+ were titrated per g of humic acid, which is in good agreement with earlier studies; they report $2.4\text{--}3.3 \text{ mmol g}^{-1}$, see Ref. 6 and references cited therein.

As the first step in the analysis of the data, we normalized the concentration of acidic groups of humic acid in molar concentration, A , so that the normalized value of Z [cf. eqn. (4)], between the two inflexion points would correspond to the highest integer q/r for the species considered. In this case we assumed a value of 2.0 between these points, which yields $10^{-3} C_A f = A$, where $f = 1.392$. If we assume that at the steady state the following reactions are involved:



with the apparent stability constant $^*\beta_{qr}$, then we may define the total concentrations of protons, H , and ligand, A , on the molar scale:

$$H = h + \Sigma q[\text{H}_q\text{A}_r] \quad (6)$$

$$A = a + \Sigma r[\text{H}_q\text{A}_r] \quad (7a)$$

The analysis of the present data is similar to that described in our previous work.⁶ Thus, we assume that the present steady-state data, as a first approximation, can be used as an approach to equilibrium, and thus be analysed as equilibrium data. The general least-squares computer program LETAGROP was used.²⁴ It should be noted that in the present analysis we tried to describe the data using as few parameters as possible, provided that the deviations are of the same order of magnitude as those expected from the analytical errors. We are aware of that some acidic group(s) may involve intra-

particle group(s), which, owing to electroneutrality, may require exchange with another positive ion in order to dissociate protons to be released into the bulk solvent. Thus, the dissociation of such a group will depend on the concentration of the bulk electrolyte as well as on the kind of ions used. The reason why apparent proton 'pseudo-equilibria' exist between acid-base groups within the particles and the bulk solvent may be due to proton transfers via proton jumps.

The analysis of our data gave the following results: $\log^* \beta_{11} = 5.79$, $\log^* \beta_{21} = 9.56$, $\log^* \beta_{12} = 8.58$ and possibly also $\log^* \beta_{31} = 11.27$. Recalculating to apparent pK values and stepwise constants, K , yields: $pK(\text{HA}) = \log K_1 = 5.79$, $pK(\text{H}_2\text{A}) = \log K_2 = 3.77$, $\log K_{12} = 2.79$ and $[pK(\text{H}_3\text{A}) = \log K_3 = 1.7]$. These constants, valid for a 0.1 M KCl medium, compare well with those of our previous study in 0.1 M NaClO_4 medium⁶ which gave the pK -values 5.82, 3.84, 1.64 and $\log^* \beta_{12} = 8.57$. This good agreement indicates that our steady-state approach is valid and that it yields well reproducible data comparable to true equilibrium data.

The present apparent pK values are in fair agreement with the pK values reported by Bolton *et al.*²⁵ and by Wahlberg and Ågren¹⁷ (see Table 1 in Ref. 6). A 'dimer' was recently reported by Bergelin *et al.*,²⁶ however, they apparently studied a soil extract and not highly purified humic acids. It is possible that $pK(\text{HA}) = 5.79$, at least partly, corresponds to an internal carboxylic group.⁶ If so, then the pK of 5.3 reported by Bolton *et al.*²⁵ is not surprising, since their ionic medium was 0.1 M LiNO_3 as compared to 0.1 M KCl in our study.

2.2. Calcium ion data $Z(pH)$ at constant B and C_A . Figure 2 shows two sets of Z -curves for the humic acid concentrations, C_A , of 1 and 2 g l^{-1} obtained by titrations in the presence of approximately constant Ca^{II} concentrations, $B = 0.13, 0.26, 0.5$ and 1.7 mM and $B = 0.26, 0.5$ and 1 mM, respectively. It follows from these data (Fig. 2) that at low pH their Z -values do not tend to coincide with those of the acid-base curve recorded in the absence of calcium ions. In studies of low-molecular-weight carboxylates, Z -data generally coincide at low pH; see, for instance Refs. 27 and 28. The reason for this deviation in the present study is most likely due to the fact that some calcium ions remain bound within intraparticle sites. Thus the calcium ions may be distributed over two phases. Since we do not know the concentration in either phase, an analysis in the form of predominating species and their stability constants from the present Z -data alone does not seem possible. On the other hand, by using the parameters extracted from the $\log(B-b)/b$ data (see the next section) we could describe five of the calcium ion titrations by theoretical Z -curves (Fig. 2) (see Section 2.3.5 below).

2.3. Data for $\log B/b(pH)$ at constant B and C_A . Figure 3 shows two sets of $\log(B-b)/b$ data recorded at steady state by the calcium-sensitive electrode for approximately constant humic acid concentration, $C_A = 1$ and 2 g l^{-1} .

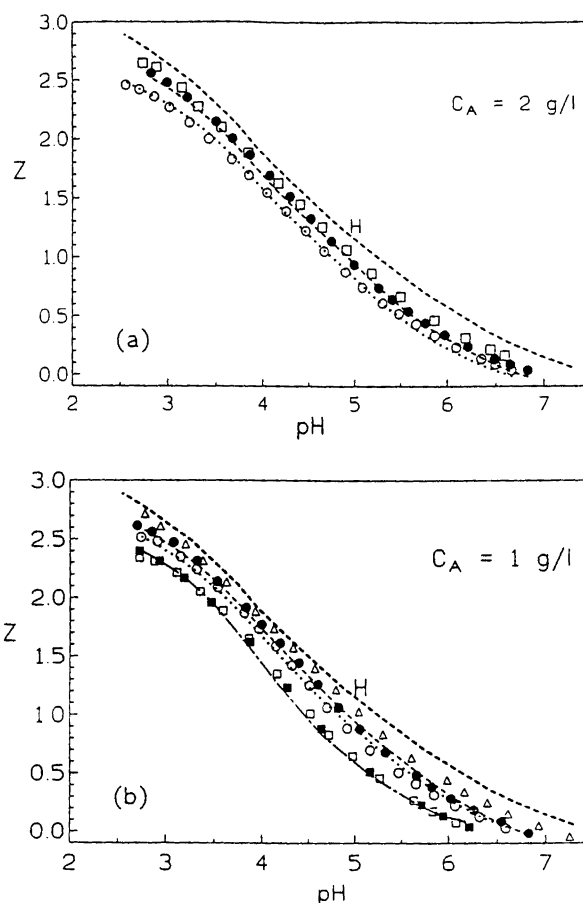


Fig. 2. Z , the average number of protons bound per C_A , plotted as a function of pH for approximately constant C_A and B . The symbols are the experimental data and the curves have been calculated for $\log \beta_{11} = 3.61$, $\log \beta_{21} = 6.7$, the acid-base stability constants given on p. 978, and for the parameters B_i , s , and T listed in Table 1; see the text on p. 981. The H -curve is recorded in the absence of calcium ions. (a) \square , $B = 0.26$ mM; \bullet and dashed curve, $B = 0.5$ mM; \circ and dotted curve, $B = 1$ mM; (b) \triangle , $B = 0.13$ mM; \bullet and dashed curve, $B = 0.26$ mM; \circ and dotted curve, $B = 0.5$ mM; \blacksquare (titration no. 1), \square (titration no. 2) and dashed-dotted curve, $B = 1.7$ mM.

These data were obtained via eqn. (2) using the relationship

$$\log B/b = (E_B - E_{Ca})/29.48$$

Here E_B is the EMF for B mM calcium ions in the absence of humic acids. B is the total molar concentration of calcium ions defined by

$$B = b + \sum p[\text{Ca}_p \text{A}_r] = b + b \sum p b^{p-1} a^r \beta_{pr} \quad (8a)$$

where b and a are the free molar concentrations of calcium ions and ligands, respectively, and where β_{pr} is the stability constant for the general reaction



For simplicity, the charge is omitted on A and the complexes. Likewise, we define the total molar concentra-

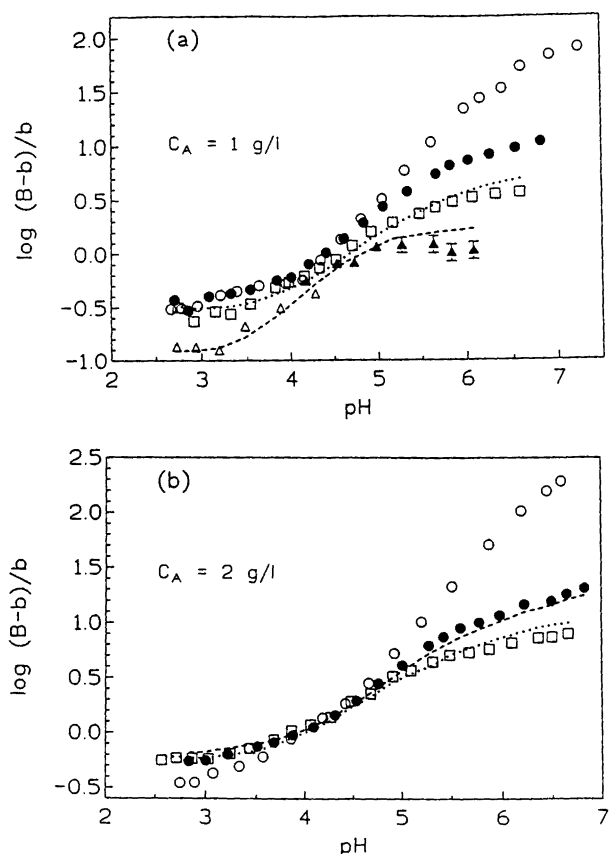


Fig. 3. Data for $\log(B-b)/b$ as a function of pH for approximately constant values of C_A and B . The symbols are experimental data and the curves are calculated for $\log \beta_{11}=3.65$, $\log \beta_{21}=6.7$, the acid-base constants given in the text on p. 978, and the B_1 -values listed in Table 1. (a) \circ , $B=0.13$ mM; \bullet , $B=0.26$ mM; \square and dotted curve, $B=0.5$ mM; \blacktriangle (titration 1), \triangle (titration 2) and dashed curve, $B=1.73$ mM. (b) \circ , $B=0.26$ mM; \bullet and dashed curve, $B=0.5$ mM; \square and dotted curve, $B=1$ mM. The experimental errors are similar to the sizes of the symbols (or less) except where error bars are given.

tion of humic acid ligands

$$A = a + \sum r[H_q A_r] + \sum r[Ca_p A_r] \quad (7b)$$

Here, A (in M) is related to C_A (in $g\ l^{-1}$) via $A = 10^{-3} C_A f$, where $f = 1.392$ (see Section 1).

For $B \ll A$ and using the results of the acid-base study, eqn. (7b) may be reduced to

$$\begin{aligned} A &\approx a + [HA] + [H_2A] + 2[HA_2] + [H_3A] \\ &\approx a(1 + hK_1 + h^2K_1K_2) \end{aligned} \quad (7c)$$

(The contributions to A for the species HA_2 and H_3A are omitted in this first approximation.) From eqn. (7c) it follows that for a constant pH and a constant A , the free ligand concentration, a , will be approximately constant. Then it follows from eqn. (8a) that each set of the data, $\log(B-b)/b$ versus pH at constant C_A , in Figs. 3a and 3b would either be expected to fall on the same curve ($p=1$), or to increase with increasing B ; in the latter case this is due to the fact that polynuclear species

($p > 1$) are formed. However, an examination of Figs. 3a and 3b clearly shows that none of these cases is valid, especially not for the data of the two lowest B -values in each set, where $B \ll A$. Instead, in both Figs. 3a and 3b, the $\log(B-b)/b$ data decrease for increasing B . This kind of result cannot be explained by any kind of equilibrium or steady-state approach, if the reactions take place in one single phase. However, the explanation is most likely that we do not have one single phase but two phases, thus supporting the results we observed from our kinetic data (cf. p. 977). It is important to note that the calcium ions present in one of these phases, the intraparticle phase of humic acid particles, are not in equilibrium with the free calcium ions present in the other phase, the bulk solvent phase (see p. 975).

2.3.1. Average composition of the complexes. Although a substantial fraction of calcium ions may have diffused into the particles to be bound there, an attempt may be made to analyse the reactions occurring on the surface of the particles within the bulk solvent phase. For low B , such a binding may mainly involve one kind of binding site. For instance, when we plot the data for $B=0.13$ mM and $C_A=1\ g\ l^{-1}$ (Fig. 3a) and those for $B=0.26$ mM and $C_A=2\ g\ l^{-1}$ (Fig. 3b) on the same diagram, we find for $pH < 6$ at constant pH:

$$\Delta \log[(B-b)/b] / \Delta \log C_A \approx 1 \quad (9)$$

Thus, when $\log(B-b)/b$ is plotted against $(pH + \log C_A)$ for these two series of data, they should fall on the same curve. As shown by Fig. 4, this is valid below pH 6, and thus in this range the main coefficients of $(p; r)$ are (1; 1) assuming complexes of the general composition $Ca_p A_r$. This follows from eqn. (8a), which after reduction ($p=1; a \ll A$) and substitution by eqn. (7c) gives

$$\begin{aligned} (B-b)/b &= a\beta_{11} \approx A(hK_1 + h^2K_1K_2)^{-1}\beta_{11} \\ &\approx A(10^{pH}) \times \text{constant} \end{aligned} \quad (8b)$$

2.3.2. Analysis of the data for $B \leq 0.26$ mM. The value of B involves a fraction which is bound within the humic

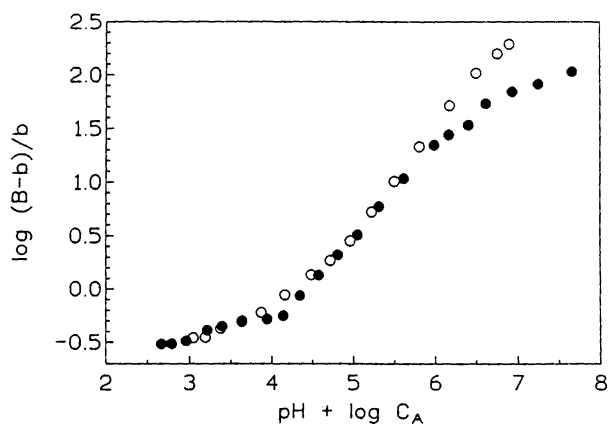


Fig. 4. Experimental data $\log(B-b)/b$ as a function of $(pH + \log C_A)$, see the text. \circ , $B=0.26$ mM, $C_A=2\ g\ l^{-1}$; \bullet , $B=0.13$ mM, $C_A=1\ g\ l^{-1}$.

acid particles, the concentration of which we do not know. However, at low pH the data of Fig. 3 tend to be independent of pH, indicating that $\log(B-b)/b$ then approaches a limiting value where $(B-b)/b = (B_i)_L/b$. The quantity $(B_i)_L$ is supposed to be the limiting fraction at low pH of intraparticle bound Ca^{2+} ions. For the two titrations of Fig. 4, we estimated the limiting value $(B_i)_L$ from the data at $\text{pH} \leq 3$ and eliminated it from B . Then the value of $\log \beta_{11}$ was estimated by extrapolation to a low pH, where the intraparticle bound Ca^{2+} ions, B_i , approaches $(B_i)_L$. For this purpose we used the function $\log F(\beta)$ obtained from eqn. (8b):

$$\log F(\beta) = \log[B - (B_i)_L - b]/b - \log A^* + \log F(h) \quad (8c)$$

where

$$A^* = A - (B - b) = A - [\text{CaA}] - [(\text{CaA})_i] \approx aF(h) \quad (7d)$$

and

$$F(h) = 1 + hK_1 + h^2K_1K_2 + 2ah^*\beta_{12} \quad (7e)$$

Here it is assumed that the small contribution to A of intraparticle bound calcium ions can be described as the 1:1 species $(\text{CaA})_i$. Since for these data $B \ll A$, the possible existence of other intraparticle species, such as $(\text{CaA}_2)_i$ or $(\text{Ca}_2\text{A})_i$, will not markedly change the value of A^* . The quantity $ah^*\beta_{12}$ in $F(h)$ is calculated via the approximation $A^*hK_1K_{12}/(1+hK_1)$, where K_1 and K_{12} are stepwise constants. The extrapolation of $\log F(\beta)$ is shown in Fig. 5, which yields $\log \beta_{11} = 3.7$.

2.3.3. Analysis of the data for $B \geq 0.5$ mM. In the first step we estimated B_i , the intraparticle concentration of Ca^{2+} ions. For these high B -values the concentration gradient of Ca^{2+} ions over the particle membrane may be sufficiently high so that the "back" exchange of protons is small, and thus the B_i -values may be assumed to be constant over the studied pH-range. The estimation was done at low pHs, where $\log(B-b)/b$ becomes approximately constant (Fig. 3). For instance, at $B = 1$ mM

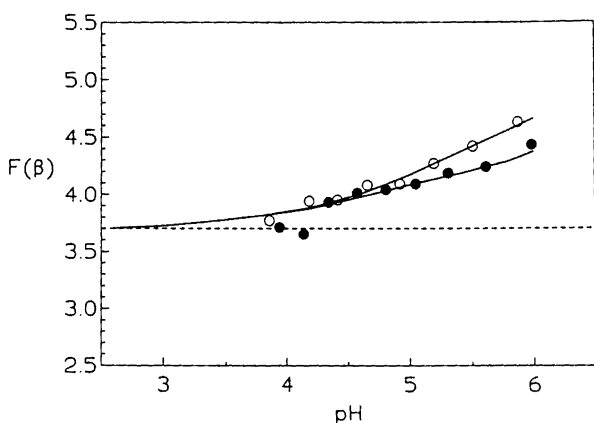


Fig. 5. Extrapolation of the function $F(\beta)$, plotted against pH, to a constant value giving $\log \beta_{11} = 3.7$. The symbols are the same as those in Fig. 4.

we obtained $B_i = 0.34$ mM, which corresponds to 0.17 mmol g^{-1} humic acids.

When we further analysed these data at high B , we found that those at $B = 1.73$ mM were less useful, since the difference $(B - B_i - b)$ was small relative to B , and thus suffered from relatively large errors. For the data $B = 1$ mM, it was indicated that a 1:1 species alone could not explain the data; most likely, a Ca_2A species coexists with CaA . On the other hand, for the data at $B = 0.5$ mM a fair agreement was obtained with the experimental data, if a CaA species was assumed. Then, the following equation was used [cf. eqn. (8c)]

$$\begin{aligned} \log Y &= \log(B - B_i - b)/b - \log A^* \\ &= \log \beta_{11} - \log F(h) \end{aligned} \quad (8d)$$

Using eqn. (7e), eqn. (8d) was normalized into the model function (cf. Refs. 29 and 30)

$$\log U = -\log[R + v + v^2 + 2RA^*vK_{12}/(R + v)] \quad (10)$$

where

$$\log Y - \log U = \log \beta_{11} + \log R \quad (11)$$

$$\text{pH} + \log v = \log K_2 \quad (12)$$

$$\log R = \log K_2 - \log K_1 \quad (13)$$

In the plot of $\log Y$ against pH the experimental data of eqn. (8d) were compared to a set of normalized curves $\log U(\log v)_R$ calculated from eqn. (10); Fig. 6 shows the result in the position of the best fit yielding $\log \beta_{11} = 3.7$. It was assumed that the acid-base constants were the same as those analysed by the Z -data (cf. Section 2.1).

2.3.4. Least-squares analysis. The results obtained graphically were subjected to a least-squares analysis using LETAGROP²⁴ as modified for the present system. The data of the two titrations at $B = 0.5$ mM and the one at $B = 1$ mM were used. In this analysis it was found that

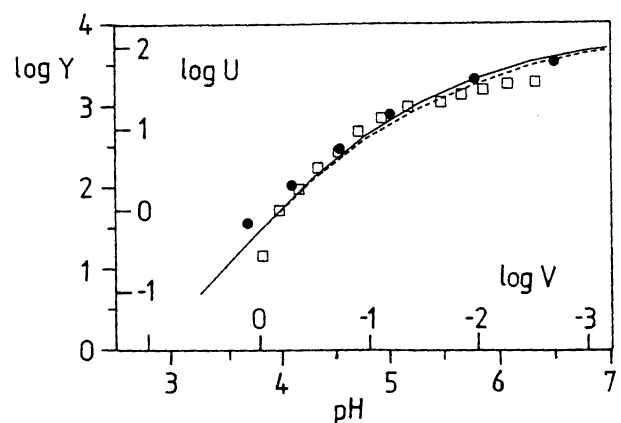


Fig. 6. Experimental data for $\log Y$ against pH [eqn. (8d)] compared with the normalized model function (fully drawn curve, $C_A = 1$ g l^{-1} ; dashed curve, $C_A = 2$ g l^{-1}) $\log U(\log v)_R$ for $\log R = -2.02$ in the position of the best fit giving $\log \beta_{11} = 3.7$. \square , $B = 0.5$ mM, $C_A = 1$ g l^{-1} ; \bullet , $B = 0.5$ mM, $C_A = 2$ g l^{-1} (every third point).

an improved agreement with the experimental data was obtained when the binuclear Ca_2A species was introduced. Other species, such as, for instance, CaA_2 , did not give any decrease in the error-square sum as compared to the combination CaA and Ca_2A . This latter combination gave $\log \beta_{11}=3.65$ and $\log \beta_{21}=6.73$.

Using these constants, we calculated the theoretical curves shown by Fig. 3; included in this figure are calculated curves for the three sets of data used in the refinement and also for $B=1.73$ mM. Equations (8a) and (7b) were used as well as the acid-base constants (see p. 978), and for each titration, a constant concentration of intraparticle bound calcium ions was assumed formally denoted as $(\text{CaA})_i$ species. It follows from Fig. 3 that the theoretical curves essentially describe the data. As expected the assumption of a constant B_i -value may not be completely fulfilled at high pH, especially for $B=1.73$ mM. Since the Z -data at this B -value are well explained (Fig. 2), we believe that this also has to do with limited precision in the $\log(B-b)/b$ data (cf. p. 980).

2.3.5. Comparison with Z -data. The apparent stability constants, β_{11} and β_{21} as well as the estimated intraparticle bound fractions of calcium ions, B_i , obtained from the calcium electrode data were also used to calculate theoretical curves for the corresponding Z -data (Fig. 2). However, for pH higher than 4.5 a clear-cut, relatively large deviation was observed (Fig. 7). This deviation was more pronounced for the lower ratio of $B/C_A=0.25$, than for the higher one, $=0.5$ mmol g^{-1} . As indicated in Fig. 7, the reason is most likely that calcium ions induce intraparticle dissociation of protons beyond those dissociating in the absence of calcium ions. Thus, indicating that for the higher pH, the true intra-

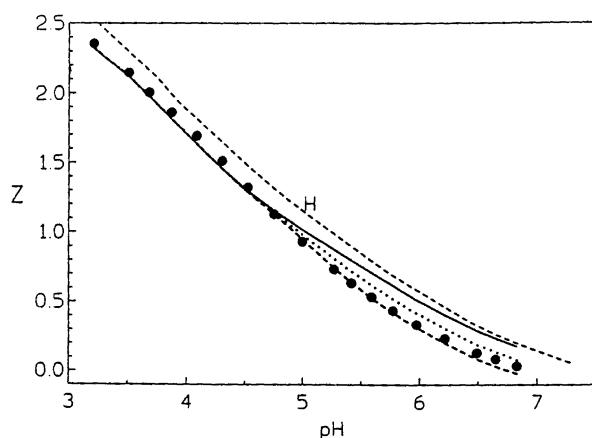


Fig. 7. Experimental data for Z against pH for $B=0.5$ mM and $C_A=2$ g l^{-1} , \bullet . The curves are calculated for the same stability constants as those given in the legend to Fig. 3, and for $B_i=0.21$ mM and $T=10.3$, assuming the release of none (fully drawn curve), one (dotted curve), and two (dashed curve) protons per B_i beyond those dissociating in the absence of calcium ions (curve indicated by H) assuming intraparticle complexes of the formal composition $(\text{CaH}_{-s}\text{A})_i$ with $s=0, 1$ and 2 , respectively.

particle species may be formally written $(\text{CaH}_{-1}\text{A})_i$ and $(\text{CaH}_{-2}\text{A})_i$ for the B/C_A ratios 0.5 and 0.25, respectively. This is illustrated in Fig. 7, where Z -data for $B=0.5$ mM and $C_A=2$ g l^{-1} are calculated assuming that none, one, and two extra protons are released per B_i . Also, it is assumed that a reversed exchange with protons occurs when the proton gradient increases at lower pH. This process, which depends on the diffusion flux densities of mainly H^+ and Ca^{2+} (see Ref. 31), was simulated by introducing an empirical parameter T (which depends on both C_A and B) and the function

$$F(T)=10^{T-2\text{pH}}(1+10^{T-2\text{pH}})^{-1} \quad (14)$$

In the curves shown in Fig. 2, the extra dissociation of one proton and two protons per B_i , formally described as $(\text{CaH}_{-1}\text{A})_i$ and $(\text{CaH}_{-2}\text{A})_i$, are assumed for the ratios $B/C_A=0.5$ and 0.25 mmol g^{-1} , respectively. In calculating Z , the term $sF(T)B_i$ was subtracted from $(H-h)$ in eqn. (4); s corresponds to the extra protons released as indicated by the $(\text{CaH}_{-s}\text{A})_i$ species. It follows from these data that the extra release of protons appears to be proportional to the inverse of the Ca^{2+} gradient across the particle membrane. Also, a more than three-fold increase of B , 1.73 mM, as compared to the B -value of Fig. 7, $=0.5$ mM, yields almost no extra release of protons, and the corresponding Z -data can be explained by a single $(\text{CaA})_i$ species in the whole pH range.

With the knowledge about the parameters s and T in mind, the β_{11} - and β_{21} -values were also refined using the Z -data for $B=0.5$ mM and $B=1$ mM. This gave $\log \beta_{11}=3.61$ and $\log \beta_{21}=6.75$; thus, values in good agreement with those obtained from the B/b -data. By using these constants the final theoretical Z -curves were calculated (Fig. 2). It follows from Fig. 2 that the three sets of Z -data are well described by these theoretical curves, as are also the Z -data at $C_A=1$ g l^{-1} for $B=0.26$ mM and $B=1.73$ mM (Fig. 2b). The remaining data at low B could not be analysed in this way (assuming a constant B_i -value for the whole pH range) owing to the fact that the intraparticle calcium ion concentration varies with pH. Nevertheless, these two titrations at $B/C_A=0.13$ mmol g^{-1} were used in the graphic analysis of the 1:1 species CaA (Figs. 4 and 5), and the estimated constant, $\log \beta_{11}=3.7$, agreed with that obtained when other sets of data were analysed.

Finally, the constants determined in this work were used to calculate the distributions of the complexes. Figure 8a shows the distribution over B and Fig. 8b shows that over A both represented as a function of pH. Note that Fig. 8 gives the ranges of existence at steady state for the proton and calcium ion binding of the humic acid particles formally denoted as the complexes H_qA_r and Ca_pA .

Discussion

The results described in the previous section has furnished support for the idea that humic acid particles in solution

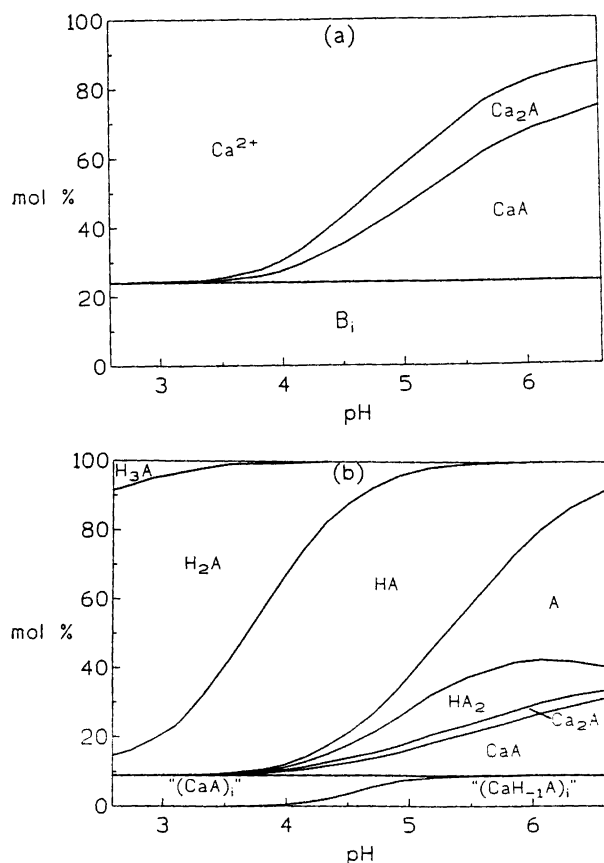


Fig. 8. Distribution of Ca^{2+} and H^+ among different complexes expressed as mol % of B (a) and mol % of A (b), where $A = 1.392C_A \times 10^{-3}$ M. The distributions refer to $B = 0.5$ mM and $C_A = 1$ g l $^{-1}$. For a certain pH, the mol % of a given complex is represented by the segment of the vertical line falling within the corresponding range. The intraparticle contribution to B is indicated by B_i (a) and as complexes with the index i, (b).

bind calcium ions both within the particles and on their surfaces. The diffusion of calcium ions into the humic acid particles is not surprising, considering that these particles mainly consist of water molecules, about 90% as indicated from the fractal dimension⁸⁻¹⁰ as well as from analysis of water in peat.¹¹ Nevertheless, this appears to be the first study in which it is shown that the metal ion interaction of humic acid particles involves a two-phase system in solution: (1) the intraparticle phase and (2) the bulk solvent phase involving the particle surfaces. Note that the humic acid particles of the present preparation dissolved into a homogenous solution (as indicated by neutron scattering), and this is valid for the present concentration ranges as well as for pH 7–2.5.

In earlier studies by Bunzl *et al.*,¹³ the metal ion diffusion into humic acid particles was explained by an ion-exchange mechanism, similar to that occurring in a synthetic ion exchanger. Then, the humic acid system seemed to obey mathematical models based on film diffusion rather than intraparticle diffusion. Our kinetic

study of calcium ions, best illustrated by Fig. 1 in Ref. 6, closely agrees with that reported by Bunzl *et al.*,¹³ the rate increases with increasing calcium concentration, which is consistent with film diffusion.

A further support for the idea that calcium ions diffuse into the humic acid particles is the data recorded by a calcium-ion sensitive electrode (Fig. 3) and the Z-data (Fig. 2). The dramatic deviation shown by the first set of data (see Section 2.3) and the clear-cut deviation shown by the Z-data (see Section 2.2) cannot be explained by what might have been expected from calcium ion reactions occurring in one single phase. These data clearly indicate that calcium ions escape into another phase, the humic acid particle phase, and that these calcium ions are not in equilibrium with the free calcium concentration in the bulk solvent.

Although the distribution of calcium ions between the two phases depends on the diffusion flux densities of the ions Ca^{2+} , H^+ , K^+ and Cl^- (see p. 975), in the present concentration ranges, the ions Ca^{2+} and H^+ are the most important for ion exchange.³¹ At high B, the gradient of Ca^{2+} may dominate over that of H^+ , so that the intraparticle bound calcium concentration can be estimated. This was done at low pH, where the binding to ligands on the surface is negligible (Fig. 8). These intraparticle-bound calcium ions were first tentatively assumed to be of the $(\text{CaA})_i$ type. However, the deviation of the Z-data for high pH (Fig. 7) indicated that complexes of the types $(\text{CaH}_{-1}\text{A})_i$ and $(\text{CaH}_{-2}\text{A})_i$ may also be involved; alternative combinations might also be possible such as, for instance, $(\text{CaH}_{-2}\text{A}_2)_i$ or perhaps $(\text{CaH}_{-2}\text{AC})_i$, (C belongs to a residue which is not titrated in the absence of calcium ions). This result is in good agreement with our previous work on the corresponding Cu^{II} system,⁶ which also involved extra proton dissociation beyond those dissociating in the absence of complex forming metal ions. A similar result was also reported from a study of cadmium ion complexes involving Z-data.²⁵ However, in that case, perhaps due to the limited contribution of metal ion binding to the Z-data (Figs. 2 and 8), Bolton *et al.*²⁵ assumed that the binding sites involved ligands on the surface of the particles. Regarding the present study, it is important to emphasize that the fairly weak complex forming calcium ion generally does not release protons from ligands which have pK-values far outside the measured pH range. Thus, the binding sites formally described as the species CaH_{-1}A and CaH_{-2}A are more an indication of the strong electric field produced by the bivalent calcium ion within the particles rather than an ability of calcium ions to form strong complexes. In principle, this field is the same for any bivalent metal ion,³¹ and thus so far Cu^{II} , Ca^{II} , and perhaps even Cd^{II} , appear to give similar results. However, so far the nature of their binding sites is unknown and they may not necessarily be the same.

In agreement with the present kinetic study involving stepwise addition of calcium ions to a humic acid solution, some kind of 'saturation' of the intraparticle bound

Table 1. Parameters used for calculating the theoretical curves in Fig. 2.^a

$C_A/g\ l^{-1}$	B/mM	B_i/mM	T	s
1.0	0.26	0.085	9.5	2
1.0	0.50	0.12	9.17	1
1.0	1.73	0.18	—	0
2.0	0.5	0.21	10.3	2
2.0	1.0	0.34	10.05	1

^aIncluded in these calculations are also the β_{11} and β_{21} constants listed in the legend to Fig. 2 as well as the acid–base constants given in the text on p. 978.

Ca^{2+} ions, B_i , seem to be reached. When going from about 1 mM to 1.73 mM, the τ -value decreases only with about 3 s (Fig. 1) and the intraparticle bound calcium ions, B_i , seem to be approximately the same, i.e. 0.17 and 0.18 mmol g^{-1} humic acid, respectively (Table 1). For $B \leq 1$ mM the B_i contribution to B is substantial, about 24–42 mol % (Table 1).

The complex formation occurring in the bulk solvent phase was analyzed in three steps. First the predominating values of the coefficients ($p; r$) for complexes of the general composition Ca_pA_r were analyzed and found to be (1; 1), (Fig. 4). Then this result was analyzed graphically giving the first value of the stability constant for CaA (Figs. 5 and 6) and hinting that a minor complex Ca_2A exists. Finally, the constants obtained graphically were improved by least squares analyses, independently involving B/b -data and Z -data giving the apparent constant for the main CaA complex, $\log \beta_{11} = 3.6$, and for the minor Ca_2A complex, $\log \beta_{21} = 6.7$.

In our previous study⁶ we obtained a similar result indicating the formation of the 1:1 CuA complex on the surface of the humic acid particles. The value of the apparent stability constant was fairly close to those reported for dicarboxylic acids, such as malonic acid, and it was suggested that the site may involve two juxtapositioned carboxylates. Also, in this study it is tempting to speculate that carboxylate groups might be the ligands involved, especially since Ca^{2+} ions generally prefer oxygen ligand atoms to other kinds of donor atoms. However, low-molecular-weight carboxylates, which, of course, are quite different from the large humic acid particles, yield somewhat lower $\log \beta_{11}$ -values:³² oxalate ranges from 1.7 to 3.0, and malonate from 1.9 to 2.5.

A comparison of the present results with those reported by other investigators is difficult owing to the fact that not one of them considered the non-equilibrium nature of the humic acid system nor the existence of a two-phase system (cf. the reviews 3–5). In some early studies there were no precautions made for the sensitivity of humic acids to oxygen.³³ Furthermore, as discussed by Sposito *et al.*,⁷ the design of the measurements as well as their representation makes it difficult for readers to find out what kind of reactions really occurred in their systems. In this latter study,⁷ on the calcium ion com-

plexes of fulvic acids, two different types of binding were reported; however, no stability constants and no composition of the complexes were given. Nevertheless, there was a hint that some complex might be of the 1:1 type, and Ca^{2+} ions showed a modest ability to release protons from undissociated groups. Thus, although there are obvious differences between fulvic acids and humic acids,² there are certain pieces of qualitative correlation between their study⁷ and some of our results.

Finally, we would like to emphasize the possible importance of the binding of the metal ions within the humic acid particles for processes occurring in soil and water systems (so far, involving calcium and copper ions).⁶ The intraparticle binding apparently gives the humic acids the capacity to store metal ions at relatively high acidity (Fig. 8), which means that even if humic matter is repeatedly subjected to acid rain, calcium and copper ions may still be available for plants, for instance via microbiological activity. As a result, the binding and storage of metal ions within the humic acid particles may be of major consideration, and this should be kept in mind when the speciation and mobility of metal ions are discussed.

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