

## Studies of Fulvic and Humic Acids 2. Protolytic Processes of Humic Substances from a Brown-Water Lake and from a Meadow Soil in 0.1 M NaCl Aqueous Solution at 25 °C

Anette Bergelin,<sup>a</sup> Olle Wahlberg<sup>b,\*</sup> and Staffan Ågren<sup>a</sup>

<sup>a</sup>Department of Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden and <sup>b</sup>Department of Physical, Inorganic and Structural Chemistry, Stockholm University, S-106 91 Stockholm, Sweden

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Proton affinity spectra for humus in lake water and in soil were recorded by a high precision emf titration technique in the pH interval  $2.5 < \text{pH} < 8.5$ . The lake water was examined without any preconcentration procedure. Extrapolated steady-state values were used as approximations to the equilibrium values. The observations could be explained by a simple model with three protonated surface sites, with the  $\text{p}k_a$ -values  $2.6 \pm 0.1$ ,  $4.4 \pm 0.1$  and  $6.7 \pm 0.1$ . Some sites were connected by hydrogen bonds, which could be observed as dinuclear surface complexes corresponding to an apparent  $\text{p}k_a = 5.4 \pm 0.1$ . The hydrogen-bonded particles have a limited solubility, with  $\text{p}k_s = 5.4 \pm 0.1$ . The humic acids from the soil were sparingly soluble in acid solution, with  $\text{p}k_s = 4.1 \pm 0.1$ . A dynamic mechanism for keeping the lake water buffered at ca. pH 5.4 is suggested. A surface potential of  $-6 \pm 2$  mV is reported for the lake humus.

The acid–base properties of humic substances have been studied by many researchers, cf. Table 1. Humic and fulvic acids are responsible for the protolytic properties of brown-water lakes. A workshop report about those acids has been edited by Perdue and Gjessing.<sup>16</sup> Humus is important for the regulation of nutrient availability in soils, which is pH-dependent.<sup>17</sup> Several models have been applied to explain the properties of humic substances,<sup>16,18</sup> of which proton affinity spectra<sup>17</sup> will be used in this study. This method has the advantage that separate functions are used for different sites, and each site is modelled by parameters for the total analytical concentration of the site and its microscopic formation constant. The observed proton affinity spectrum is explained by the superposition of the spectra for the different sites. The identified protolytic functions are subtracted in the data analysis, until the background noise level is reached. Perdue<sup>16</sup> gives an overview of current models to describe the acid–base properties of DOC. He emphasises that the present models can predict the degree of dissociation as a function of pH, but not the concentrations of DOC and its dependence of the ionic strength or the interaction with metal ions. We encountered two major problems in describing humic substances in natural water. One is the

change over time. Humic substances in water are dynamic systems.<sup>19</sup> Such a system can in favourable cases be described as a steady-state or a quasi-equilibrium state. The addition of a small amount of acid or base to the lake water, or a minor change of the total concentration of humic substances, causes a perturbation of the system. The relaxation to a new steady-state can take several hours. In a previous report, the steady-state rates were determined as a function of pH and total concentration of humic substances.<sup>19</sup> The difficulties in predicting DOC concentrations could be related to the tendency of humic substances to precipitate. Proton affinity spectra can be used to study this problem, since the presence or absence of solid particles can be seen in the graph.

### Objectives

In this study the best available technique is used to determine the acid–base properties of humic substances, by direct titrations of water from an oligotrophic lake and water solutions of humic substances extracted from a meadow soil. We intended to study the pristine lake water: only sodium chloride was added to give a constant ionic strength of 0.1 M, for collection of high-precision data. We plan, however, to collect data at lower ionic

\* To whom correspondence should be addressed.



strength in a future investigation; the ionic strength of the natural water studied is about 1 mM.

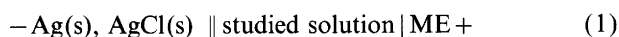
### Symbols, units and activity scale

Concentrations are expressed in mol dm<sup>-3</sup>, and the concentration of H<sup>+</sup> will be written as  $h$ . pH =  $-\log(\gamma_H[H^+])$ , where  $\gamma_H$  is the activity coefficient. Site no.  $i$  will be referred to as  $Di$ . The fully protonated site HD $i$  is chosen as the zero point for the proton balance. The concentration of the protonated site is written  $di = [HDi]$ . The interaction of a site with protons will be described as a complex species  $(Di)_rH_{p+r}$  with the concentration  $di_{pr}$ . The sites will also be named Site A, Site B, etc.  $H_{tot}$  and  $Di_{tot}$  denote the total concentrations. A normalised variable  $u = \beta_{-11}h^{-1}$  is introduced to simplify the calculations. The activity coefficients of the low-molecular species are assumed to be constant. This is justified here, since sodium chloride is in at least 50-fold excess. In this study  $\gamma$  is set to unity. Therefore, the constants reported are valid in 0.100 M NaCl. The concepts fulvic and humic are operationally defined.<sup>20</sup> We refer to smaller molecular sizes with the former and to larger molecules with the latter word.

### Experimental

**Chemicals and analyses.** Strong acids and bases were prepared and analysed as described by Li *et al.*<sup>21</sup> Samples of water were taken directly from Snuggan, which is a brown-water lake situated 15 km north of Stockholm. The lake water was passed through a 0.45  $\mu$ m filter. Soil humus has been extracted from a meadow at Kungsängen 5 km south of Uppsala, Sweden. The soil humus was dissolved in 5 ml distilled water and dialysed against 10 mM EDTA for 1 h and against distilled water for 24 h (cut-off = 8000 Da). The water from Snuggan contains a mixture of fulvic and humic acids with the former in excess, while high-molecular humic acids predominate in the soil samples.

**Measurements.** Sodium chloride and hydrogen chloride were added to make solutions with 0.1000 M NaCl and ca. pH 2.5. The experiments were performed as titrations, adding 0.1148 M NaOH solution from a buret to the solution containing the humic substances. The equipment has been described elsewhere.<sup>19</sup> We measured the emf of the following cell:



ME is either a glass or a hydrogen electrode. The hydrogen electrodes were only used for calibration. The solution in the salt bridge and in the studied solution both contained 0.1 M NaCl. pH was calculated from:

$$E = E^\circ + 59.155 \log h + jh \quad (2)$$

The liquid junction-potential is approximated by the function  $jh$ , where  $j$  is a constant. We found typically

$E^\circ = 369.18$  mV and  $j = -462$  mV M<sup>-1</sup>. Altogether 12 000 data points were measured and evaluated.

**Computer programs.** The following computer programs were used: 1. MATHCAD, MathSoft Inc. USA. 2. GRAPHER, Golden Software Inc. USA. 3. OFFICE, Microsoft USA. 4. LETAGROP,<sup>22</sup> revised version KTH Sweden (least squares). 5. SOLGASWATER<sup>23</sup> Umeå Sweden (species modelling).

### Data treatment and results

In the calculations described below extrapolated steady state values were used. They were measured at 12-min intervals for a period of at least 3 h after the additions of acid or base to the humate solution.

**Thermodynamic model.** The studied surface reactions can be written:



where  $Di$  is the surface site type no.  $i$ . The protonated sites HD $i$  and H<sup>+</sup> were chosen as components. The mass balance equations are written as:

$$Di_{tot} = di + \sum r di_{pr} \quad (4a)$$

$$H_{tot} = h + \sum p di_{pr} \quad (4b)$$

with

$$di_{pr} = \beta_{pr} h^p di^r \quad (4c)$$

for dissolved species and

$$l = \beta_{pr}^* h^p di^r \quad (4d)$$

for sparingly soluble species.

The average number of H<sup>+</sup> dissociated from surface site no.  $i$  is written:

$$Zi = p \beta_{pr} h^p di^r / (di + \beta_{pr} h^p di^r) \quad (5a)$$

or

$$Zi = -u / (l + u) \quad (5b)$$

with  $r = 1$  and  $p = -1$  for a singly protonated site.

**Polyelectrolytical behaviour.** Humic substances are known to have electrically charged surfaces.<sup>20</sup> We have assumed that the surface is neutral when all sites are protonated. The components were chosen so that  $Zi$  is a measure of the fractional charge of the surface site  $i$ , cf. eqns. (5a) and (5b). The planar approximation of the surface was used to estimate the effect of the surface charge on the data.

$$h_{\text{surface}} = h_{\text{bulk}} \exp(-\Psi F / RT) \quad (6a)$$

with

$$\Psi F / RT = Z \Psi^\circ / 25.68 \quad (6b)$$

$\Psi$  is the surface potential and  $Z$  is the fraction of the protons dissociated from the surface sites. We define  $\Psi = \Psi^\circ$  for  $Z = 1$ .

*Proton affinity spectrum.* The total number of protons bound to the system ( $H-h+k_w h^{-1}$ ) as a function of  $\log h$  was observed. Since the data points were collected at small  $\log h$  intervals, we can use the quotient below to approximate the derivative:

$$Y_{\text{obs}} = d(H-h+k_w h^{-1})/d\log h \approx [\Delta(H-h+k_w h^{-1})/\Delta\log h] \quad (7a)$$

Assuming  $i$  protonated sites on dissolved species, eqns. (4)–(6) can be used to derive a function  $Y$ , of the normalised variable  $u$ :

$$Y_{\text{calc}} = \ln 10 \times \Sigma [Di_{\text{tot}}u/(1+u)^2] \quad (7b)$$

where the summation is made over the different sites. The function  $Y_{\text{obs}}(\log h)$  shows a series of more or less overlapping peaks (Fig. 1). Each peak refers to a protonated site  $i$  with the peak maximum at  $\text{pH} = \text{p}K_a$ . The area under the peak is a measure of  $Di_{\text{tot}}$ , the total concentration of the surface site  $i$ . When a peak has been identified, it is subtracted to reveal the hidden peaks. The presence of solid particles can be detected in the proton affinity spectrum if they interact with the dissolved species.

*Analysis of the proton affinity spectrum.* The original proton affinity spectra are shown in Figs. 1–3. Discontinuities were observed at ca. pH 3 and at ca. pH 8, and they were interpreted as interactions with solid particles. As seen in Fig. 3, the humic acids from the soil are precipitated in acid solutions. The humic acids dissolve when pH is raised and protons are dissociated, either at ca. pH 2.6 or at ca. pH 4.1. The negative values

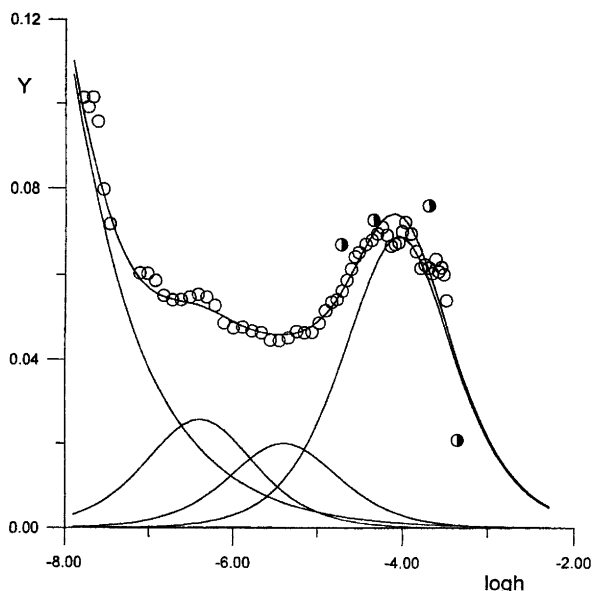


Fig. 1. The proton affinity spectrum for the lake water. The solid curves were calculated with MATHCAD for the model (9). The lake water was first acidified with HCl, and then titrated with NaOH (○). The half-filled symbols represent a back titration.  $\text{DOC} = 28 \text{ mg dm}^{-3}$ .

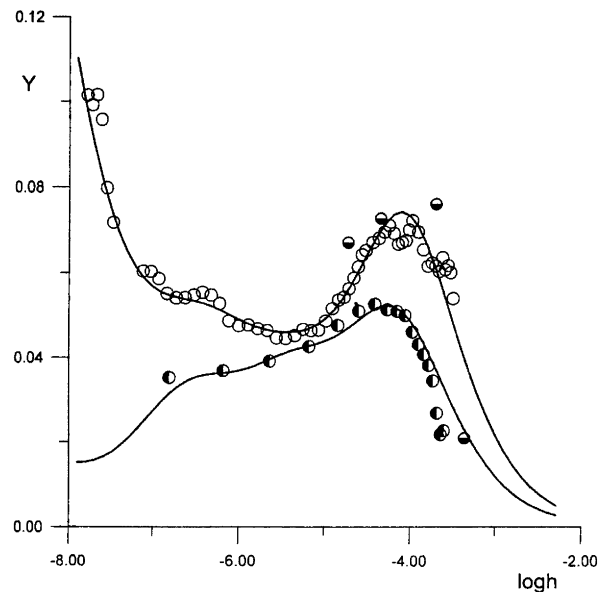


Fig. 2. The proton affinity spectrum for the lake water. The solid curves were calculated with MATHCAD for the model (9). The sample in the upper curve was passed through a  $0.45 \mu\text{m}$  filter, and that of the lower curve was also passed through a cation exchanger before the titration.  $\text{DOC} = 28 \text{ mg dm}^{-3}$ .

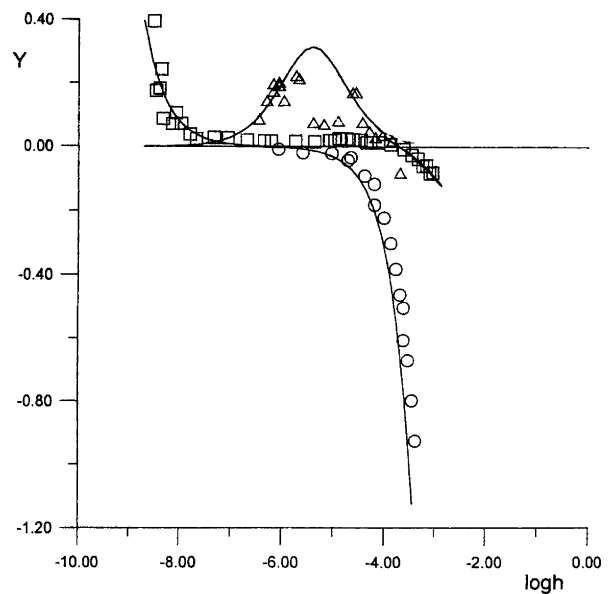


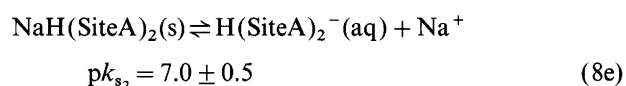
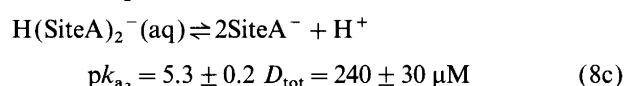
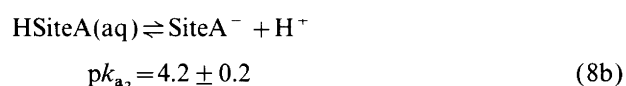
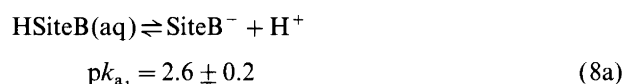
Fig. 3. The proton affinity spectra for the soil humus. The solid curves were calculated with MATHCAD for the model (8). The curves correspond to  $116 \text{ mg DOC dm}^{-3}$  (□),  $28 \text{ mg DOC dm}^{-3}$  (△) and  $7 \text{ mg DOC dm}^{-3}$  (○).

of  $Y$  mean that the solid particles contain less protons than the dissolved species. Different compositions,  $(Di), H_{p++}$ , of the solids were tested, cf. eqn. (3). The data could be best explained by assuming the presence of  $\text{HD}(s)$  in acid solution. From eqns. (4) and (5) we derived  $Y_{\text{calc}} = -\beta_{11}^* u$ , for the reaction  $\text{HD}(s) \rightleftharpoons \text{D}^- + \text{H}^+$ . The soil humus and the lake humus show similar behaviour at ca. pH 8, and from eqns. (4)

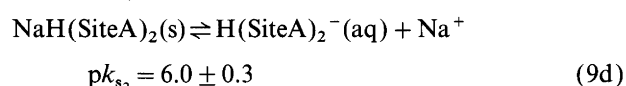
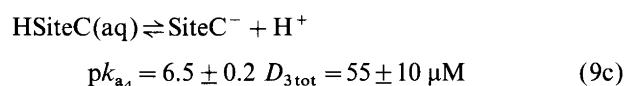
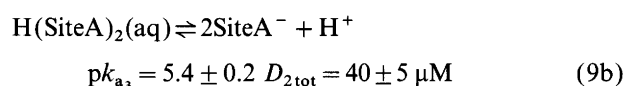
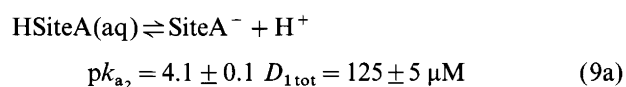
and (5) we derived  $Y_{\text{calc}} = -lu^{-1/2}$ , with  $l = \beta_{-12}^* \beta_{-11}^{1/2}$  for the reaction  $\text{NaD}_2\text{H}(\text{s}) \rightleftharpoons 2\text{D}^- + \text{H}^+ + \text{Na}^+$ . We had to assume that sodium is involved in the reaction to balance the charges. Sodium is a part of the constant ionic medium, but was not accounted for in the calculations, thus the constants are valid in 0.100 M NaCl. Subtraction of the solid species from the affinity spectrum reveals the peaks at pH 4.1, 5.4 and 6.7. They were identified and subtracted to give the residual  $Y$ . The scatter in the residual  $Y$  is a few  $\mu\text{M}$  of  $\text{H}^+$ , which is the precision of the measurements. The polyelectrolytical surface charge would make the peaks broader, but no such effect could be observed.

*Apparent quasi-equilibrium constants.* The apparent  $\text{p}k_{\text{a}}$ -values, obtained from the proton affinity spectrum, give very useful information about the system. They were obtained by graphical fitting of the model to the data.

Soil humus:



Lake humus:

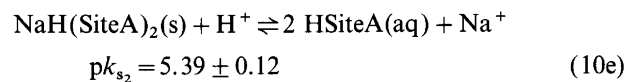
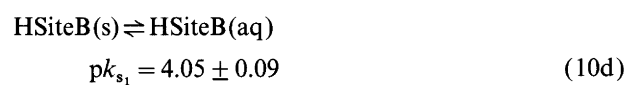
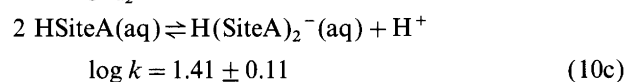
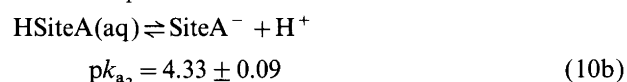
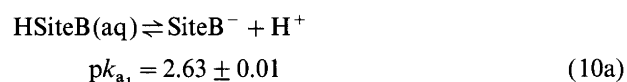


The same quasi-equilibrium constants were obtained for the two kinds of humus. The parameters  $\text{p}k_{\text{a}_1}$ ,  $\text{p}k_{\text{a}_2}$  and  $\text{p}k_{\text{a}_4}$  describe the surface protonation, while  $\text{p}k_{\text{a}_3}$  reflects a dimerisation of the surface sites. The solubility of the apparent dimers is given by  $\text{p}k_{\text{s}_2}$ . The limited solubility in acid solution is modelled by  $\text{p}k_{\text{s}_1}$ . Other metal ions could influence the protolysis. The lake water contained 0.12 mM  $\text{Ca}^{2+}$ , 0.02 mM  $\text{K}^+$ , 0.05 mM  $\text{Mg}^{2+}$  and 0.02 mM  $\text{Al}^{3+}$ . One sample of lake water was passed through a cation exchanger to remove the free metal

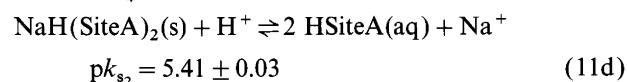
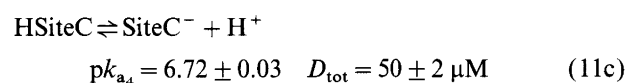
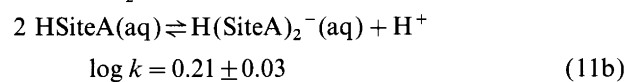
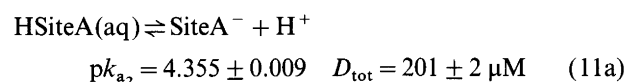
ions, leaving 11  $\mu\text{M}$   $\text{Al}^{3+}$  and 8  $\mu\text{M}$   $\text{K}^+$  in the solution (cf. Fig. 2). Aluminium and potassium are obviously bound to the humic substances. We tried to minimise this effect in the present study, and within the limits of error we could not detect any influence of aluminium or potassium on the protolysis. We also found 5–10  $\mu\text{M}$  iron in our humate solutions.

*The refined model.* The apparent equilibrium constants are used to model the different buffer maxima. However, we want to use our precise data for a more detailed analysis, assuming that the equilibrium approximation holds. The relevant timescale here is hours to weeks. Within this time the aggregates may change in size or conformation. We have varied the total concentration of the humic material in order to estimate the degree of aggregation. A problem is the precipitation of solid phases, which limits the range of concentrations of dissolved species. The result from the graphical analysis of the proton affinity spectrum was used as a starting point for the least-squares treatment. We tested models with different combinations of dissolved and solid complexes. The total concentration of the reacting sites was 250  $\mu\text{M}$ . The standard deviation of  $\text{H}^+$  is less than 1  $\mu\text{M}$ . A surface potential  $\Psi^{\circ} = -6 \pm 2$  mV gave the best fit with the present data. The zeta potential of the lake humus was measured to be  $-5$  mV at ca. pH 5.5. Below are listed the reactions and constants of the final quasi-equilibrium models valid at 25 °C and in 0.1 M NaCl solutions. The same constants were obtained with different samples, but the amount of protons titrated differed.

Soil humus:



Lake humus:



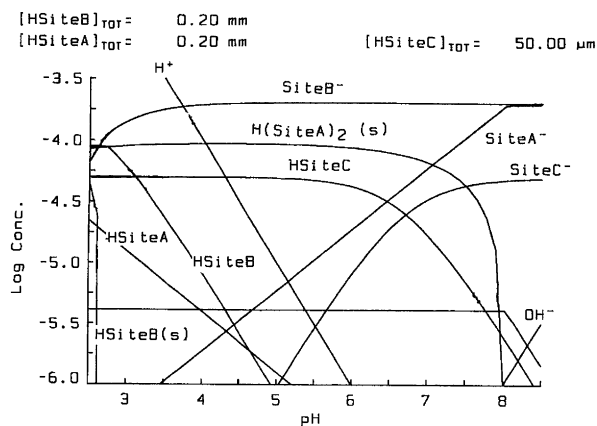


Fig. 4. Species distribution diagram for the soil humus studied, calculated with SOLGASWATER. The tentative reactions and constants are given in eqns. (10a)–(10e) and (11c).

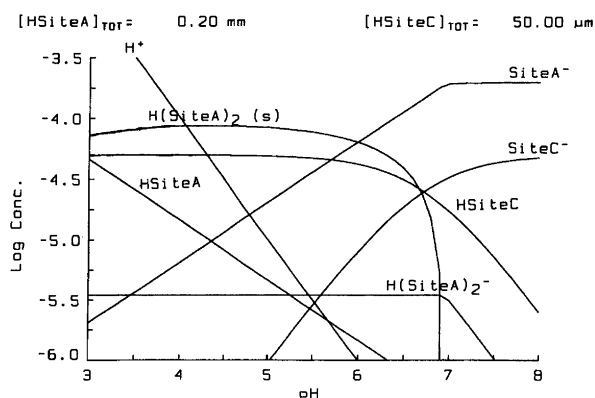
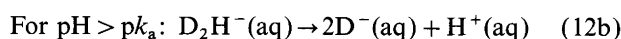
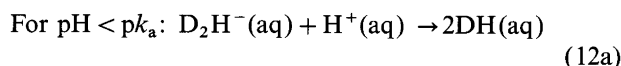


Fig. 5. Species distribution diagram for the lake humus studied, calculated with SOLGASWATER. The tentative reactions and constants are given in eqns. (11a)–(11d).

The errors given are  $3\sigma$ , where  $\sigma$  is the standard deviation. The small errors indicate a high precision of the data. However, systematic errors may influence the data. The models are tentative. Figures 4 and 5 show the species distributions as a function of pH. We could not calculate  $pK_{a4}$  from the soil humus data, but HSiteC was assumed to be present in small amounts. The dinuclear complex  $H(\text{SiteA})_2(\text{aq})$  gave the best fit for the lake water and the soil water. The graphical analysis gave the apparent  $pK_a$ -values in eqns. (8c) and (9b), while the least-squares analysis was used to calculate the dimerisation equilibrium constants in eqns. (10c) and (11b). The formation constants of the dimers are different for the two types of humus. The aggregation seems to be higher in the soil humus than in the lake humus, which is expected since the former contains more high-molecular humic acids.

*The dynamic buffer system in Lake Snuggan.* It has been found earlier that the steady-state rate changes sign at pH 5.4, close to the maximum of the peak for  $D_2H(\text{aq})^-$ .<sup>19</sup> Protons are consumed below pH 5.4 but produced at higher pH-values. This would be consistent

with a change of protons involved in hydrogen bonding:



The dynamic molecular mechanism involves time-dependent particle interactions, but can be approximately described by an apparent  $pK_a = 5.4$ .

## Discussion

It was necessary to use both kinetic and quasi-equilibrium processes to explain our observations. The protonation of surface carboxylate groups is probably rapid, while various changes of the aggregates may be slow. The water in Lake Snuggan is buffered by the changing particles according to eqn. (12). However, the timescale may be weeks. Three buffer maxima were observed for the lake humus, at ca. pH 4.4, 5.4 and 6.7. The soil humus precipitated in acid solution, cf. Fig. 3, which is in accordance with the operational definition of humic acids.<sup>20</sup> It is interesting to note the agreement between our apparent  $pK_a$ -values and those reported by Paxeus *et al.*<sup>3</sup> They preconcentrated their samples and performed rather quick titrations lasting only 1 h. The acid-base properties of the soil humus and the lake humus in this report are also similar, even if the data seem very different, depending on the distribution between dissolved and precipitated material (cf. Figs. 1–3).

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